Novel Germline and Somatic Mutations of the MSH2 Gene in Hereditary Nonpolyposis Colorectal Cancer. *D.C. Ding[1,2], R.L. Huang[3], T.Y. Chu[1], K.S. Hwang[3]* 1) Dept of Obstetrics and Gynecology, Buddhist Tzu Chi General Hospital, Hualien, Taiwan, R.O.C; 2) Graduate Institute of Medical Science, Tzu Chi University, Haulien, Taiwan, R.O.C; 3) Dept of Obstetrics and Gynecology, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan, R.O.C.

The non-epigenetic somatic second hits of the mismatch repair genes in hereditary nonpolyposis colorectal cancer (HNPCC) were not experimentally characterized before. We describe a HNPCC family in which the proband was found with synchronous cancers of endometrium, ureter, and bladder. A novel germline IVS 12 -2A>G splice acceptor site mutation of the MSH2 gene, 4 bases downstream to the known IVS12 -6T>C polymorphism site, was identified. The allelic specific second hits in different primary tumors were analyzed. In an in-situ transitional cell carcinoma, the wild type allele was lost by loss of heterozygosity (LOH), leaving a transcript skipping the exon 13. In the invasive endometrial carcinoma, LOH of the germline mutant allele occurred. This allowed the discovery of a somatic c.1637 delT mutation at exon 10 of the wild type allele. Replication error phenotype was evident in both of these two tumors. The novel splice acceptor site mutation underscores the importance of the canonical sequences around the splice acceptor site in RNA splicing. To our knowledge, this is the first discovery of a non-deletion somatic mutation of the wild type allele of the mismatch repair gene in HNPCC tumor.

Long QT, Syndactyly, Cardiac Defect, Joint Contractures and Stroke: Expanding the Clinical Spectrum of Timothy Syndrome, or a New Syndrome? *J. Gillis*[1,2], *G. Gross*[2], *D. Chitayat*[1,2] 1) Dept. of Medical Genetics, University of Toronto, Toronto, ON, Canada; 2) The Hospital for Sick Children, Toronto, ON, Canada.

Timothy Syndrome (TS) is a rare disorder characterized by cardiac arrhythmia, congenital heart disease, syndactyly, facial anomalies and neurologic sequelae including seizure, mental retardation, hypotonia and autism. Previous reports have shown that TS results from a de novo missense mutation in the $Ca_v1.2$ L-type calcium channel (CACNA1C) gene. Of the few cases reported, most present in the newborn period with no other known affected family members. Despite elucidation and characterization of the causative gene, the mode of inheritance remains elusive, and the prognosis is poor. We report a male newborn, born to a healthy, non-consanguinous couple, following an uncomplicated pregnancy and delivery, who presented with marked ventricular repolarization delay with several self-limited episodes of torsades des pointes and prolonged QTc interval on ECG. On clinical exam, he had wide fontanelles, split sutures and facial dysmorphism including: high forehead, frontal bossing, deep set eyes, micrognathia and fleshy low-set ears. Others features included short neck, sloped shoulders and syndactyly of the fingers and toes, ulnar deviation of the 2nd-5th fingers and dorsiflexion contractures of both hands and bilateral dorsiflexion of both feet. Subluxation of both hips was confirmed by ultrasound. He developed seizures, suffered an acute left hemispheric infarct and further episodes of arrhythmia. Rate control was achieved with beta-blocker. On early follow up, he demonstrates high intelligence and is advanced with his developmental milestones. There was no evidence of immune deficiency. Results of mutation analysis of the CACNA1C gene are pending. Collagen studies are being done to rule out concurrent connective tissue disease. Most of the findings in our patient are consistent with Timothy Syndrome. However, the contractures, facial dysmorphism and stroke have not yet been reported with this condition. This may be other manifestations not yet reported, or a new syndrome.

Molecular differences in breast tumors from African American and Caucasian women. *R.E. Ellsworth[1], J.A. Hooke[2], C.D. Shriver[2]* 1) Clinical Breast Care Project, Windber Research Inst, Windber, PA; 2) Clinical Breast Care Project, Walter Reed Army Medical Center, Washington, DC.

Breast carcinomas display different characteristics in African American women (AAW) compared to Caucasian women (CW), including earlier onset and less favorable clinical outcome. The less favorable prognosis in AAW has often been attributed to socioeconomic status, including access to quality-health care, however, these differences in outcome may also have a genetic contribution. To assess whether breast tumors from AAW differ at the molecular level from those from CW, a number of molecular markers, including ER, PR, HER2, Ki67, and p53 were examined in invasive tumor specimens from AAW (n=66) and CW (n=282) treated at Walter Reed Army Medical Center (WRAMC), an equal-access health care facility. When examined independently, breast tumors from AAW had significantly ($P<0.05$) higher frequencies of the following markers compared to those from CW: ER negative (46% vs. 20%) and PR negative (53% vs. 27%) negativity, and positive HER2 status (44% vs. 27%). AAW more frequently had the triple negative (ER-, PR- and HER2-) phenotype ($P<0.005$) than CW. Ki67 levels did not differ significantly between the two groups, however, differences in p53 status were significant, with 52% of tumors from AAW having positive p53 status compared to 30% of tumors from CW. Because 100% of the AAW patients with invasive breast cancer received regular screening mammograms, differences in tumor phenotype and outcome were likely not attributable to under utilization of early medical interventions. Together, these data suggest breast tumors in AAW differ at the molecular level from those in CW.

An infertile male with apparent 45,X turned out to have 45,X,der(Y)t(Y;13)(q11.1;q11),-13; Clinicopathologic and cytogenomics studies. *Y.X. Cui[1], X.Y. Xia[1], L.J. Pan[1], Y.H. Wang[1], J. Xu[2], Y.F. Huang[1]* 1) Department of Reproduction and Genetics, Jinling Hospital, Nanjing University School of Medicine, Nanjing, Jiangsu, China; 2) Cytogenetics Laboratory, London Health Sciences Centre and University of Western Ontario, Canada.

A 25-year-old Chinese man with a history of 3 years of infertility was diagnosed with azoospermia. His testes were slightly soft and marked small (8 ml, bilaterally vs.19.87.1 ml for a normal adult Chinese). He had normal male external genitalia, relative poor beard and slightly bilateral gynecomastia. His intelligence and development were normal. His serum testosterone was 7.9nmol/L by radioimmunoassay (vs. 9.4-37.0 nmol/L for a normal adult Chinese). His FSH, LH, estradiol, and prolactin concentrations were within normal ranges. Biopsy of the left testis showed the presence of only Sertoli cells and absence of germ cells in all seminiferous tubules examined. Initial routine cytogenetics of both lymphocytes and testicular fibroblasts suggested a karyotype of 45,X with a bit of uncertainty about the proximal regions of one chromosome 13. PCR using STSs including sY14 indicated the presence of SRY gene in the genome. FISH using probes wcp13, SRY, and X/Y CEP showed the presence of SRY and Y centromere in the questionable chromosome 13. The parents had a normal karyotype. The patient's karyotype was reinterpreted to be 45,X,der(Y)t(Y;13)(q11.1;q11),-13.ish der(Y)(SRY+, DYZ3+, wcp13+). This is an unbalanced karyotype with deletion of 13pter-q11 and Yq11-qter including the 3 azoopermia factor regions AZFa, b and c. PCR using 16 STSs in AZFa (interval 5C), AZFb(interval 5P-6A), and AZFc(interval 6D-6F) mapped the breakpoint to the interval 5B below sY82. DNA polymorphism analysis showed the chromosome X is of maternal origin. We propose that Y;13 recombination likely occurred during paternal meiosis. In literature, there are 9 cases of 45,X male with Y-acrocentrics (14, 15, 21, 22) translocation involving Yq11. To our knowledge, this is the first reported case with a 45,X,der(Y)t(Y;13) (q11.1;q11),-13, which will be useful for the phenotype/molecular karyotype correlation.

Co-occurring asthma and externalizing symptoms: Genetic and environmental influences by child gender and reporter. *R.P. Goin-Kochel[1], J.L. Silberg[2], L.J. Eaves[2]* 1) Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Virginia Institute for Psychiatric & Behavioral Genetics, Virginia Commonwealth University, Richmond, VA.

Background and Purpose: Prior work has often detected a relationship between asthma and externalizing symptoms in children, and one study concluded that the association was based on a common genetic etiology. However, this work relied solely on parental report of childrens symptoms and did not have the statistical power to analyze data separately by child sex. The purpose of the current project, then, was to help clarify the nature of the relationship between these two phenotypes. **Methods**: We examined the relationship between asthma and externalizing symptoms among a population-based sample of adolescent twins (n = 1014) to assess the relative influence of both shared and trait-specific genetic and environmental influences. Maternal reports of asthma and both child and maternal reports of externalizing behaviors from the Virginia Twin Study of Adolescent Behavioral Development (VTSABD) were analyzed for boys and girls separately using structural equation modeling within the classic twin-study design. **Results**: Twin correlations were higher in MZ versus DZ twins for the two traits. For girls, the relationship between asthma and externalizing symptoms was best explained solely by additive-genetic factors, whereas for boys, this relationship was best accounted for by individual specific-environment effects. These findings were observed within both maternal and child reports, although parameter estimates differed between the two. **Conclusions**: Our results suggest different etiologies for co-occurring asthma and externalizing behaviors between the sexes. They further highlight the importance of considering gender differences, particularly in the context of adolescence, where different developmental trends may be at work, as has previously been reported for asthma. Future studies may elucidate which of these phenotypes presents first, potentially influencing the development of the second, by examining these data longitudinally, beginning at the earliest ages possible, when asthma is most acute.

Reciprocal Chromosomal Translocations, 47,XY, t(11;14) +der(14) t(11;14) (q25;q24.1), 46,XY,t(1;4) (q11-12;q11), 46,XY,t(6;9) (p21;q34) and 45,XY, der(13;15) (q10;q10). Four Mexican Pediatric Cases Report. *J. Aparicio[1,6], M. Barrientos[2], M.L. Hurtado[3], C. Gil[4,6], R. Ruiz[5], W. San Martin[4], C. Salinas[7]* 1) Dept Genetics,; 2) Endocrinology,; 3) Cytogenetics,; 4) Estomatology,; 5) Pediatrics, Hosp para el Nino Poblano, Puebla; 6) Estomatology, Benemerita Universidad Autonoma de Puebla, Mexico; 7) Estomatology and Genetics, univ of South Carolina, USA.

INTRODUCTIÓN. The reciprocal translocations should not be in relation to fenotipical malformations, since there should not be DNA alteration. however, fenotipical malformations, mental retardation and neoplasic proceses have been reported.CLINICAL CASES. Pacient 1: An adolescent male with a fibro-osseous lesion of the jaws, a non familiar gigantiform cementoma, radiologically and histopathologically, with a flat fascies and minor phenotypic alterations. A de novo reciprocal translocation between chromosomes 1 and 4. Pacient 2: A 9 years male with mental retardation, facial malformations, polidactilia, hypertricosis, small genitalia and obesity, with a translocation, 46,xy,t(6;9). both parents with a normal cariotype. Pacient 3: A newborn male with partial trisomy of chromosome 14 (14q24) and a translocation t(11;14), with , lobar neumony, hidrocele, septicemia, interauricular septal defect, cleft palate and a dismorfic phenotipe. Patient 4: A 10 years male, with oligodoncy and hyperkinesis with a 45,xy,der(13;15). Has an affected carrier mother 45,xx der(13;15). CONCLUSIONS. some chromosomal translocations has been reported as primary etiological factor for different kind of clinical malformations and celular neoplasias. t(1;4), t(6;9), t(11;14) and t(13,15) has been thought to be in relation to cell lymphoma, leukemia and other kind of neoplasias, originating from pre-immune b-cells in the mantle zone of the primary follicles in secondary lymphoid organs. in b-prolymphocytic leukaemia, in plasma cell leukaemia, and in chronic lymphocytic leukaemia, in multiple myeloma and also in immunosupresion events. it is important to study such chromosmal translocations in afected children in order to get an earlier treatment for a better quality of life.

Risck factors associated with a late Dental Exfolation in a small Mexican town with a High Consanguinity Degree. *S.A. Arias[1], J. Aparicio[1,2], G. Quintanar[1], M.A. Parra[1]* 1) Estomatology, Benemerita Universidad Autonoma de Puebla; 2) Genetics, Hospital para el Niño Poblano, Puebla, Mexico.

INTRODUCTION. The formation of human dentition begins as early as the 6th week in utero, during which, tooth buds of the primary (deciduous) teeth develop at 10 specific sites in the developing maxilla and mandible. Primary teeth begin to calcify at about 3 to 4 months in utero, and the enamel of all crowns is completed by 10 months after birth. Beneath the primary teeth, 20 permanent (succedaneous) teeth develop. As root development takes place in the permanent teeth underneath, this causes exfoliation of the primary teeth. Osteoclast formation is stimulated, which results in the resorption of the roots of the primary teeth and their subsequent loss. MATERIALS AND METHODS. Several families with late dental exfoliation and duplication (poliodoncy) was observed in a small Mexican Town. The families were estudied by performing radiological, metabolic, chromosomal studies and genetic pedigree to evaluated a mendelian inheritance on the population due to consanguinity. DISSCUSION. With the exfoliation of primary teeth and replacement by permanent teeth, the child enters a mixed-dentition stage. Permanent teeth erupt in the following sequence: lower central incisor and first molars at about age 6 to 7, followed by the upper central incisor and lateral incisors, the canines and premolars, second molars, and finally third molars during late teens up to the early twenties. Disorders of tooth eruption and positioning are common pediatric dental problems that present clinically as maloclusion or abnormal alignment of the dentition. Delayed eruption of all teeth is indicative of developmental delay, hormonal abnormalities, and nutritional or systemic disturbances Failure of eruption of single or small groups of teeth suggests local causes such as malpositioning of teeth, supernumerary (extra) teeth, retained primary teeth, or cysts. CONCLUSIONS. All genetic studies were normal in relation to late exfoliation, however some families had also chidren with albinism, where future studies will be also perfomed to find out the relation with consanguinity to provide genetic counseling.

Novel contiguous gene deletions and duplications in patients with developmental delay, mental retardation, and dysmorphology. *S. Aradhya[1], M. Manning[1,2], A.M. Cherry[1]* 1) Clinical cytogenetics, Stanford University, Stanford, CA; 2) Division of Medical Genetics, Stanford University, Stanford, CA.

Developmental delay is often seen in conjunction with mental retardation and dysmorphic features. This broad phenotype is observed in approximately 1-3% of children and is most often due to chromosomal abnormalities, although it is often difficult to recognize the specific genomic imbalance with conventional methods. We have studied a cohort of 20 patients with developmental delay in an effort to identify the underlying genetic causes. Some of these patients also had mental retardation and dysmorphic characteristics. Conventional cytogenetic analysis by karyotyping and sub-telomere fluorescence in situ hybridization (FISH) had previously yielded normal results for these patients. The Human Genome Project has encouraged the development of highly sensitive assays to detect quantitative changes in genetic material. Using whole genome microarrays, we identified eleven unique mutations of which nine were deletions and two were duplications. The mutations ranged in size from 280kb to 3 Mb. Virtually all of the patients also carried large-scale deletion and duplication polymorphisms, some of which were new. The mutations were confirmed by FISH and were shown to have occurred *de novo* in the respective families. All of the cytogenetic alterations found in our patients, with the exception of one, were large enough to encompass several genes, suggesting that diffuse phenotypes like developmental delay are due to contiguous aberrations in a significant fraction of these patients. Our results also underscore the strength of high-resolution genomic arrays in diagnosing cases of unknown genetic etiology. Eight of the eleven mutations have not been previously reported and represent novel genetic syndromes. Our findings add to an increasing number of genetic disorders caused by large-scale deletions or duplications. As genes in the respective mutation intervals are further studied, these new clinical entities will provide valuable insight into mechanisms that regulate prenatal and neonatal development.

Heterochromatin variations may cause changes in the quality of sperm. *A. Amiel[1,2], F. Sardos-Albertini[1], B. Bartoov[2], M.D. Fejgin[1,3]* 1) Genetics Institute, Meir medical center, Kfar-Saba, Israel; 2) Bar Ilan University; 3) Tel Aviv University.

Teratospermia is characterized by the presence of spermatozoa with abnormal morphology. It has been shown that infertile men with abnormal sperm parameters have an increased rate of sperm aneuploidy and that poor sperm morphology is associated with numerical chromosome abnormalities of the spermatozoa. In this study we evaluated the karyotype of 5 control healthy men, 10 men with teratospermia and 13 men with azoospermia. Also, the disomy rate of chromosomes 8,9,18, X and Y in sperm cells taken from the normal and teratospermic groups. Results: All teratospermic men and 8 of the azoospermic group showed a structural variation of 9qh + (46,xy,9qh+), compared to none in the controls. One patient with azoospermic was 47,xxy. The disomy rate of the group with teratospermia was significantly higher with all probes analyzed compared to control group ($p<0.001$). Our findings raise the possibility that the variation in the constitutive hetrochromomatin of some chromosome interfere in the meiosis process such as the exact pairing of the homologue chromosomes.

six years benefits of Deflazacort treatment for Mexican Pediatric patients with Duchenne muscular dystrophy. *R. Ruiz[1], J. Aparicio[2], A. Ortiz[3], M.A. Cubillo[4], F.E. Romero[5]* 1) pediatric Rheumatology; 2) Medical Genetics; 3) Orthopedics; 4) Rehabilitation; 5) Nutrition, Hospital para el niño Poblano, Puebla, Mexico.

INTRODUCTION. Duchenne muscular dystrophy (DMD) (also known as muscular dystrophy - Duchenne type) is an inherited disorder characterized by rapidly progressive muscle weakness beginning in the lower part of the body including pelvis later affecting all the body DMD is the most common form of muscular dystrophy It usually affects male patients, but in rare cases it can also be observed in female patients. It is considered an X-linked recessive inherited disease. A milder form of this disease is known as Becker Muscular dystrophy (BMD). In BMD, most of the symptoms are similar to Duchenne, but the onset is diefrent. MATERIAL AND METHODS. A group of 25 pediatric patients were diagnosed as Duchenne muscular dystrophy by enzimatic tests (CPK, DHL and Aldolasa), muscular biopsy and clinical syntoms includings Gowers sign. All of them were treated with deflazacort and their different clinical evolution among them was then analyzed. Weigth, size, arterial measure and glucose were monitored every three months for control CONCLUSIONS. DMD is named after the French neurologist Guillaume Benjamin Amand Duchenne (1806-1875), who first described the disease in the 1860s. One third of the cases are known to be caused by development of spontaneous mutations in the dystrophin gene. Boys with DMD develop weak muscles because the muscle fibers become distrophic, due to mutations in the dystrophin gene, which encodes a cell membrane protein in myocytes. Deflazacort dose concentration was used acording to clinical evolution, if muscular strenght decreased, the dose was used at 0.3mg/kg. This study shows that Deflazacort has an important impact on health, a better quality of life and few side effects were observed during the treatment among the patients. However, a near future study will be performed by comparing these results with a control group.

Knockdown of estrogen receptor alpha gene expression by siRNA in human breast cancer cell line. *M. El-Zawahri[1], Y. Luqmany[2], A. Al-Azmi[1]* 1) Dept Biological Sci, Fac Sci, Kuwait Univ,; 2) Det Pharmaceutical Chemistry, Fac Farmacy, Kuwait Univ, Kuwait.

The role of estrogen receptor alpha (ER) in breast cancer has been highlighted by numerous studies. Consequently, inhibition of ER is one of the major strategies for prevention and treatment of breast cancer. However, failure to overcome development of endocrine resistance, arising despite continued expression of tumor ER, limits this approach. The aim of this study is to establish a breast cancer cell line containing a permanent source of small interference RNA (siRNA) which specifically inhibits the production of ER protein, to produce a model system to investigate loss-of-function of ER. Three siRNA constructs (PI-III) targeting different sequences of human ER, and a scrambled sequence were cloned into the pRNA-U6.1/Neo GenScript vector. MCF7 breast cancer cells were transformed with 2 or 4ug of each plasmid (6 and 24h exposure), using lipofectamin or Xtreme reagent conjugates. Transformants were rescued by growth in G418 selection medium. ER mRNA levels were determined by Real Time RT-PCR of extracted RNA, and ER protein by Western blotting; normalization was achieved by simultaneous analysis of -actin. Presence of plasmid DNA in transformants was verified with primers targeting various regions of the vector. Stably transfected cells maintaining antibiotic resistance over several passages were established by continuous culture. Linearized anti-ER-siRNA construct PII most effectively down-regulated ER mRNA (as evidenced by Real-Time RT-PCR analysis) and protein in these cells as compared to G418 resistant transformants containing scrambled siRNA; complete knockdown was not observed. Whereas 4g produced more transformants, 24h exposure did not increase transformation efficiency. In conclusion, Our three anti-ER-siRNA vector constructs silenced its target mRNA specifically and we have successfully established a long term culture of MCF7 breast cancer cells that exhibit decreased expression of ER. This is hoped to provide a model system in which to study aspects of endocrine resistance. (Supported by Kuwait University Grant YS 01/04).

Genetic conversations - a bridge towards delivery of genetic services in medical subspecialties. *M. Cloutier[1], C. Shuman[2], R. Weksberg[2], D. Chitayat[2,4], F. Miller[3]* 1) Mol & Med Gen, U Toronto; 2) Clin & Metab Gen, SickKids, Toronto; 3) McMaster U, Hamilton; 4) Mount Sinai Hospital, Toronto.

The advent of genomic medicine, with the increasing availability of genetic testing, poses major challenges for the delivery of genetic services. Primary care providers, medical specialists and nurses are becoming increasingly involved in the provision of genetic information and in the interpretation of genetic test results. The objective of this study was to gain insight into pediatric cardiologists and cardiac specialist nurses views on the provision of genetic services (genetic testing and genetic counseling) for their patient populations, both currently and for the future. Semi-structured interviews were conducted with non-genetics pediatric specialists in cardiology, including staff cardiologists, nurses and fellows. The qualitative analysis of the transcribed interviews was guided by a joint grounded-theory and hermeneutic approach and revealed the following key themes: 1. Genetics is an essential subtext to the provision of cardiac care; 2. A desire to share cardiogenetic care and integrate the role of genetic counselors in cardiology; 3. Genetic counseling or genetic conversations? Genetic conversations provide factual genetic information related to a patients condition. This differs from genetic counseling which, in addition to providing genetics education, allows for the contextualization of genetic information to help patients make decisions and adapt to the psychological, familial and social issues that stem from the risk or condition in the family. We propose a provisional theory of a genetic counseling continuum as a model to describe the types of genetic counseling activities that occur in non-genetics pediatric specialties. These results have implications for enhancing our understanding of the types of genetic counseling activities provided by non-genetics specialists and for policy formulation regarding the development of genetics programs in medical subspecialties. Recommendations are proposed for post-graduate training programs. This study informs the broader literature on the future shape and scope of genomic medicine in a pediatric setting.

Analysis of LOH effect on TCO gene within Iranian population with Familial Non- Medullary Thyroid Carcinoma. *H. Atashi Shirazi[1], M. Hedayati[1], A. Shafiee[2], M. Daneshpour[1], F. Azizi[1]* 1) Dept Genetics, EMRC of Shahid Beheshti University of Medical Science , Tehran,I.R.Iran; 2) Milad hospital, Tehran, I.R.Iran.

Introduction: Familial non-medullary thyroid carcinoma (FNMTC) is mostly sporadic and account for ~ %3-7; of thyroid cancers. Its inheritance is reported as autosomal dominant. Papillary and follicular thyroid carcinoma is two main variants of FNMTC. One of genes which involve in susceptibility to FNMTC is TCO (19p13.2). LOH studies have been used to identify sites harboring tumor suppressor genes involved in tumor initiation or progression. Aim:The aim of the study was to permit LOH analysis of TCO gene in Iranian FNMTC patients. Material & Methods: For LOH analysis, totally 56 papillary and follicular carcinoma subjects and their family selected. Peripheral blood genomic DNA was extracted from affected and non affected FNMTC subjects, according to standard salting out protocol and amplified using PCR technique. Finally LOH identified on acrylamide gel electrophoresis after staining. Results: Sixteen follicular and forty papillary carcinoma samples were analyzed. LOH observed at %5.4 of the follicular and % 17.9 of papillary carcinoma cases. Briefly, the obtained data related to analyzed microsatellite markers were: %10.7 D19S916, %12.5 D19S413, %41.1 D19S391, %1.8 D19S568 and %3.6 D19S865. LOH was absent in all normal samples. Papillary carcinomas displayed higher rate of LOH than follicular carcinoma. Discussion: This study data revealed that more than %20 of Iranian FNMTC showed LOH, and it is more frequent in papillary than follicular carcinoma. The predominant microsatellite marker in LOH searching related to D19S413. So probably there is difference in mechanisms controlling chromosomal stability in these two forms of papillary and follicular cancers.

The North Carolina - Louisiana Prostate Cancer Project (PCaP): Methods and design. *J. Bensen* Lineberger Cancer Ctr-North, Univ North Carolina, Chapel Hill, NC.

The North Carolina - Louisiana Prostate Cancer Project (PCaP) is a multidisciplinary population-based case-only study designed to investigate social, individual, and tumor-level causes of racial differences in prostate cancer (CaP) aggressiveness. PCaP includes nine projects; two are designed specifically to examine genetic variation in known hereditary CaP susceptibility genes and identify new genes associated with CaP aggressiveness. The PCaP population-based sample consists of incident CaP cases from North Carolina (NC) and Louisiana (LA) including 1,000 African Americans and 1,000 Caucasian Americans. Study nurses administer structured questionnaires and collect blood, adipose tissue, urine and toenail samples during an in-home visit. Clinical data are abstracted from medical records, diagnostic biopsies are reviewed and assayed, and tissue microarrays are constructed from prostatectomy samples. CaP aggressiveness is classified based on PSA, clinical stage and Gleason grade. Preliminary data was analyzed through August, 2005, by which time over 400 study visits were completed with 122 and 95 NC and 88 and 101 LA, African Americans and Caucasian Americans, respectively. Participation exceeded 70% at both sites through August, 2005 with an average time from diagnosis to study visit of 3 months. Of eligible participants interviewed, 98% consented to blood, urine and toenail donation, and 81% of African Americans and 91% of Caucasian Americans consented to donation of adipose tissue. Ninety-nine percent of patients provided consent for medical records retrieval and abstraction, and for tumor block retrieval and analysis. Preliminary data demonstrated between-and within-group differences in patient characteristics, screening and treatment by race and state. Preliminary data from this well-characterized cohort support the feasibility of this comprehensive study to help determine the focus of public health efforts, including those related to identification of genetic risk factors, to reduce racial disparities in CaP mortality. Supported by the Department of Defense Prostate Cancer Research Program.

Germline analysis of the ACVR1B gene as a candidate gene for ovarian failure. *H. Dixit[1], K.L. Rao[1], J. Nair[2], V.V. Padmalatha[1], M.K. Kanakavalli[1], M. Deenadayal[3], N. Gupta[4], B.N. Chakrabarty[4], L. Singh[1]* 1) Centre for Cellular and Molecular Biology, Hyderabad, Andhra Pradesh, India; 2) Vellore Institute of Technology, Vellore, Tamil Nadu, India; 3) Infertility Institute and Research Centre, Hyderabad, Andhra Pradesh, India; 4) Institute of Reproductive Medicine, Kolkata, West Bengal, India.

An amenorrhoea (>6 months) with elevated FSH levels before 40 years is defined as premature ovarian failure (POF). Elevation of FSH levels results in depletion of ovarian follicles. Activins up-regulate FSH production by activating its Type II and Type I receptors located on gonadotrophs. Activated Type I receptor (ACVR1B) sends intracellular signaling for FSH upregulation. The loss of function mutations in this gene cause carcinomas due to loss of growth inhibitory effect of activin. We hypothesize that gain of function mutations in this receptor gene may cause ovarian failure due to constitutive or increased FSH production. These mutations can result in either activin independent form of receptor or its increased sensitivity for activin binding. Case control study was performed by DNA sequencing analysis for coding regions (9 exons) of ACVR1B gene. A total of 196 ovarian failure cases including 133 POF cases, 63 pri. amenorrhoea (PA) cases and 200 control were recruited. All individuals were chromosomally normal and their recruitment followed strict medical norms. Sequence analysis of ACVR1B gene revealed 7 novel variants and 3 documented variants. Three novel missense variants c.392T>G, c.505T>G, c.581G>A were exclusively associated with patients while completely absent in controls. The c.392T>G (p.Iso131Ser) variant was revealed in 7/133 POF cases (p value=0.0015) and 4/63 PA cases (p value=0.003). The c.505T>G (p.Cys169Gly) was present in a POF patient. The c.581G>A (p.Gly194Glu) variant was present in 4/133 cases of POF (p value=0.025). Preliminary analysis showed gain of function nature of these variants. Frequency of an intronic variant c.332-155_332-154insA was higher in patients (12.2%) than controls (5%) (p value=0.012). This is first report presenting gain of function mutations in ACVR1B gene associated with ovarian failure.

Identification of Biological Markers in Amniotic Fluid of Trisomy 21 during Pregnancy by Proteomics. *D. Cha[1], K. Kim[2], C. Lee[1], S. Lee[1], J. Chang[1], K. Lee[1], J. Kim[1]* 1) Dept OB/GYN, Kangnam CHA Hosp, Seoul, Korea; 2) Dept molecular biology, KonKook Uni., Seoul, Korea.

Objectives : To identify the proteoms in amniotic fluid for the early detection of trisomy 21. **Methods** : Amniotic fluids from 3 patients (range 16-18 weeks) with Downs affected pregnancies were matched for gestational age and fetal sex with the amniotic fluid from 3 unaffected pregnancies. Total protein concentration was determined by the Bradford protein assay. Acetone-precipitated albumin/IgG-depleted/containing samples were separated on 4~20% gredient SDS-PAGE and the protein bands stained by coomassie blue. The amount of 25ug of protein was loaded in a lane of SDS-PAGE. The gel percentage was 4~20. After in-gel digestion, the sample was analyzed by LC-MS/MS. **Results** : Analyses were focused on regions corresponding to molecular masses between 6.5 and 250 kDa. 20 proteins were detected after albumin IgG depletion in the amniotic fluid of unaffected pregnancies. There were 22 proteins detected after albumin/IgG depletion in the amniotic fluid of trisomy 21. Proteomic profiling identified the differential expression of 11 proteins in the affected pregnancies. 4 proteins such as actin alpha 2 and serine proteinase inhibitor were increased and 7 proteins such as alpha-1-acid glycoprotein 2 precursor and prostaglandin D2 synthase were decreased in trisomy 21. **Conclusion** : Proteomics in the amniotic fluid is a valuable approach to develop new potential biological markers for the early screening of trisomy 21.

Hypertensives show downregulated expression of adrenoceptor genes in arterial tissue. *J.R. Dungan[1, 2], C. Yucha[3, 2], Y. Conley[4], J. Johnson[5], S. Kneipp[2], T. Langaee[5]* 1) Center for Aging, Duke Univ., Durham, NC; 2) Nursing, Univ. of Florida, Gainesville, FL; 3) Nursing, Univ. of Nevada-Las Vegas, Las Vegas, NV; 4) Nursing, Univ. of Pittsburgh, Pittsburgh, PA; 5) Center for Pharmacogenetics, Univ. of Florida, Gainesville, FL.

Objective: Differential expression of adrenoceptor genes has been implicated in the pathobiology of hypertension and may help explain some health disparities. This pilot study explored relative differences in $_{1A}$- and $_{2}$-adrenoceptor gene expression in human arterial tissue between people with and without hypertension. **Methods:** Relative levels of RNA of the investigated genes were measured in internal mammary artery tissue samples obtained from consented subjects who had coronary artery bypass surgery at local hospitals in Gainesville, FL. The extracted RNA was analyzed with Real-Time, reverse-transcription polymerase chain reaction. **Results:** The study included 41 subjects. Hypertensive subjects showed 3.92-and 2.05-fold reductions in $_{1A}$- and $_{2}$-adrenoceptor gene expression, respectively, compared to normotensives ($p < 0.05$).Comparing Caucasian hypertensives and normotensives produced over 4- and 5-fold reductions in hypertensives $_{1A}$- and $_{2}$-adrenoceptor gene expression ($p < 0.05$); however no significant differences were found between African American and Caucasian hypertensives. **Conclusions:** The downregulation of both investigated adrenoceptor genes in hypertensives suggests alterations at the level of transcription in the pathobiology of hypertension. To our knowledge, this is the first investigation exploring these particular genes and methods in *human* tissues, especially with regard to examining race. Interpretation of results is cautioned due to some limitations. Gene expression accounts for both genetic and environmental factors in disease. Translational research that involves gene expression is likely to benefit practitioners and patients in the future with personalized screening and management tools. This study was supported by NINR (#1F31NR009148-01), AHA (#01415124B, partial), & . Shands Hospital & the Malcom Randall Veterans Administration Medical Center also provided resource and facility support.

A *de novo* unbalanced t(7;13). *A. ABOURA[1], S. KANAFANI[2], AC. TABET[1], M. BENKHALIFA[3], E. PIPIRAS[1], B. BENZACKEN[1, 2], C. LEONARD[4], P. LANDRIEU[5], A. VERLOES[6]* 1) UF de Cytogénétique, Hôpital Robert Debré, Paris France; 2) Service de Cytogénétique, Hôpital Jean Verdier, Bondy France; 3) Genetics Laboratory, Laverrière France; 4) Laboratoire de Cytogénétique, Hôpital Bicêtre, France; 5) Service de Neurologie Pédiatrique, Hôpital Bicêtre, France; 6) UF de Génétique Moléculaire ,Hôpital Robert Debré, Paris France.

A one year old girl is addressed for a psycho-motor delay. Her parents are healthy and unrelated. Before birth, her first screening ultrasonography showed an increased nucal translucency. An amniocentesis, revealed a normal 46,XX prenatal karyotype. At birth, her clinical examination was normal. At 4 month old, a developmental delay appeared and increased at one year old, as revealed by the Denver test. A cranio-facial dysmorphia was noted with a wide forehead, long face, large nose, very small low implanted and in posterior rotation ears, heavy cheeks and a double chin. A high resolution karyotype showed one doubtful 13qter. CGH, then CGH array of 2600 clones were performed. The latter with a resolution of 1 Mb (Spectral Genomics), revealed a 7qter duplication and a 13qter deletion. A 2 colour painting WCP 7 and WCP 13 identified a reciprocal exchange between the terminal region of the long arms of one chromosome 7 and 13. A 2 colour subtelomeric probes 7 qter and 13 qter showed 1 fluorescent spot on 13qter, and 3 fluorescent spots of another colour on 7 qter. The karyotypes of the parents were normal. The proband has an unbalanced *de novo* karyotype: 46,XX.ish der(7)t(7;13)(qter+;qter-)(BACs+,BACs-),rev ish enh(7)(qter)dim(13)(qter). The microsatellites analysis for chromosomes 7 and 13 showed that the duplicated material on chromosome 7 is maternal whereas the deleted material on chromosome 13 is paternal. A set of BACs is hybridized to better explore the imbalance. We discuss the possible mechanisms of this rearrangement and the phenotype-genotype correlation.

Absence of the lysosomal enzyme Acid Sphingomyelinase protects against liver fibrosis following Bile Duct ligation. *C. Brown, J. Murray, A-M. D'Angona, J. Serriello, K. Karey, A. Vitsky, K. Sampath, L. Andrews, J. McPherson*
Pharmacology & Toxicology, Genzyme Corp, Framingham, MA.

The lysosomal enzyme, acid sphingomyelinase (ASM) is believed to play a role in apoptotic hepatic injury (Garcia-Ruiz et al. 2003). Indeed, ASM knockout mice are protected against liver damage elicited by TNF. Whether the protective effect of ASM is specific for this TNF biased liver injury model or extends to other acute models of liver disease such as Bile duct ligation (BDL) is currently unknown. With surgical ligation of the bile duct chronic hepatocellular apoptosis and inflammation results in additional markers of liver damage such as liver fibrosis and necrosis. We examined the role of ASM in the development of hepatic fibrosis by investigating whether the susceptibility to BDL-elicited liver fibrosis differs between ASM transgenic and wild type mice. Wild type mice (C57/Bl), heterozygotes with partial ASM levels or ASM Knockout mice (ASMKO) were subjected to Bile duct ligation or a sham surgical procedure. Seven days post surgery all excised livers were examined histologically for liver fibrosis and serum analysis performed to investigate potential alterations in liver function. Animals subjected to bile duct ligation demonstrated hepatomegaly and significant liver injury characterized by increased bile duct hyperplasia, extensive liver fibrosis, necrosis and inflammation. Sham surgery animals, where the abdomen was opened and liver manipulated, lacked these histological changes. Animals subjected to BDL had significantly elevated levels of serum gamma glutamyl transpeptidase, aspartate aminotransferase, bilirubin and cholesterol indicative of compromised liver function. Histologically liver fibrosis and bile duct hyperplasia were significantly improved in ASM KO and heterozygote mice compared to wild type mice however this was not reflected in serum markers, likely due to complication of liver function by the disease phenotype. In conclusion the absence of ASM appears to protect against fibrotic hepatic damage elicited by bile duct ligation however this protection while evaluable histologically did not appear to be reflected at the functional level.

Identification and replication of GATA2 polymorphisms associated with familial early onset coronary artery disease. *J.J. Connelly[1], C. Haynes[1], L. Wang[1], S.H. Shah[1,2], J.E. Cox[1], D. Crosslin[1], A.B. Hale[1], T. Wang[1], S. Nelson[1], D.C. Crossman[3], C.B. Granger[1], J.L. Haines[4], C.J.H. Jones[5], J.M. Vance[1], P.J. Goldschmidt-Clermont[6], W.E. Kraus[2], E.R. Hauser[1], S.G. Gregory[1]* 1) Department of Medicine and Center for Human Genetics, Duke University, Durham, NC; 2) Department of Medicine and Division of Cardiology, Duke University, Durham, NC; 3) University of Sheffield, Sheffield, United Kingdom; 4) Vanderbilt University, Nashville, TN; 5) University of Wales College of Medicine, Cardiff, United Kingdom; 6) Miller School of Medicine, University of Miami, Miami, FL.

The transcription factor GATA2 plays an essential role in the establishment and maintenance of adult hematopoiesis. It is expressed in hematopoietic stem cells, as well as the cells that make up the aortic vasculature, namely aortic endothelial cells and smooth muscle cells. We have shown that GATA2 expression is predictive of location within the thoracic aorta; location is suggested to be a surrogate for disease susceptibility. The GATA2 gene maps beneath the Chromosome 3q linkage peak from our GENECARD early onset coronary artery disease study. Given these observations, we investigated the relationship of several known and novel polymorphisms within GATA2 with coronary artery disease. We identified five SNPs that were significantly associated with early onset coronary artery disease in our family based sample set (GENECARD). These results were validated by identifying significant association of two of these SNPs in an independent case-control sample set that was phenotypically similar to the GENECARD families. These observations identify GATA2 as a novel coronary artery disease susceptibility gene and suggest that the study of this transcription factor and its downstream targets may uncover a regulatory network important for coronary artery disease inheritance.

A Score Test for Linkage Analysis of Ordinal Traits Based on IBD Sharing. *R. Feng*[1], *H. Zhang*[2] 1) Biostatistics, University of Alabama at Birmingham, Birmingham, AL; 2) Biostatistics, Yale University, New Haven, CT.

Statistical methods for linkage analysis are well established for both quantitative and binary traits. However, numerous diseases including cancer and mental illness are rated on discrete, ordinal scales, and the genetic heritabilities and/or candidate genes for many of the diseases have been documented. Recently, a latent variable model has been developed to analyze pedigree data with ordinal traits from which increased power of detecting linkage is revealed when the ordinal, rather than dichotomized, traits are fully used. The challenge with the latent variable model is that the likelihood is usually very complicated and as a result, the computation is intensive and sometimes prohibitive. We derived a score statistic based on the identity by decent (IBD) sharing information within pedigrees, which greatly alleviates the computation burden. Using the new test statistic, the latent variable model for ordinal traits can test linkage for large pedigrees while adjusting for non-genetic covariates. Using simulation studies, we examined the asymptotic distribution of the test statistic and the power of our proposed test under various genetic models. We then applied our method for the Collaborative Study on the Genetics of Alcoholism (COGA) and performed a genome scan to map susceptibility genes for alcohol dependence. We found strong linkage signals on chromosomes 4, 13 and 18.

Double male syndrome with Tetralogy of Fallot , report of the second case. *M. AL-Atwi[1], H. Al-Girim[1], W. Eyaid[2], I. Amir[3]* 1) pediatrics Department, King AbdulAziz Hospital, AlAhsa, Eastern, Saudi Arabia; 2) pediatrics Department, King Fahad Hospital, Riyadh,central, Saudi Arabia; 3) Cytogentics Department, King Fahad Hospital, Riyadh,central, Saudi Arabia.

The patient was a product of full term uneventful pregnancy presented at age of 8 weeks with ten days history of cyanotic spells his clinical examination was unremarkable except for a loud systolic murmur with normal hearts sounds and normal femoral pulses bilaterally. He has normal penis with bilaterally descends testis. His echocardiography was compatible with Tetralogy of Fallot (TOF). The patient was referred to our cardiac center where he was operated for TOF repair. Because of his congenital heart disease a chromosomal analysis was done which revealed the presence of an extra chromosome X as well an extra chromosome Y in all the 20 analyzed cells giving 48,XXYY chromosomal constitution . Chromosome number 22 was also investigated by the fluorescence in situ hybridization technique (FISH) which showed no evidence of abnormality involving the critical region of DiGeorge syndrome on chromosome 22q . The combination of 48,XXYY with TOF was first reported in 1995 by Meschede et al . Our report and others suggest that congenital heart disease may occur in the 48,XXYY syndrome more frequently than currently appreciated.

Genomic DNA isolated from semen better diagnostic and prognostic marker of AZF status. *R. Dada[1], R. Kumar[1], R. Kumar[2], K. Kucheria[1]* 1) Dept Anatomy, All India Inst Medical Sci, New Delhi, India; 2) Dept Urology, All India Inst Medical Sci, New Delhi, India.

Deletions of Azoospermia factor (AZF)genes on Yq are pathogenetically involved in male infertility. AZFgene consists of 3 non overlapping loci AZFa,AZFb and AZFc and these are involved in germ cell development and differentiation. The aim of this study was to establish if AZF status of DNA isolated from blood reflects the Yq microdeletions status of DNA isolated from germ cells which are of different embryonic origin. Seventy five infertile males with idiopathic oligozoospermia were included in this study. Cytogenetic and semen analysis was done in each case. Testicular Fine Needle aspiration Cytology was collected whenever possible. Of the 75 cases, 5 cases were identified as Klinefelter mosaics, 1 cases had D/D group robertsonian translocation. In cytogenetically normal cases (n=69) microdeletion analysis was done) from DNA isolated from blood and semen using STS-PCR approach using primers sY84, sY86 (AZFa); sY127, sY134 (AZFb); sY254, sY255 (AZFc. The STS was considered as absent after 3 amplification failures. Of the 69 cases 2 cases showed deletion of at least one of the AZF loci from blood DNA and 5 cases showed deletion from semen DNA. The DNA isolated from blood had AZFc deletions which were also detected in semen DNA and both these cases had depressed spermatogenesis on testicular cytopathology. The semen DNA in addition showed 1 case with AZFb and 2 cases with large deletions spanning AZFb and AZFc loci. Two cases with AZFb and AZFc had hypospermatogenesis and the cases with AZFb deletion had maturation arrest. As majority of infertile couples opt for Assisted Reproduction, genetic analysis is a must in these cases. Till date majority of centers offer microdeletions status from DNA isolated from blood. However this study clearly highlights the need to do Yq microdeletions analysis from semen DNA in all infertile couples as this is a better diagnostic and prognostic marker than Yq screening from genomic DNA isolated from blood.

Low-income Americans understandings of the relationship of genes and behavior in producing health outcomes.
C. Condit, M. Gronnvoll, J. Landau, C. Groscurth, T. Harris, L. Shen, L. Wright Speech Communication, Univ Georgia, Athens, GA.

To assess the understandings held by low income African Americans and Whites in the U.S. of the relationships of genes and environment in producing human health outcomes, 40 in-depth interviews were conducted. Results suggest most participants encode genes and health behaviors in separate verbal neural networks linked solely through the concept of health outcomes, rather than having models that integrate the two verbal neural networks. Activation of the genetics track leads to a deterministic discourse about health causation, but this does not generally lead to fatalistic beliefs, because participants shift readily to the behaviors track to imagine effective health prevention activities. In addition, a large sub-group harbors an additive model of the relationship between genes and environment. This model imagines risk-conferring alleles as placing individuals on a higher platform, so that health behaviors are useful in lowering risk levels. This model may provide the basis for integrating conceptions of genetics into health campaigns focused on prevention. Although no participants clearly articulate a multiplicative or interactive model of the relationship between genes and environment, a widely shared model of how heart disease occurs may provide the platform for introducing that model. Barriers include understandings of genes as equivalent to having a disease (after the model of viruses) and a belief that behavior-induced damage cannot be erased by behaviors.

Prioritary indications for prenatal karyotyping in developing countries: a study in Brazil. *R. Gus[1,2], M.T.V Sanseverino[1,2], J.A.A Magalhaes[2], R. Giugliani[1]* 1) Genetics Dept, Hosp Clinicas Porto Alegre, Porto Alegre, RS, Brazil; 2) Fetal Medicine Group, Obstetrician Dept.

In the last decades the study of fetal chromosomes became a very important tool for prenatal diagnosis. In developing countries, like Brazil, this test is not widely offered, specially because abortion due to fetal disease is not legally allowed. The purpose of this study was to describe the Fetal Medicine Group experience in Brazil, trying to give priorities to the indications for such exam. Despite prenatal diagnosis is very limited here, we have been providing this test free of charge to at-risk pregnancies since 1989. In a genetic counseling session, the risks, methods and approaches were explained to the family. Accordingly to each situation, the best procedure was indicated. CVS and AF were mainly obtained for cell culture. The cultures remained at the CO2 incubator with Amniomax medium for few days, and then harvested and G-banded for chromosome analysis. From 1989 to 2005, 874 fetal karyotypes were performed and abnormal results were found in 69 (7,8%) cases. The three most frequent indications were: advanced maternal age, fetal malformation detected at ultrasound, and previous child with Down syndrome. Despite advanced maternal age was the most frequent indication for prenatal diagnosis (233 cases and 10 abnormal karyotypes), the majority of the aberrant karyotypes was found in the group where the indication was a severe fetal malformation detected at ultrasound (21.5% of 167 cases). On the other hand, although the history of a previous child with Down syndrome was a frequent indication (123 cases), we did not find any positive case on the prenatal analysis performed in this group. This means that, for this last indication, the risk of complications due to the procedure (0,5%)was greater than the risk of recurrence of Down syndrome, in our sample. Considering the limitations to offer prenatal tests in our conditions, we propose that the indication of prenatal diagnosis for couples who had a previous child with Down syndrome should be reconsidered, and higher priority given to the pregnancies where a malformation is detected on the ultrasound scan.

A statistical approach to predict the presence of a *CHD7* mutation in patients with suspected CHARGE syndrome. *J.P. Ferraro[1], M.S. Williams[2], C.M.A. van Ravenswaaij[3], L.H. Hoefsloot[3], M.C.J. Jongmans[3], M.A. Hefner[4], S.R. Lalani[5], S.D. Fernbach[5], A.A. Safiullah[5], J.W. Belmont[6]* 1) Department of Medical Informatics, Intermountain Healthcare, Salt Lake City, UT; 2) Clinical Genetics Institute Intermountain Healthcare Salt Lake City, UT; 3) Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 4) Division of Medical Genetics, Department of Pediatrics, Saint Louis University School of Medicine, St. Louis, MO; 5) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 6) Department of Pediatrics, Baylor College of Medicine, Houston, TX.

The purpose of this study is to apply statistical models to determine the predictive accuracy of the various CHARGE syndrome diagnostic criteria, singly and in combination, for finding a mutation in the *CHD7* gene in patients with the clinical diagnosis of CHARGE syndrome.

Clinical information from 196 patients who underwent *CHD7* mutation analysis was obtained from the two testing centers. Twenty clinical characteristics were identified, as well as gender and mutation status. Several statistical approaches including binomial logistic regression, step-wise regression and Bayesian model averaging were used to identify the best combination of characteristics to predict *CHD7* positive status. In addition, the individual power of the characteristics to predict a *CHD7* positive-mutation was also calculated.

Analysis of the individual factors lead to some surprising findings. Neither choanal atresia, long recognized as an important feature of CHARGE syndrome, nor characteristic CHARGE ear were found to be a significant predictor of presence of a mutation in *CHD7*. The single feature with the best overall predictive performance was pituitary dysfunction. Three features; coloboma, temporal bone anomaly and cranial nerve dysfunction singly and in combination had very high predictive power for presence of a mutation. Additional combinatorial analyses as well as methods to approximate missing data will be modeled and presented.

Inductive screening of -thalassemia in a large consanguineous Pakistani family. *S.M. Baig[1,2], M. Amin-Ud-Din[3], H. Hassan[1], A. Azhar[1], J.M. Baig[1], M. Aslam[1], J.A. Qureshi[1], T. Zaman[1]* 1) Human Molecular Genetics Laboratory, National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan; 2) Molecular Biology Laboratory, Nuclear Medicine Oncology and Radiotherapy Institute (NORI), Islamabad, Pakistan; 3) Human Genetic Research Society, Biology Department, Government College, D.G. Khan, Pakistan.

Pakistan has one of the highest numbers of children with transfusion dependent thalassemia in the world due to high frequency of the gene, consanguineous marriages, high birth rate, and large population size. In this report, we present retrospective inductive screening of a large consanguineous Pakistani family for -thalassemia gene, a strategy for identification and counseling carriers of hemoglobinopathies in developing countries for prevention of the disorders caused by recessively inherited genes. In this family with an index case, 27 members were screened for -thalassemia and 12 (44.4%) were found to carry the mutant gene, a very high carrier rate as compared to just over 5% gene frequency of -thalassemia in the general population of Pakistan. Screening was carried out in the available living members of the index families by measuring the Hb A2 and red cell indices using automated Hb electrophoresis system and hematology analyzer. Amplification refractory mutation system (ARMS-PCR) was used to identify the specific -thalassemia mutation. This report emphasizes the need of mandatory screening of families with index case in particular to identify the carriers for disease prevention through genetic counseling and prenatal diagnosis program. This approach is more practical, feasible and cost effective as compared to population screening.

Islet-specific glucose-6-phosphate catalytic subunit-related protein (IGRP) is a candidate gene for plasma glucose. *D. Fradin[1], F. Letourneur[2], P. Bougneres[3]* 1) INSERM U561, Paris, France; 2) ICGM, Paris, France; 3) INSERM U561 and Department of Pediatric Endocrinology, Paris, France.

Plasma glucose (PG) is at the center of energetic metabolism in humans, and is implicated as a nutrient in multiple physiological processes at the cell and organ level. PG is highly regulated in order to be maintained in narrow limits, in contrast with other fuels such as free fatty acids, ketone bodies or amino-acids, whose range of physiological variation is very wide. There is however an individual variability of glucose level, which depends on genetic and environmental factors. Higher PG level within the normal range is an independent risk factor for type 2 diabetes among young men. We have tested a common variant of IGRP (Islet-specific Glucose-6-phosphatase catalytic subunit-Related Protein) as a potential candidate gene of glucose homeostasis. IGRP is located in the endoplasmic reticulum (ER) and expressed exclusively in pancreatic islets. This enzyme catalyzes the conversion of glucose-6-phosphate (G-6-P) to glucose. An association study analyzing the Val219Leu variant of IGRP was conducted in 638 obese Caucasian juveniles with glycaemia. We found that the Val219Leu variant of IGRP was significantly associated with PG levels. Obese children who were Val219 homo- or heterozygotes had significantly greater plasma glucose concentrations at 30, 60, 90 and 120 min following oral glucose ingestion (7.66mM 0.06, 6.91mM 0.08, 6.4mM 0.06, 5.96mM 0.05 respectively) than subjects with the Leu/Leu genotype (7.13mM 0.11, $p=1.10^{-4}$; 6.46mM 0.13, p=0.011 ; 5.91mM 0.11, $p=7.10^{-4}$; 5.72mM 0.08, p=0.038). These findings suggest that IGRP influence the individual variability of glucose homeostasis. Because the physiological role of IGRP has been established by only one paper (Petrolonis et al. 2004), these results should await for replication and functional characterization of the gene and of its variant.

Detection of known and novel human syndromes by high-density oligonucleotide array comparative genomic hybridization (HOaCGH). *Y.S. Fan, D. Barbouth, P. Jayakar, H. Zhu, S. Sacharow, L.J. Elsas* The Dr. John T. Macdonald Foundation Center for Medical Genetics, University of Miami Miller School of Medicine, Miami, FL.

Genomic imbalance is a major cause of morphological and developmental disorders. Fluorescent in situ hybridization (FISH) has been used for diagnosis of some of the over 40 known dysmorphic syndromes and 3% of idiopathic mental retardation associated with subtelomeric rearrangements. Microarray-based comparative genomic hybridization (aCGH) has shown large-scale DNA copy number variations in the human genome. BAC clone-based arrays targeting to the known microdeletion syndromes and the 41 subtelomeric regions (TaCGH) have been used as a diagnostic tool for clinical cases. We have assessed the clinical utility of three platforms of aCGH: a targeted array (TaCGH), a BAC-clone based array for the whole genome with a ~1M interval (BaCGH) and a high-density oligonucleotide array containing 44,000 probes representing over 30,000 mapped human genes (HOaCGH). Using HOaCGH, we have precisely determined the size and gene involvement of two cases with known microdeletion syndromes (Miller-Deiker; DiGeorge) and fully characterized a new neocentric supernumerary marker chromosome derived from Xp22.3pter. While the number of cases in our study is rapidly growing, we have at this time compared the results of TaCGH and HOaCGH on 32 new patients with developmental delay and dysmorphic features. We have identified a 1p36.3 deletion and 4 novel microdeletions: del(15q11.2q11.2)(0.3Mb, 7 genes), del(15)(q24.1q24.2) (3.04Mb, 56 genes), del(19p13.1p13.2)(3.46 Mb, 101 genes), del(21)(q21.3qq22.12)(6.18Mb, 99 genes). We also identified a 7.47 Mb duplication at Xp11.22p11.23(107 genes). Thus, clinically relevant large genomic alterations have been revealed in 6 out of 32 patients (19%) and 5 of them (83%)are not in the regions targeted by TaCGH. Our study suggests that high density whole genome array CGH is a powerful diagnostic tool for unexplained developmental disorders and further molecular studies of these disorders will lead to new genomic mechanisms of disease.

Chiari type I malformation: phenotypic definition by MRI and significant linkage to 15q21.3 and 9q22.31 with a high-density SNP genomic screen. *A.L. Boyles[1], D.S. Enterline[1], P.H. Hammock[1], D.G. Siegel[1], S.H. Slifer[1], L. Mehltretter[1], J.R. Gilbert[1], D. Hu-Lince[2], D. Stephan[2], B.J. Iskandar[3], T.M. George[1], T.H. Milhorat[4], M.C. Speer[1]* 1) Duke University Medical Center, Durham, NC; 2) Translational Genomics, Phoenix, AZ; 3) University of Wisconsin-Madison Medical School, Madison, WI; 4) The Chiari Institute, Great Neck, NY.

Chiari type I malformation (CMI) is clinically characterized by herniation of the tonsils of the cerebellum below the foramen magnum into the top of the spinal column. CMI presents with myriad of neurological symptoms, including chronic and often unremitting pain, and increases the risk of syringomyelia. Although the etiology is unknown, a small posterior fossa (PF) volume relative to total cranial volume is widely thought to cause the herniation. MRIs from 114 affected individuals and their family members were used to evaluate correlations between 10 cranial morphologies and heritabilities were estimated in a subset of 99 MRIs from 32 families. Support for the small PF theory was provided by the correlations between PF delineating structures and high heritability of PF volume (0.955, p=0.003), thereby confirming evidence for a genetic factor in this condition, supporting previous reports of familial clustering.

A genome wide linkage screen with a 10k SNP chip in 23 families with 71 CMI patients identified two significantly linked regions. At 15q21.1-22.3 two-point LOD scores maximized at 3.3 identifying a 13cM region with multipoint scores LOD scores over 1. This region contains a biologically plausible CMI gene, fibrillin-11. Mutations in this gene are the major cause of Marfan syndrome and it is also linked to Shprintzen-Goldberg syndrome which can include CMI in the phenotype. On chromosome 9 multipoint LOD scores maximized at 3.05 highlighting a 40cM region (9q21.33-33.1) with LOD scores over 1 containing an 8.5cM region with multipoint LOD scores over 2. This linkage evidence further confirms a genetic component to CMI and warrants further exploration with fine mapping and investigation of plausible candidate genes.

The potential of Mad1 and Menin in breast cancer therapeutics. *L. Andrews, T.E. Mott, S.M.O. Phipps, J.B. Berletch, T.O. Tollefsbol* Biology, Univ Alabama at Birmingham, Birmingham, AL.

Expression of the catalytic subunit of telomerase, hTERT, is closely linked with the bypass of cell cycle checkpoints in normal and malignant cells. The purpose of this study is to investigate the effects of tumor suppressors, Mad1 and Menin, in inhibiting the expression of telomerase in breast cancer cells. We have introduced Mad1 and Menin in a Tet-responsive vector into estrogen receptor positive and negative breast cancer cell lines. TRAP assays, soft agar analysis of tumorigenicity, mRNA expression of hTERT, growth curve studies, and immunoblotting analyses were performed. The results have confirmed that introduction of these tumor suppressors is sufficient to inhibit telomerase activity, decrease proliferative potential, reduce anchorage independence, and alter epigenetic regulatory and cell cycle checkpoint mechanisms, thereby reducing the carcinogenic phenotype of these cells. Information gained in these investigations could have great potential in the development of therapies for breast cancer.

An interphase fluorescence in situ hybridization (FISH) assay for the detection of 3q26.2/EVI1 rearrangements in myeloid malignancies. *D. Bobadilla[1], E. Enriquez[1], G. Alvarez[1], P. Gaytan[1], D. Smith[2], M.L. Slovak[1]* 1) Cytogenetics, City of Hope National Med Ctr, Duarte, CA; 2) Biostatistics, City of Hope National Med Ctr, Duarte, CA.

Chromosome rearrangements involving band 3q26.2 are associated with an unfavorable prognosis and a highly aggressive subgroup of myeloid malignancies. These apparently balanced rearrangements result in ectopic EVI1 and/or MDS1/EVI1 expression and may be difficult to detect in poor quality metaphases. In this report, we describe the development of a dual-color FISH assay for the detection of 3q26.2 aberrations. A series of BAC clones encompassing and flanking the EVI1 gene covering ~2 Mb were evaluated in total but narrowed to a set of two probe combinations extending 1.2 Mb; one probe set encompasses the EVI1 gene (RP11-82c9) and extends telomeric (RP11-362k14) while the second probe set covers the EVI1 gene (RP11-82c9) and extends centromeric (RP11-24L16). Each probe was mapped to normal metaphase chromosomes to determine their exact location and probe hybridization characteristics, before evaluation with the inv(3)(q21q26.2) positive Kasumi-4 cell line, 10 known negative samples and 33 patient samples with known 3q26.2 rearrangements by conventional cytogenetics collected at various treatment time points. Using this probe combination, normal interphase cells show two fusion signals corresponding to co-localization of the two probes on the EVI1 locus, whereas positive samples showed clear separation of the dual color probes, including 8 positive samples with variant signal patterns for a 3% cutoff for negativity despite the vast breakpoint heterogeneity observed. Interestingly in AML and MDS, the inversion breakpoints are usually 3 to EVI1 whereas 3q26.2 translocation breakpoints were more frequently 5 to EVI1, and most inv(3) CML samples showed a breakpoint within the EVI1 gene. However, two 3q26.2 translocation samples were found to have breakpoints 3 to EVI1. This study demonstrates that despite the extensive breakpoint heterogeneity observed with 3q26.2 aberrations, a robust FISH assay is achievable and effective for the detection of 3q26.2 abnormalities in myeloid malignancies.

A haplotype in FFAR1 (GPR40) predisposes individuals to Type 2 diabetes. *P. Banerjee[1], L.S. Wood[1], M.L. Millham[2], D.S. Lee[3], A.S. Robertson[2], J. Johnson[4], K.L. Houseknecht[2]* 1) Pharmacogenomics, Molecular Profiling, Pfizer Global Research and Devlopment, Groton, CT; 2) Department of Cardiovascular, Metabolic & Endocrine Diseases, Pfizer Global Research and Devlopment, Groton, CT; 3) Statistics, Pfizer Global Research and Devlopment, Groton, CT; 4) Metabolex, Inc., Hayward, CA.

FFAR1 (GPR40) is a G-protein coupled receptor highly expressed in pancreatic beta cells in rodents and humans and facilitates glucose-induced insulin secretion in response to mid to long-chain fatty acids in vitro. It has been reported that thiazolidinedione drugs can activate FFAR1 in some cells. To further examine the function of this gene in vitro, we stably transfected FFAR1 clones into HEK293 cells. Upon fatty acid exposure, cells expressing FFAR1 exhibited mobilization of intracellular Ca2+ stores quantified by fluorometric imaging plate reader (flipr). We found a significant, differential functional response to thiazolidinedione drugs (but not fatty acids) among cell lines expressing FFAR1 with the Arg and His alleles of the SNP, rs2301151 [Arg211His}. To further investigate the functional consequences of this polymorphism, we examined whether rs2301151 and other SNPs in the FFAR1 gene region are associated with the increased risk of developing Type 2 diabetes. We genotyped 3 SNPs (rs12975589, rs2301151 and rs387083), in 276 Caucasian Type II diabetic subjects and 1989 non-diabetic controls. The results of logistic regression modelling for each marker identified rs387083 as having a moderate association with diabetes ($p=0.0283$, unadjusted for multiple comparisons) after adjusting for BMI. BMI was a highly significant ($p<0.0001$) covariate associated with probability of developing diabetes. Based on the LD results, haplotypes were estimated for rs2301151 and rs387083 and examined for a haplotype-trait association. The results of the haplotype trend regression (HTR) test indicate that the A-G haplotype is associated with an increased risk of diabetes (p-value=0.0115, adjusted for BMI). These results suggest that a haplotype in the FFAR1 region might contribute to susceptibility towards developing diabetes in Caucasian subjects.

The effects of cytogenetic factors on the survival of a child with ALL. *H. Cangul[1], T. Yakut[1], T. Gulten[1], A. Meral[2], B. Baytan[2]* 1) Department of Medical Genetics Uludag University School of Medicine, Bursa, Turkey; 2) Department of Child Hematology, Uludag University School of Medicine, Bursa, Turkey.

Acute lymphoblastic leukemia (ALL) is the most common form of childhood leukemias and constitutes 80% of all leukemia cases under the age of 15. Cases with ALL respond well to therapy. The qualitative and quantitative detection of specific chromosomal abnormalities has an important merit as a prognostic marker besides age, leukocyte count, and immunophenotyping in estimating prognosis, assessing the clinical status of the cases before and after therapy, and in monitoring minimal residual disease. The rearrangements t(9;22) and t(4;11), and hypodiploidies indicate poor prognosis whereas hyperdiploidy favors a good one. The case presented here was diagnosed at 13 months of age and stratified into the high-risk group based on age, leukocyte count, and immunophenotyping. In molecular cytogenetic analysis by FISH, whereas the translocations of t(9;22), t(4;11), t(15;17), and t(8;21) were negative, the copy numbers of chromosomes 8 and 21 were increased. The case was treated according to standard protocols but died within one and a half years. Although hyperdiploidy suggests a good prognosis, the findings of our case show that potential good prognostic effects of trisomies 8 and 21 failed to counter-balance the poor prognosis in a patient clinically stratified into the high-risk group. This provokes further studies with large series to evaluate the relationship between prognosis and cytogenetic characteristics in leukemia patients.

Two Novel SMAD4 Mutations and Nonclassical Pathology in Juvenile Polyposis Syndrome. *C. Cremin[1], T. Moyana[2], E. Tomiak[3], M.T. Geraghty[3]* 1) Hereditary Cancer Program, BC Cancer Agency, Vancouver, BC, Canada; 2) Department of Pathology, Ottawa Hospital, Ottawa, Canada; 3) Department of Genetics, Children's Hospital of Eastern Ontario, Ottawa, Canada.

We describe two patients with novel SMAD4 mutations, who had quite dissimilar manifestations of Juvenile Polyposis Syndrome (JPS). The first patient presented with anemia and endoscopic examination showed numerous polyps restricted to the colorectum. The second patient also presented with symptoms of anemia but was found to have numerous polyps restricted to the stomach. Pathologic examination of the resected stomach showed profuse polyposis. In both cases, the histology of the polyps was consistent with juvenile polyposis but with nonclassical features, as illustrated in this report. Analysis of genomic DNA revealed that both patients had truncating mutations in the SMAD4 gene. Taking our current findings in conjunction with previous reports that demonstrated an association between gastric polyposis and SMAD4 mutations, as well as reports that documented nonclassical juvenile polyp morphology in individuals with a SMAD4 mutation, we would like to suggest that SMAD4 gene analysis should be considered for cases of severe gastric polyposis, regardless of polyp histology.

Improvement in swallowing in Miglustat-treated Niemann-Pick disease type C children. *Y.H. Chien, W.L. Hwu, N.C. Lee, L.K. Tsai, A. C. Huang* Dept Medical Genetics, Natl Taiwan Univ Hosp, Taipei, Taiwan.

Niemann-Pick disease type C (NP-C) is a neurodegenerative disease characterized by impaired intracellular trafficking and sequestration of macromolecules. Studies in an animal model of NP-C disease showed that miglustat delayed the onset of neurological dysfunction, increased life span, and reduced ganglioside accumulation and neuropathological changes in those mice. Preliminary result for a clinical trial also suggested a positive effect of miglustat. Two children, age 14 and 9 years, with abnormal cholesterol esterification and filipin staining were enrolled in our study. Milgustat dosages, based on their body surface, were 150mg and 100mg three times daily, respectively. The efficacies were evaluated by neurological examination, videofluoroscopic studies (VFS) for swallowing assessments, MMSE, Purdue Peg Board, liver and spleen organ volumes, chitotriosidase, and quality of life. There was no weight loss or unpreventable gastro-intestinal disturbances during treatment. The initial VFS of the older boy showed oropharyngeal dysphagia with significant symptomatic aspiration. After 6-months treatment, aspiration decreased significantly. He was able to walk and write again after the treatment. The younger patient also had improvement in oral dysphagia. He further showed improvements in MMSE, Purdue Peg Board, and quality of life. Their liver and spleen volumes and chitotriosidase levels remained stationary during this period. The older boy suffered from migratory, diffuse pain over face, throat, back, joints and feet, though electrophysiologic studies failed to demonstrate the presence of peripheral neuropathy. The management included dose reduction to 100mg three times a day and analgesics, but the latter might have in turn led to memory loss and hallucination. Therefore, miglustat seems to be able to reverse some of the symptoms in NP-C patients, but more experiences are certainly required.

Partial rescue of truncating mutations in the *ATRX* gene through unusual intra-exonic splicing. *R.J. Gibbons[1], N. Malik[1], C. Fisher[1], A. Fryer[2], D.R. Goudie[3], I.D. Krantz[4], J. Traeger-Synodinos[5], E. Kanavakis[5]* 1) MRC Molecular Hematology Unit, Weatherall Institute of Molecular Medicine, John Radcliffe Hosp, Oxford, UK; 2) Clinical Genetics, Alder Hey Childrens Hospital, Liverpool, UK; 3) Human Genetics Dept of Pathology, Ninewells Hospital and Medical School, Dundee, UK; 4) Division of Human Genetics, The Childrens Hospital of Philadelphia, Philadelphia, PA 19104, USA; 5) Medical Genetics, University of Athens, St. Sophias Childrens Hospital, Athens 11527, Greece.

Nonsense mutations or frameshifting deletions or insertions are often predicted to lead to protein truncation and when they lie upstream of important functional domains they are commonly presumed to be null alleles. We have identified, in 5 unrelated families, 4 such mutations within the *ATRX* gene. They lie within 24bp of each other in the middle of a 3kb coding exon. Surprisingly, protein analysis by western blot showed that in addition to the predicted truncated protein product, some apparently full-length protein was also encoded by the mutant alleles. Analysis of the ATRX transcripts revealed a previously unsuspected pattern of alternate splicing within this large exon which was present in both normal and mutant cell lines. This leads to the in frame loss of 87bp of sequence and the omission of these mutations from the alternate spliceform. To date, all predicted truncating mutations in ATRX have been shown to be rescued to some degree. It seems likely that true null alleles in the human are lethal as has been demonstrated for the ATRX knockout in mouse.

Creating meaning from the mania: Identifying potential candidate genes for bipolar disorder using fine mapping, expression arrays and association analysis in an Irish bipolar disorder sample previously linked to 14q21-32. *S.E. Dobrin[1], F. Cassidy[2], C. Zhao[1], J.C. Badger[1], S. Roche[2], P. McKeon[2,3]* 1) Center for Human Genetics, Marshfield Clinic Research Founation, Marshfield, WI; 2) Smurfit Institute of Genetics, Trinity College Dublin, Dublin 2, Ireland; 3) Department of Psychiatry, St Patrick's Hospital, Dublin 8, Ireland.

We previously carried out a 10cM whole genome scan in a sample of 60 Irish bipolar affective disorder (BPAD) affected sib pairs (ASPs). The most significant result was on chromosome 14 at 76cM (peak marker D14S588 (68.21MB), multipoint NPL=3.28, under the narrow, BPAD type I, disease model). The region of the chromosome with nominal p-values <0.05 was quite substantial, extending from 47cM to 122cM; therefore, we undertook a fine-mapping analysis of this region. 100 SNP markers, at a 0.6cM resolution, were analyzed in an extended sample of 88 ASPs. Linkage analysis was carried out using Genehunter Plus which resolved our original linkage peak to 3 separate peaks. The most significant NPL score was at rs1885590 (66.8MB) which is located within intron 2 of the MPP5 gene (multipoint NPL=3.21, narrow disease model) and is very close to the original peak. An additional 80 SNPs are being genotyped to further resolve this region to 0.2cM. All markers were also tested for association in 125 bipolar type I trios. Four SNPs had p-values <0.05: rs229646 (64.26MB), rs2885128 (75.85MB), rs2148564 (93.14MB), and rs2010649 (94.87MB). rs229646 is located within intron 8 of KIAA0599 (pleckstrin homology domain containing, family G (with RhoGef domain) member 3) and rs2148564 is located in intron 22 of KIAA1409. We also used the data from a separate whole genome expression array study on post-mortem brain samples provided by the Stanley Foundation to identify potential candidate genes from the original linkage peak region of chromosome 14. Here we present the complete results from our linkage, association, and expression analyses detailing the novel candidate genes under investigation.

Mapping of small RNAs in the human ENCODE regions. *C. Borel, M. Gagnebin, C. Gehrig, E. Kriventseva, E. Zdobnov, S.E. Antonarakis* Dept Genetic Medicine, Univ Geneva Medical Sch, Geneva, Switzerland.

Large-scale efforts have significantly contributed to the current understanding of the complexity of the human transcriptome. Yet, most experiments have been focused on transcripts longer than 100 bp, missing shorter RNAs. In this study, we examined the expression of small RNAs in the ENCODE regions, representing approximately 1%; of the human genome from 44 loci. We used the Affymetrix ENCODE tiling arrays that interrogate expression of every 22nt using overlapping 25mer probes to map the size fractioned small RNAs (from 19 and 50nt) in 4 different cell lines (HepG2, HelaS3, GM06990, SK-N-SH). The labelling of those small RNAs was performed with polymerase A and a labelled poly(A)-tail was synthesized. We observed about 7500 small RNA signals. Only 8% of which overlap exons of annotated Gencode genes, whereas the majority map to intergenic (35%) and intronic (51%) regions. The data analysis also showed that i) 35% of the 3or 5 UTR exhibit evidence of transcription of small RNA, ii) 20% of small RNA is cell type specific, iii) 25% of signals show evidence of overlapping transcription on both strands of the genome. We performed the same experiment after stimulation of SK-N-SH cells by Retinoic Acid (6uM, 48 hrs). We found that 17% of the small transcripts are differentially expressed (5 fold) and responded to an induced developmental program. This is consistent with a biological function of these transcribed regions. As we expect some of these small RNAs to be microRNAs, we used comparative sequence analysis to detect evolutionarily conserved and stable RNA stem-loop structures characteristic to microRNAs precursor genes. A high fraction of the predictions overlapped with the detected expression, and we selected some of the candidates for testing by northern-blot.

Investigation of HSA21 microRNAs expression in Down Syndrome. *M. Gagnebin, C. Borel, S. Deutsch, S. E. Antonarakis* Dept genetic medical, Univ Geneva Medical Sch, Geneva, Switzerland.

Down Syndrome is a disorder due to an extra copy of chromosome 21. Our understanding of the molecular pathogenesis is remarkably poor since it is not clear how the extra copy of HSA21 leads to a wide range of phenotypes. We investigated a new class of functional sequences: microRNAs (miRNAs), that act primarily as post-transcriptional repressors of target genes through 3UTR interactions. We have used quantitative real-time PCR to accurately measure the expression of miRNAs. Due to their small size (~22nt) and the absence of poly(A) tail, a specific assay was designed to reverse transcribe each mature miRNA with specific stem loop primers. We analysed the expression of the 4 known HSA21 miRNAs (miR-125b, miR-let7c, miR-155, miR-99) and 5 non-HSA21 miRNA in lymphoblastoid cell lines from 14 normal and 14 trisomy 21 unrelated individuals. Only miR-155 is significantly overexpressed (2 fold) in the trisomic lymphoblastoid cell lines. We analysed also the expression of those miRNAs in different tissues of one set of monozygotic twins discordant for trisomy 21. We observed a significant overexpression of 1) miR-Let7c in trisomic heart, 2) miR-125 in trisomic brain, 3) miR-125, miR-155, miR-99 in trisomic kidney. This tissue specificity supports the conclusionis that miRNA expression is controlled by tissue-specific regulatory mechanisms. We conclude that HSA21 microRNAs are overexpressed in trisomy 21 individuals in a tissue dependant manner. We are currently investigating the identification of the target transcripts that are functionally regulated by these specific miRNAs and may contribute to a cellular phenotype.

Kinship Estimation by Machine Learning Approach. *J. Ge[1,2], M.B. Rao[1,2], R. Chakraborty[1,2]* 1) Dept Biomed Engineering, Univ Cincinnati, Cincinnati, OH; 2) Center for Genome Information, Dept. Environmental Health, Univ Cincinnati, OH.

Kinship estimation, otherwise called determination of biological relationships between individuals, is relevant in genetic epidemiological investigations, forensic case-works, paternity testing, and victim identifications in mass disaster cases. Two genetic factors, genotyping error and population substructure, causes inaccuracies of kinship estimation from genotype data. Genotyping errors reduce the precision of likelihood estimation, and population structure mimics excess homozygosity in comparison to the Hardy-Weinberg expectations in the population. Generally traditional maximum likelihood methods, either based on single locus (i.e., scores derived from Identity-by-State, IBS) or multipoint linkage information (i.e., Markov Chain based models), perform well in distinguishing common relationships, but to reach a satisfactory precision for close relationships, at least 200 markers (i.e., half genome-scan) are required. Loss of information (e.g., use of only mean IBS scores), attendant to the traditional methods can be minimized by using Machine Learning algorithms (MLAs), as MLAs can utilize other features of IBS scores, offering opportunity for precise estimation of kinship with lesser number of genetic markers. In our study, two learning methods, Support Vector Machine (SVM) and Decision Tree (DT), are used. Theoretically SVM provides the best generalization ability on unseen data, compared with other classification methods, and DT explicitly gives the significance of inferred kinship. Extensive simulation experiments with unlinked loci showed increased efficiency of kinship estimation, compared with traditional methods even in the presence of genotyping errors and population substructure. However, for linked loci the gain of efficiency is more modest. Using real datasets encompassing most of the common relationships, empirical gain of efficiency of MLAs in comparison to the traditional methods are also evaluated. (Research supported by a grant form the Department of development, State of Ohio, and NIH grant GM 41399).

CRYBA4, a Novel Human Cataract Gene that is also Involved in Microphthalmia. *G. Billingsley[1], S. Santhiya[2], A.D. Paterson[1, 6], K. Ogata[3], S. Wodak[3], S.M. Hosseini[1], M.S. Manohar[2], P. Vijayalakshmi[7], P.M. Gopinath[8], J. Graw[4], E. Héon[1, 5]* 1) Program of Genetics & Genomic Biology, The Hospital for Sick Children, Research Institute, Toronto, ON, Canada; 2) Dr ALM Postgraduate Institute of Basic Medical Sciences, Department of Genetics, University of Madras, Taramani, Chennai, India; 3) Center for Computational Biology, The Hospital for Sick Children Research Institute; 4) GSF-National Research Center for Environment and Health, Institute of Developmental Genetics, Neuherberg, Germany; 5) Department of Ophthalmology and Vision Sciences, The Hospital for Sick Children, University of Toronto; 6) Department of Public Health Sciences, University of Toronto; 7) Arvind Eye Hospital and Post Graduate Inst. of Ophthalmology, Madurai, India; 8) Science Centre, Manipal Academy of Higher Education, Manipal, India.

Genetic analysis of a large Indian family affected with AD congenital lamellar cataract allowed us to identify a novel cataract gene, CRYBA4. Following a genome wide screen, linkage analysis identified a maximum LOD score of 3.20 (=0.001) with marker D22S1167 of the -crystallin genes cluster on chromosome 22. A c.317TC sequence change, which segregated with the disease status, was identified in CRYBA4 exon 4. To date, CRYBA4 was the only gene in this cluster not associated with either human or murine cataracts. The change, predicted to substitute the highly conserved hydrophobic Phe 94 by hydrophilic Ser, was not observed in 239 healthy control individuals. Protein modeling suggested a significant reduction in the intrinsic stability of the mutant crystalline monomer, which would impair its ability to form the association modes critical for lens transparency. Considering that CRYBA4 associates with CRYBB2 and that CRYBB2 has been implicated in microphthalmia, mutational analysis of CRYBA4 was performed in microphthalmia patients (n=32). We identified a c.242TC (Leu69Pro) sequence change in CRYBA4 exon 4 in one patient, which is predicted to disrupt the CRYBA4 -sheet, most probably leading to a structure with reduced stability. This is the first report associating mutations in CRYBA4 with cataractogenesis or microphthalmia.

Laryngeal Anomalies in Van Den Ende-Gupta Syndrome. *O.A. Abdul-Rahman[1], J.D. Carron[2]* 1) Department of Preventive Medicine, University of Mississippi Medical Center, Jackson, MS; 2) Deparment of Otolaryngology and Communicative Sciences, University of Mississippi Medical Center, Jackson, MS.

In 1992, Van den Ende et al. first reported a multiple congenital anomaly syndrome characterized by blepharophimosis, arachnodactyly, and congenital contractures in a Brazilian girl born to consanguineous parents. In 1995, Gupta et al. compared this entity with Marden-Walker syndrome and noted the absence of psychomotor retardation. Several additional patients have been reported with Van Den Ende-Gupta syndrome (VDEGS) broadening the phenotype to include cleft palate, dysmorphic facial features with a beaked nose and mildly crumpled ears, elbow deformities, and enlargement of the cerebellum. We present a 12 month-old African-American female born to non-consanguineous parents who was diagnosed with VDEGS based on the presence of blepharophimosis, beaked nose, crumpled ears, arachnodactyly, and congenital contractures of the hands, elbows, and knees. The patient also had chronic rhinorrhea and was referred for an otolaryngologic evaluation. Direct laryngoscopy identified an unusual laryngeal malformation characterized by large, globular cuneiform cartilages, shortened aryepiglottic folds, a tightly coiled epiglottis, and laryngomalacia. Due to stridor and prolapse of the redundant cuneiform cartilages, supraglottoplasty was performed with removal of the cartilages. Release of the aryepiglottic fold was also performed to separate the epiglottis from the arytenoid cartilages. We propose expanding the phenotype of VDEGS to include laryngeal anomalies and recommend direct laryngoscopy with appropriate intervention in VDEGS patients presenting with chronic rhinorrhea or stridor.

Dp71ab/DAPs complex composition changes during the differentiation process in PC12 cells. *J. Romo-Yáñez[1], V. Ceja[1], R. Ilarraza[1], R. Coral[2], F. Velázquez[3], D. Mornet[4], A. Rendón[5], C. Montañez[1]* 1) Depto. Genética y Biología Molecular, CINVESTAV, Mexico; 2) U. de Inv. Méd. en Genética Humana, CMN siglo XXI, México; 3) Depto. Bioquímica, UANL, México; 4) U. de Montpellier1, Lab. Physiol. des Interactions, EA 701, Institut de Biologie, France; 5) INSERM EMI 99-18, Lab. Physio. Mol. et Cell. de la Retine, France.

Dystrophin Dp71 has been identified as the major DMD (Duchenne Muscular Dystrophin) gene product in brain. Dp71 interacts with DAPs (Dystrophin Associated Proteins), as well as cytoskeletal actin. Dp71 mRNA is alternatively spliced generating different isoforms, one of them, Dp71ab lacks exons 71 and 78. PC12 cells express several Dp71 isoforms including Dp71ab. To gain insight into the function of Dp71 dystrophins, we identified DAPs that interact with Dp71ab during neuronal growth factor (NGF) induced differentiation of PC12 cells. DAPs expression was analyzed by RT-PCR, Western blot and indirect immunofluorescence coupled to confocal microscopy. RT-PCR analysis showed mRNA expression of all sarcoglycans. Inmunofluorescence analysis confirmed the expression at the protein level for these proteins, except -sarcoglycan. The colocalization analysis showed , and -sarcoglycans strongly colocalized with Dp71ab in undifferentiated PC12 cells; these colocalizations decreased in the differentiated state and showed a strong colocalization with -sarcoglycan. Western blot analysis revealed that the expression of -dystroglycan, 1-syntrophin, 1- and -dystrobrevins were not affected during NGF differentiation. In order to identify proteins interacting with Dp71ab, coimmunoprecipitation assays were performed; Dp71ab forms a complex with -dystroglycan, 1-syntrophin, -dystrobrevin, -, - and -sarcoglycans in undifferentiated PC12 cells. In differentiated PC12 cells, the complex composition changes since Dp71ab associates only with -dystroglycan, 1-syntrophin, -dystrobrevin and -sarcoglycan. Interestingly, neuronal nitric oxide synthase protein associates with Dp71ab-DAPs complex during NGF treatment, raising the possibility that Dp71ab may be involved in signal transduction events during neuronal differentiation.

Identification of a novel region for AIS on chromosome 12p with an autosomal recessive model of inheritance. *P. Giampietro[1], C. Raggio[2], C. Zhao[1], D. Dorshorst[1], S. Dobrin[1], J. Weber[4], R. Blank[3]* 1) Marshfield Clinic, Marshfield, WI; 2) Hospital For Special Surgery, New York, NY; 3) University of Wisconsin, Madison, WI. GRECC Service, William S. Middleton VAMC, Madison, WI; 4) Prevention Genetics 3700 Downwind Drive Marshfield, WI.

Identification of a novel region for AIS on chromosome 12p with an autosomal recessive model of inheritance P. Giampietro1, C. Raggio2, C. Zhao1, D. Dorshorst1, S. Dobrin1, J. Weber4, R. Blank3. 1) Marshfield Clinic, Marshfield, WI; 2) Hospital For Special Surgery, New York, NY; 3) University of Wisconsin, Madison, WI. GRECC Service, William S. Middleton VAMC, Madison, WI; 4) Prevention Genetics 3700 Downwind Drive Marshfield, WI.
Adolescent idiopathic scoliosis (AIS) is defined as a lateral curvature of the spine of 10 or greater which affects children during their most active growth phase. It is a complex, common,genetically heterogeneous condition, with genetic and environmental influences. The estimated frequency of AIS is 2-3% in the general population. Autosomal dominant with variable penetrance, multifactorial and X-linked dominant modes of inheritance have been suggested. Previous studies have demonstrated genetic heterogeneity for AIS with linkage to 17p11, 19p13.3, 6q, distal 10q and 18q. No causative genes have yet been identified. Molecular hypotheses include connective tissue matrix alterations, neuromuscular imbalance and altered vestibular function. Multiple-point linkage analysis was performed on 54 samples from 7 multi-generation AIS families.Phenotype was determined by clinical evaluation and radiographic analysis. Genotyping was performed with 400 microsatellite markers at a density of 10 cM, on average.Using multipoint analysis option in Genehunter with a disease frequency of 1-3%, HLOD scores of 3.45 and 1.94 were obtained under both autosomal recessive model and dominant model at markers GATA49D12 and GATA4H03, respectively. The markers are 6 cM apart and lie in a gene rich region at approximately 31cM and 13.4Mb on chromosome 12p. Fine mapping studies are in progress to narrow down this region.

Bayesian Networks for Modeling SNP Markers. *H. Dong*[1], *L. Jin*[1], *M. Xiong*[1,2] 1) Genetics, Fudan University, Shanghai, China; 2) Biostatistics, University of Texas Health Science center at Houston.

Mutations of DNA sequence are a major force for the occurrence of SNPs. Sequence evolution causes variations of SNPs in populations. SNPs are nonrandomly assembled along the genome. The dependence and structural complexity of SNPs are essential dynamic properties of SNPs in population. However, most studies of SNPs variation have ignored the structural properties of SNPs. The concepts of pair-wise linkage disequilibrium (LD) and haplotype block only partially characterize the structure complexity of SNPs. Similar to our society in which we live in a world of networks, the term "network" also turns out to be a central notion in investigation of variation of SNPs in population. Bayesian network is a graphic model that efficiently encodes the joint probability distribution for a large set of variables and hence is also suited for modeling SNPs. In this report, we develop a general mathematical framework for studying variation of SNPs. The marker network can be represented by a directed acyclic graph in which nodes represent markers and arcs represent probabilistic dependence between the markers. The algorithms for learning structure of SNPs network and estimating parameters in the network have been developed. The relationship between the LD and network structure, and the haplotype blocks and network structure have been revealed. The overall properties of dependence among SNPs and limitations of pair-wise LD for modeling dependence of SNPs have been investigated. The proposed algorithms for learning Bayesian networks of SNPs are applied to SNPs data sets from African, Caucasian and Chinese populations. It turn out that Bayesian networks of SNPs will open a new field in network approach to studying the pattern of LD among SNPs, population structure and evolutions.

A novel DNAI1 mutation in Kartagener syndrome. *I. Gutierrez-Roelens[1], K. Cefle[2], M. Vikkula[1]* 1) Lab Human Molecular Genetics, Christian De Duve Inst, Brussels, Belgium; 2) Division of Medical Genetics, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey.

Kartagener syndrome (KS) (MIM 244400) is a genetically heterogeneous disorder, characterized by nasal polyposis, bronchiectasis and situs inversus. The disease is inherited as an autosomal recessive trait. Kartagener syndrome and Primary Ciliary Dyskinesia (PCD) are associated with the same respiratory symptoms, but situs inversus is specific to KS. Situs inversus, clearly indicates embryonic disruption of normal left-right patterning. A link between cilia and left-right determination has been suspected in man based on the pathophysiology of PCD and KS. Both disorders result from anomalies of cilia structure (missing or abnormal dynein arms, abnormal radial spokes and missing central pair of microtubules). To date, four dynein genes (DNAI1, DNAH5, DNAH7, and DNAH11) have been identified to be mutated in PCD and KS. In this study, we report a consanguineous family of Turkish origin, composed of two unaffected parents, and four children, two of whom present Kartagener syndrome. Genome wide SNP screening for homozygosity using the 50K SNP chip allowed us to identify a linked region on chromosome 9p21.2-q21.13 where the DNAI1 gene is located. Direct sequencing of the DNAI1 gene permitted us to identify a c.625C>T nucleotide change, which creates a premature stop codon (p.Arg209X) in the 5-coding region. The four unaffected individuals of the family are heterozygous for the C/T alleles, whereas the two affected children are homozygous for the T allele. The c.625C>T substitution generates truncated polypeptides that probably could not play a role in outer dynein arm assembly leading to a loss-of-function of the cilia. (vikkula@bchm.ucl.ac.be) (http://www.icp.ucl.be/vikkula).

Post-marketing surveillance of oral miglustat treatment in patients with Type 1 Gaucher disease. *B. Bembi[1], D. Hughes[2], I.N. van Schaik[3], B. Schwierin[4], C.E.M. Hollack[3]* 1) Metabolic Diseases Unit, Pediatric Hosp B Garofolo, Trieste, Italy; 2) Royal Free and University College Medical School, London, UK; 3) Academic Medical Center, Amsterdam, The Netherlands; 4) Actelion Pharmaceuticals, Allschwil, Switzerland.

A non-interventional, web-based post-marketing surveillance programme was implemented to collect comprehensive baseline and ongoing clinical information on miglustat (Zavesca) use in Type 1 Gaucher disease (GD1), focusing on neurological manifestations. This study aims to enhance the awareness of safety precautions and to stimulate the appropriate monitoring of miglustat use in individuals with GD1. Descriptive statistics were used for the data analysis. From March 2003 to the end of March 2006, information was available on the first 62 GD1 patients (61% females) prescribed miglustat in 13 European countries (44 centres). Overall exposure to miglustat represented a cumulative period of 79 patient-years, with a mean exposure SD of 1511 months. Mean age SD was 4316 years. At baseline, 44 (71%) patients were previously treated with enzyme replacement therapy (ERT) and mean duration SD of treatment was 64 years. Neurological assessment information was available for 51 (82%) patients. Amongst these, 30% displayed neurological manifestations (11% tremor, 8% neuropathy, 8% memory problems, 6% cognitive abnormalities). Twenty (32%) patients had bone pain. In the follow-up period, no adverse events (AEs) were reported in 28 (45%) patients. AEs led to discontinuation in 11 (18%) patients mainly due to gastrointestinal disturbances, with 8 of the 11 cases occurring during the first 6 months treatment. Tremor was reported in 9 (14%) patients, memory problems in 2 (3%) patients. Bone pain was observed in 2 osteoporotic patients showing skeletal symptoms at baseline. Overall, data from the post-marketing surveillance of miglustat in GD1 raised no new safety concerns, particularly no peripheral neuropathy. Web-based post-marketing surveillance is an effective tool for comprehensive safety monitoring of patients receiving miglustat in an international clinical setting.

Analysis of Polymorphisms in Complement Factor H (*CFH*) and Hypothetical *LOC387715* in Mexican Mestizos with Advanced Age-related Macular Degeneration. *A. Contreras[1], I. Silva-Zolezzi[1], A. Hidalgo[1], A. Inchaustegui[1], R.A. Cano Hidalgo[2], J.C. Zenteno Ruiz[2], R. Ayala Ramirez[2], H. Perez-Cano[2], S. March[1], E. Graue[2,3], G. Jimenez-Sanchez[1]* 1) National Institute of Genomic Medicine, Mexico; 2) Fundacion de Asistencia Conde de Valenciana IAP, Mexico; 3) School of Medicine, UNAM, Mexico.

Age-related macular degeneration (AMD) is the most common cause of central blindness in elderly population, it is characterized by poor vision in the central field due to progressive destruction of the macular area. Molecular mechanisms underlying AMD are poorly understood. Recently, several studies identified 2 genes associated to AMD with major but independent contributions: Hypothetical *LOC387715* in whites and Complement Factor H (*CFH*) in non-hispanic whites. More than 80% of the Mexican population is considered Mestizo, resulting from the admixture of any of 62 ethnic groups with the Spaniards in the last 500 years. To evaluate participation of these 2 *loci* in the Mexican Mestizo population, we genotyped 4 SNPs previously associated with AMD in 53 unrelated Mexican Mestizos with advanced AMD and 1088 unrelated controls. These SNPs were, 1 in *LOC387715* (rs10490924) and 3 in *CFH,* 2 intronic SNPs (rs1410996, rs380390) and the Y402H variant (rs1061170). Our results are consistent with a significant association of the *LOC387715* variant with advanced AMD in Mestizo patients from Mexico City (O.R. 8.555, C.I. [4.157-17.609], p-value=1.146×10^{-11}). No significant association was found for any of the *CFH* SNPs analyzed in our sample. Our results suggest that *LOC387715* could be an important contributor to advanced AMD in the Mestizo population, similar to data reported for white patients (Jakobsdottir J et al., 2005, *Am J Hum Genet* 77: 389-407). Interestingly, we did not find association to *CFH*. This may be due to sample size or most likely, to a different molecular mechanism of this complex disease in the Mexican Mestizo population. To confirm these results and identify additional *loci* associated with susceptibility to AMD in the Mexican Mestizo population, we are conducting a genome-wide association study in a larger sample of these patients.

A prospective study of neurological manifestations and other comorbidities in adult Type 1 Gaucher disease. *M. Beck[1], L. Marodi[2], M. Biegstraaten[3], I.N. van Schaik[3], C. Niederau[4], D. Hughes[5], P. Giraldo[6], C.E.M. Hollack[3]* 1) Pediatric Dept, Univ Mainz, Mainz, Germany; 2) University of Debrecen, Debrecen, Hungary; 3) Academic Medical Centre, Amsterdam, The Netherlands; 4) Klinik fur Innere Medizin, Universitat Essen, Dusseldorf, Germany; 5) Royal Free Hospital, London, UK; 6) Miguel Servet University Hospital, Zaragoza, Spain.

Gaucher disease (GD) is a complex disorder and comprehensive information on its natural history is relatively limited. Central and peripheral nervous symptoms have been reported as secondary manifestations of Type 1 Gaucher disease (GD1). To further investigate these findings, a multinational (5 countries, 6 centres) prospective study has been set up and is currently ongoing with the endorsement of the European Working Group on Gaucher Disease (EWGGD). The objective of this study is to determine whether neurological symptoms are present in GD1. The primary endpoints are the prevalence and incidence of peripheral neuropathy (PN) in GD1 patients who have not received miglustat. Diagnosis of PN will be confirmed based on analysis of symptoms and electrodiagnostic assessments validated by an independent central assessor. Secondary endpoints are incidence of peripheral neuropathies over 2 years, and changes from baseline of various clinical and biological parameters such as neurological and neuropsychological status, skeletal symptoms, plasma electrophoresis and quality of life. Descriptive statistics will be used for data analysis. This study will recruit up to 100 patients with GD1, either untreated or treated by enzyme replacement therapy. As of April 2006, seventy-four patients have been enrolled. Analysis of baseline data is currently ongoing. This first set of analyses will provide information on several aspects of the natural history of GD1, with a special focus on the prevalence of peripheral and central neurological manifestations. The comprehensive information provided by this study will contribute towards a better understanding and management of this disease.

All is heterogeneous: Surveying clinical, genetic, and allelic heterogeneity in autosomal recessive congenital ichthyosis. *K.M. Eckl[1,4], J. Kurtenbach[1], M. Nätebus[1], W. Küster[2], H. Traupe[3], A. Önal Akan[1,4], H.C. Hennies[1]* 1) Dermatogenetics, Cologne Ctr for Genomics, Univ Cologne, Cologne, Germany; 2) TOMESA Clinics, Bad Salzschlirf, Germany; 3) Dermatology, Univ Münster, Münster, Germany; 4) Faculty Mathematics and Natural Sciences, Univ Cologne, Cologne, Germany.

Autosomal recessive congenital ichthyoses (ARCI) form a group of rare, severe disorders of keratinization. Phenotypes and genotypes are extremely heterogeneous. Underlying genes were described at five loci. This project started in 2002 is the first long-term ARCI study that focuses on molecular mechanisms and cellular structures. We describe molecular and clinical findings in 300 families with ARCI originating from Middle Europe, Turkey, and India. We and others showed that one third of patients carry mutations in *TGM1*. In *ALOXE3* and *ALOX12B* coding for epidermal lipoxygenases 12R-LOX and eLOX-3 we identified 20 novel point mutations in 26 families, including 15 missense mutations. Mutated genes were expressed in HEK-293 cells, total protein was isolated and incubated with the genuine substrates, and enzymatic activity was determined. Analysis of reaction products demonstrated lack of enzyme activity. The only active mutant c.434G>A in *ALOXE3* was a splice-site mutation as verified in a cell-based mini-gene assay. This work showed that missense mutations in *ALOX12B* or *ALOXE3* lead to enzyme inactivation and the ARCI phenotype. Until now, we analysed 182 ARCI patient samples and 144 control samples for mutations in *ichthyin*, recently found to be mutated in ARCI patients. Five different mutations on 40 chromosomes of 31 unrelated patients were identified. However, two mutations previously found in patient samples were also identified on 26 control chromosomes. *Ichthyin* was cloned for functional studies, a polyclonal antibody was generated. Mutation analysis of the most recently identified genes *ABCA12* and *FLJ39501* is under way. Genotype/phenotype correlation studies showed that mutations in *ALOX12B* or *ALOXE3* result in a mild form of ARCI. This is in contrast to the findings in patients with mutations in *TGM1* which give rise to severe forms of ARCI in many cases but can also be associated with self-healing collodion baby.

Mendelian Randomisation using ApolipoproteinAV and lipoprotein lipase genotypes provides no evidence that increased plasma triglyceride concentrations are causally associated with insulin resistance. *T.M. Frayling[1], K.J. Ward[1], M.S. Sandhu[2], M.N. Weedon[1], R. Harbord[3], B. Shields[1], B. Knight[1], J. Luan[2], Y. Ben-Shlomo[3], A. Jeffrey[1], T. Wilkin[1], S. Ebrahim[4], N. Timpson[3], D. Lawlor[3], G. Davey-Smith[3], N. Wareham[2], A.T. Hattersley[1]* 1) Peninsula Medical Sch, Exeter, UK; 2) Cambridge, UK; 3) Bristol, UK; 4) London, UK.

Mendelian randomization can be used to dissect the causal direction of two correlated phenotypes. Insulin resistance and triglyceride concentrations are important predictors of type 2 diabetes and cardio-vascular disease. These traits are highly correlated in the normal population but it is not known if high triglyceride concentrations cause increased insulin resistance or vice versa. We analysed three polymorphisms associated with triglyceride concentrations: the S19W and -1131T/C variants in ApolipoproteinAV and the S447X variant in lipoprotein lipase, in 4949 subjects from five UK studies. We hypothesized that if triglyceride concentrations causally altered insulin resistance, participants with increasing numbers of triglyceride raising alleles would have increased insulin resistance. The 19W, -1131C and S447 alleles were associated with, 0.30 (0.22-0.38, $p=2.6x10^{-13}$), 0.27 (95%CI: 0.19-0.36, $p=5.9x10^{-10}$) and 0.2 (0.13-0.26, $p=7.7x10^{-9}$) SD increases in triglyceride concentrations respectively (using logged values). Using all three variants, participants with 1, 2, 3, and 4 triglyceride raising alleles had triglyceride concentrations 0.19, 0.43, 0.71 and 0.93 SD higher than subjects with 0 alleles respectively($p = 1.7x10^{-29}$, trend). There was no association between increasing numbers of triglyceride raising alleles and insulin resistance, as measured by HOMA (p=0.38, trend, using logged values). Measures of insulin resistance by genotype were significantly lower than expected given the association between genotypes and triglyceride concentrations and the association between triglyceride concentrations and insulin resistance ($p<0.0001$). Our study is the first to use two genes in a Mendelian randomization experiment and shows that triglyceride concentrations are unlikely to causally alter insulin resistance in the general UK population.

Detection of a cryptic deletion in a patient with a known duplication of 12q using CGH microarray analysis: Difficulties associated with genotype/phenotype correlations. *N. Champaigne, J. McKenna III, M.J. Gambello* Dept Pediatrics, Univ Texas, Houston, Houston, TX.

We report the results of comparative genomic hybridization array analysis of a patient with a known de novo duplication of 12q. The patient was born with dysmorphic features and multiple congenital anomalies. His clinical findings consisted of small palpebral fissures, flat nasal bridge, bulbous nose, pointed chin, ambiguous genitalia, and duodenal atresia secondary to an annular pancreas. His medical course was further complicated by acute renal failure with a renal biopsy revealing a diffuse mesangial sclerosis variant of congenital nephritic syndrome. Chromosome analysis by G-banding showed additional chromosomal material on the distal end of 12q. FISH studies revealed a duplication of 12q24.32 to 12q24.33. Parental karyotypes were normal. The medical literature was reviewed for similar duplications for potential genotype/phenotype correlations and revealed several cases with variable breakpoints and clinical features ranging from mild to severe. There were no cases associated with congenital nephritic syndrome. To further characterize the breakpoints, analysis with the Agilent human genome 44k oligomicroarray was performed. Microarray data revealed that the duplication actually spanned 12q24.31, 12q24.32 and 12.q24.33 and that there was an unappreciated deletion of proximal 12q24.31. This case highlights the difficulty of using previous case reports to interpret the significance of rare chromosomal rearrangements, as well as to predict the long-term consequences for patients and their families.

Correction of a mitochondrial fatty acid oxidation (FAO) disorder by fibrate: relation to genotype. *S. Gobin-Limballe[1], F. Djouadi[1], F. Aubey[1], S. Olpin[2], S. Yamaguchi[3], R.J. Wanders[4], T. Fukao[5], J. Bastin[1]* 1) CNRS UPR9078, Faculté Necker, Paris, France; 2) Sheffield Children's Hospital, Sheffield, UK; 3) Shimane School of Medicine, Shimane, Japan; 4) Academic Medical Center, Amsterdam, The Netherlands; 5) Gifu University, Gifu, Japan.

We previously showed that fibrates could restore FAO in patient cells harboring defects in Very-Long-Chain-AcylCoA-Dehydrogenase (VLCAD; mitochondrial ?-oxidation), by stimulating residual enzyme activity. Given the large number of VLCAD gene mutations known, we investigated the relation between the nature of the mutations and the response to drug. We used a panel of 41 VLCAD-deficient fibroblast lines with distinct genotypes, representing 50 different mutations. Cells were cultured under standard conditions and 400M bezafibrate was added in the culture medium 2 days before experiments; FAO was then measured using tritiated palmitate. Untreated VLCAD-deficient cells exhibited FAO rates much lower (-30 to -90%) than control. In 60% of the genotypes tested, bezafibrate induced a marked increase in FAO, and complete correction of FAO was reached in 12 cell lines. Altogether, our data identified three groups: - Group 1 -severely deficient cells with nonsense mutations, or missense mutations affecting residues essential for catalysis (G222, G441, R469), these were drug-resistant - Group 2 - missense mutations that showed a response to bezafibrate, although probably leading to enzyme instability or altered catalytic properties as drug-induced FAO rates remained lower than control - Group 3 -, about 30% of cell lines, harbored genotypes that permitted full restoration of FAO by bezafibrate, comprising mild mutations (V283A, G441D, R615Q), that probably do not greatly affect the catalytic site and/or enzyme stability. We will now characterize changes in VLCAD mRNA and residual enzyme levels induced by bezafibrate, as a function of genotype. These data should provide valuable information on structure-function relationships for a future 3-D enzyme model. Furthermore, this study might open to clinical applications by identifying VLCAD mutations and VLCAD deficient patients that are potentially responsive to bezafibrate.

Search for environmental and genetic factors of Congenital Heart Disease in Sao Miguel Island, Azores (Portugal). *R. Cabral[1,2], R. Anjos[3], L. de Fez[1], P.R. Pacheco[1,2], C. Pereira Duarte[4], L. Mota-Vieira[1,2]* 1) Mol Genetics & Pathology Unit, Hospital Divino Espirito Santo, Azores Islands; 2) Instituto Gulbenkian de Ciencia, Oeiras; 3) Pediatric Cardiology Department, Hospital of Santa Cruz, Carnaxide; 4) Pediatric Department, Hospital Divino Espirito Santo, Azores Islands, Portugal.

Congenital heart disease (CHD) is the most frequent of all clinically significant birth defects. Recently, we demonstrated that in Sao Miguel Island the CHD prevalence is relatively high: 9.16 per 1000 live births. Considering that half of the population lives in small rural localities and the internal migration is reduced, aspects that increase endogamy and inbreeding, we performed a structured family questionnaire which includes: 1. queries for CHD risk factors (maternal diabetes mellitus, alcohol and drug abuse by the mother during pregnancy, viral infections of the fetus and genetic conditions), and 2. a detailed family history to construct the ascending genealogy until the 3th generation. To that end, 162 CHD families were contacted by phone and/or letter, 106 (65.4%) of which accepted to participate. We identify 37 (34.9%) multiplex families (with 2 to 5 patients), 6 (5.7%) consanguineous families, and 6 (5.7%) multiplex families with consanguinity. Particular attention will be given to 2 consanguineous families: a sib-sib marriage (F=0.25) and one with 5 CHD siblings (F=0.0039). Moreover, we observe that 61 (57.0%) cases have maternal risk factors, of which 7% has 3 or more risk factors. In order to carry out molecular genetic analysis, a biobank consisting of DNA and RNA from patients and parents was built after informed consent. The first mutation analyzed was the C677T of the MTHFR gene in 469 healthy individuals from Sao Miguel, being 84 (17.2%) homozygous for the mutation (TT) and 221 (45.5%) heterozygous (CT). From these observed genotypes, the C677T allele frequency in Sao Miguel population is 41.5%, the second highest value in Europe. A comparative analysis will be performed for this mutation in the CHD patients, as well as for other candidate genes such as NKX2.5, TBX5 and GATA4. Funded by FCT and DRCT (Azores), Portugal.

Understanding diseases associated with mutations in the GNE gene, coding for UDP-GlcNAc 2-epimerase/ManNAc kinase. *C. Ciccone, D. Krasnewich, R. Klootwijk, I. Manoli, S. Sparks, W.A. Gahl, M. Huizing*
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The GNE gene, on 9p12-13, encodes the bifunctional enzyme UDP-GlcNAc 2-epimerase (GNE) /ManNAc kinase (MNK), which catalyzes the first two committed, rate-limiting steps in the biosynthesis of sialic acid. Sialic acid is a negatively charged sugar, typically found as the terminal sugar on glycoconjugates, where it plays a role in a variety of cellular signaling functions. Feedback inhibition of GNE by the final product, CMP-sialic acid, tightly regulates sialic acid biosynthesis. GNE mutations underlie two distinct disorders. Dominantly inherited sialuria is characterized by dramatically *increased* sialic acid levels, developmental delay, coarse facies, and hepatomegaly. Patients have a missense mutation in the allosteric site of GNE, leading to loss of feedback-inhibition by CMP-sialic acid, cytoplasmic accumulation of sialic acid, and urinary excretion of gram quantities of free sialic acid. Recessively inherited Hereditary Inclusion Body Myopathy (HIBM) is characterized by *decreased* sialic acid production. HIBM is a recessive disorder of adult onset with progressive muscle weakness and relative sparing the quadriceps muscles. HIBM patients harbor two GNE mutations within the GNE or NMK domains and outside of the enzymes allosteric site. Our technical ability to quantitate monosaccharides using HPLC/pulsed amperometric detection has allowed us to study the biochemical pathology of GNE mutations in detail. We developed individual GNE and MNK enzymatic assays and determined reduced activities in cultured fibroblasts of patients with HIBM missense mutations in either or both the GNE and MNK enzymatic domains. This loss of enzyme activity impairs sialic acid production and interferes with proper sialylation of glycoconjugates. Understanding the function and regulation of the sialic acid biosynthesis pathway permits the development of diagnostic tests and therapies for HIBM (see abstract of Huizing et al.) and sialuria (see abstract of Klootwijk et al.).

Variants in TGF1 are associated with severe lung disease in homozygous F508 cystic fibrosis patients. *L.A. Bremer, S.M. Blackman, L.L. Vanscoy, J.M. Collaco, K.E. McDougal, G.R. Cutting* McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.

Pulmonary function varies among cystic fibrosis (CF) patients with the same CFTR genotype, indicating that other factors contribute to phenotype variation. A recent case-control study showed that the transforming growth factor beta-1 (TGF1) gene may be a modifier of CF lung disease severity (Drumm, et al). The goal of this work was to evaluate the association of SNPs in TGF1 with CF lung disease severity using transmission tests. Trios selected from the CF Twin and Sibling Study were genotyped for two SNPs in TGF1: C-509T and C+29T (codon 10). Transmission disequilibrium test (TDT) was performed in patients dichotomized for two longitudinal pulmonary phenotypes (BayesFEV1%pred and AvgFEV1CF%). Because body mass index is known to be correlated with FEV1, association of TGF1 with average BMI z-score (AvgBMIZ) was also studied. Twin and sibling analysis indicates that heritability of these three phenotypes ranges from 0.5-0.8. Patients were assigned affected status if they fell below pre-determined threshold levels: BayesFEV1%pred, <68% (severe); AvgFEV1CF%, below 50th percentile (severe); AvgBMIZ, <-0.7 (severe). TDT demonstrated statistically significant association between both the -509T and +29C alleles and severe lung disease (BayesFEV1%pred: 53 trios, p=0.011 and 0.039; AvgFEV1CF%: 96 trios, p=0.025 and 0.017). No transmission distortion was seen in trios classified as having mild lung disease (136 and 160 trios) or in those classified by AvgBMIZ (168 severe trios). Patients stratified according to CFTR genotype (F508/F508 vs. other) demonstrated significant over-transmission of the -509T and +29C alleles in patients with severe CF lung disease (-509T: BayesFEV1%pred, 25 trios, p=0.011; AvgFEV1CF%, 50 trios, p=0.029; and +29C: AvgFEV1CF%, 50 trios, p=0.013). Transmission distortion was not observed in non-F508 homozygotes (28 and 46 trios). This family-based study, along with a case-control study (Drumm, et al), implicate TGF1 as a modifier of CF lung disease severity, in a context dependent on CFTR genotype.

Expression profiling in families with Mitochondrial Myopathy and Sideroblastic Anemia (MLASA) and homozygous mutation in the Pseudouridylate Synthase I (PUS1) gene. *Y. Bykhovskaya, E. Mengesha, N. Fischel-Ghodsian* Medical Genetics Institute, Ahmanson Department of Pediatrics, Steven Spielberg Pediatric Research Center, Cedars-Sinai Medical Center and David Geffen School of Medicine at UCLA, Los Angeles, CA.

MLASA is a progressive oxidative phosphorylation disorder that affects muscle and erythroid cells. Molecular basis of MLASA was recently identified to be the homozygous point mutation C656T (R116T) in the catalytic domain of the PUS1 gene. Biochemical analysis revealed a lack of pseudouridylation at the expected sites in mitochondrial and cytoplasmic tRNAs. In order to further elucidate intracellular pathways through which the homozygous mutation leads to tissue-restricted phenotype, we performed microarray expression analysis of EBV-transformed lymphoblasts from four patients with MLASA, heterozygous parents, an unaffected sibling homozygous for the normal PUS1 allele and three unrelated controls. Expression signals of 47,296 RefSeq annotated and putative transcripts from human expression beadchip microarray were analyzed using BeadStudio and GeneSpring programs. Genes coding for proteins involved in DNA transcription and its regulation, and metal binding proteins, demonstrated major differences in expression between patients and all other individuals with normal phenotype. Genes coding for ribosomal proteins differed significantly between individual with at least one copy of the mutated PUS1 gene and wild-type controls, suggesting a potential compensation mechanism. Specific analyses of pseudouridylation and iron metabolism related genes revealed several differentially expressed genes. Real-time RT-PCR results for selected transcripts were consistent with microarray results. These finding outline several potential genes and pathways involved in the pathogenesis of MLASA in affected tissues, as well as potential compensatory pathways in unaffected tissues, which will be further investigated in a mouse knockout model of MLASA. We gratefully acknowledge support from NIH/NIDDK grant RO1-DK74368.

Recessive Lethal Form of Osteogenesis Imperfecta Caused by Null Mutations in CRTAP. *A.M. Barnes[1], W. Chang[1], R. Morello[2], W.A. Cabral[1], E. Makareeva[3], N. Kouznetsova[3], S. Leikin[3], M.A. Weis[4], D.R. Eyre[4], T.E. Uveges[1], J.J. Mulvihill[5], U.T. Sundaram[6], B. Lee[2], J.C. Marini[1]* 1) BEMB, NICHD/NIH, Bethesda, MD; 2) Baylor Coll. Med., Waco, TX; 3) SPB, NICHD/NIH, Bethesda, MD; 4) U. Wash., Seattle, WA; 5) Children's Hosp. of Oklahoma, Oklahoma City, OK; 6) Dept. Human Genetics, MCV, Richmond, VA.

CASP (cartilage-associated protein), encoded by *CRTAP*, is required for prolyl 3-hydroxylation of fibrillar collagens; loss of its function causes defective osteoid formation and severe osteoporosis in mice. We have identified the first 3 patients with mutations in the coding region of *CRTAP*. All 3 have a severe recessive form of osteogenesis imperfecta (OI), which is lethal in the first year of life. Long bones are extremely osteoporotic and deformed, with prenatal fractures and abnormal modelling. Proband 1 is homozygous for an IVS1+1G>C mutation; his parents were second cousins. The alternatively spliced forms each contain a PTC in intron 1. Proband 2 is homozygous for Gln276Stop in exon 4. Proband 3 is a compound heterozygote of a 16 nt duplication in exon 1, which leads to a PTC in exon 2, and a Met1Ile substitution. Expression of *CRTAP* was quantitated in proband fibroblast total RNA by real-time RT-PCR; probands 1, 2 and 3 had 0, 5-12 and 20-22% CRTAP expression, respectively, compared to normal control. Proband fibroblast collagen is fully overmodified on SDS-Urea-PAGE, as is found in cases of OI with collagen structural defects. Proband secreted collagens have 28-45% more hydroxylysine residues than normal collagen, which is consistent with collagen Tm ~1ºC greater than normal. Mass spectrometry of proband collagen tryptic peptides revealed absence of prolyl-3-OH at the unique Pro986 site in the 1(I) chain of type I collagen in probands 1 and 2, and 20% of normal hydroxylation in proband 3. Absence of CASP in all probands and presence in the parents was demonstrated on Western blot with murine anti-CASP antibody. These recessive null mutations for CRTAP reveal that prolyl 3-hydroxylation of type I collagen is crucial for bone formation; absence of prolyl-3-hydroxylation of types V and IV collagen may also be important.

Project among African Americans to Explore Risks for Schizophrenia (PAARTNERS): Recruitment and Assessment Methods. *M.H. Aliyu[1], M.E. Calkins[2], C.L. Swanson[1], P.D. Lyons[3], R. Savage[1], R. May[1], H.W. Wiener[4], B. Devlin[5], V.L. Nimgaonkar[5], R.C. Go[4]* 1) Psychiatry, University of Alabama at Birmingham, Birmingham, AL; 2) Psychiatry, Neuropsychiatry Section, University of Pennsylvania, Philadelphia, PA; 3) Neurology, University of Virginia, Charlottesville, VA; 4) Epidemiology, University of Alabama at Birmingham, Birmingham, AL; 5) Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA.

The Project among African-Americans to Explore Risks for Schizophrenia (PAARTNERS) is a multi-site, NIMH-funded study that seeks to identify genetic polymorphisms that confer susceptibility to schizophrenia among African Americans by linkage mapping and, possibly, admixture mapping. Because deficits in certain dimensions of cognitive ability are thought to underlie liability to schizophrenia, the project also examines cognitive abilities in individuals affected by schizophrenia and their extended family members. We aim to recruit a sample of 1260 African American families, all of whom have at least one proband with schizophrenia or schizoaffective disorder. The data collection protocol includes a structured Diagnostic Interview for Genetic Studies, Family Interview for Genetic Studies, focused neurocognitive assessment, medical records review, and the collection of blood or buccal cells for genetic analyses. We have currently completed study procedures for 93 affected sib pairs, 403 case-parent trio and 13 multiplex families. A total of 289 probands have completed the best estimate final diagnosis process and 1153 probands and family members have been administered the computerized neuropsychological battery. This project lays the foundation for future analysis of cognitive and behavioral endophenotypes. The novel integration of diagnostic, neurocognitive and genetic data will also generate valuable information for future phenotypic and genetic studies of schizophrenia.

Mutation screening of the PTEN gene in patients with autism and macrocephaly. *C. Betancur[1], G. Cai[2], P. Chaste[1], G. Nygren[3], J. Goldsmith[2], J. Reichert[2], H. Anckarsäter[3], M. Råstam[3], M. Leboyer[1], C. Gillberg[3], A. Verloes[4], J.D. Buxbaum[2]* 1) INSERM U513, Université Paris XII, Creteil, France; 2) Mount Sinai School of Medicine, New York, USA; 3) Department of Child and Adolescent Psychiatry, Goteborg University, Goteborg, Sweden; 4) Clinical Genetics Unit, Hôpital Robert Debré, Paris, France.

The tumor suppressor gene PTEN, located on 10q23, is mutated in a spectrum of disorders (Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, juvenile polyposis...) collectively referred to as PTEN-hamartoma tumor syndrome. Clinical features include progressive macrocephaly, mucocutaneous changes (hemangiomas, lipomas, tricholemnomas, pigmented spots), fibrocystic breast disease, cerebellar hamartomas (known as Lhermitte-Duclos disease) and colon polyps. Hypotonia, macrocephaly, and in some cases mental retardation and/or seizures may be present. Breast, thyroid and uterine cancers are common in adults. PTEN mutations have been described in a few patients with autism and macrocephaly. In this study, we screened PTEN for mutations and deletions in 88 patients with autism spectrum disorders and macrocephaly (head circumference > 2 SD) recruited by the Paris Autism Research International Sibpair (PARIS) study or by Mount Sinai School of Medicine/Autism Genetics Resource Exchange. Mutation analysis was performed by direct bidectional sequencing of all exons, flanking regions and promoter. Deletions were searched by MLPA. No exonic or whole gene deletions were observed. We identified a previously undescribed de novo missense mutation in exon 8 (p.D326N) in a 5-year-old boy with autism, mild mental retardation and severe, progressive macrocephaly (OFC > 10 SD). Study of 15 polymorphic markers excluded non-paternity. Clinical features included postaxial hexadactyly of the feet (operated) and 3 café-au-lait spots. These findings indicate that molecular screening of PTEN should be considered in patients with autism and marked macrocephaly. Because of tumoral risk, specific medical follow-up need to be proposed to patients with PTEN mutation, as well as genetic counseling and carrier screening in the relatives.

Complement Factor H Polymorphism Tyr402His and Cuticular Drusen. *M.A. Grassi[1,6], J.C. Folk[1], T.E. Scheetz[1,4,5], C.M. Taylor[1], V.C. Sheffield[1,2,3,4,5], E.M. Stone[1,3,4,5]* 1) Ophthalmology, University of Iowa, Iowa City, IA; 2) Pediatrics, University of Iowa, Iowa City, IA; 3) Howard Hughes Medical Institute, Chevy Chase, MD; 4) Center for Bio-informatics and Computational Biology, Iowa City, IA; 5) Carver Family Center for Macular Degeneration, Iowa City, IA; 6) Heed Ophthalmic Foundation, Cleveland, OH.

The c.1204T>C, p.Tyr402His allelic variant in the complement factor H (CFH) gene is associated with a 3-fold increased risk for age-related macular degeneration (AMD). A high frequency of the histidine allele has also been noted in patients with membranoproliferative glomerulonephritis type II. We sought to determine the histidine frequency in patients with the cuticular drusen phenotype of AMD. 50 individuals were identified who met the criteria for the cuticular drusen phenotype using a standard threshold photograph. DNA samples were genotyped using a PCR based NIaIII restriction digest assay. 700 individuals with typical AMD and 252 controls were also genotyped. Fishers exact test was used to analyze the significance of allele frequency differences. The histidine variant was present in 70% (0.70+/-0.05) of the cuticular cohort; 55% (0.55+/-0.01) of the more typical AMD cases; and 34% (0.34+/-0.02) of controls. The association between the cuticular drusen phenotype and the histidine allele was highly significant [p=0.0034, OR 2.0 (1.21-3.07) vs. AMD cases; p=3.02e-11, OR 4.54 (2.79-7.50) vs. controls]. Genotype distribution between the three groups was similarly significant [p=0.0004]. The cuticular drusen phenotype is highly associated with the Tyr402His variant of the CFH gene. The significantly higher histidine allele frequency in this group compared to the typical AMD cohort suggests that the complement cascade may play a greater role in the pathogenesis of the cuticular drusen sub-type than in AMD as a whole.

Genotyping of factor VIII by TTGE (Temporal Temperature Gradient Gel Electrophoresis) and I-PCR (Inverse-Polymerase Chain Reaction) followed by sequencing in a family segregated with severe hemophilia A. *S.P. Chang[1,2], M. Chen[1,3], D.J. Lee[1], M.C. Shen[1,4,5]* 1) Department of Medical Research, and Center for Medical Genetics, Changhua Christian Hospital, Changhua, Taiwan; 2) Department of Bioscience Technology, Chang Jung Christian University, Taiwan; 3) Department of Obstetrics and Gynecology, and Department of Medical Genetics, National Taiwan University Hospital, Taipei, Taiwan; 4) Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan; 5) 5. Department of Internal Medicine, Changhua Christian Hospital, Changhua, Taiwan.

We devised a novel strategy of combining TTGE (temporal temperature gradient gel electrophoresis) and I-PCR (inverse-PCR) to detect a novel frameshift mutation, c.3331-3333del A, of the human factor VIII gene in a Taiwanese family segregated with severe hemophilia A. TTGE was regarded to be unable to detect mutations smaller than 3-bp size variation (whether deletion or addition). Here we developed a modified strategy of TTGE in this sex-linked recessive disorder by mixing the wild-type allele with the DNA we wished to examine in a 1:1 ratio of quantity and successfully hunted the 1-bp deletion. This report adds a novel mutation of human factor VIII gene and also provides a technically interesting modification to the existing mutation screening strategies for sex-linked recessive disorders.

Localization of a Prostate Cancer Predisposition Gene to a 416,515 bp Region on Chromosome Xq28 in Utah High-Risk Pedigrees. *L.A. Cannon-Albright, J.M. Farnham, N.J. Camp* Biomedical Informatics, University of Utah, Salt Lake City, UT.

Previously we observed significant linkage confirmation for HPCX in Utah high-risk prostate cancer pedigrees (LOD=2.74; p=0.0002). Here we describe our localization efforts using additional genetic markers and statistical recombinant mapping. Linkage analyses were performed using MCLINK, a Markov chain Monte Carlo method, with the Smith parametric model. Classical multipoint LODs were calculated for each pedigree. The most likely haplotype configuration for each pedigree was also determined. It is known that genetic heterogeneity is extremely problematic for localizing linkage regions. Noise from unlinked pedigrees can shift linkage evidence and mislead fine-mapping efforts. Here we have focused on linked pedigrees only. We identified 32 high-risk extended Utah pedigrees with pedigree-specific LOD>0.588 (p=0.05) between DXS1200 at the q-telomere; inspection of the haplotype configurations suggested that twenty of these had at least three prostate cancer cases sharing a segregating haplotype. These 20 pedigrees were used for localization. For each pedigree, the multipoint LOD graph was used to infer recombinants, such that a sharp decrease in the LOD (>0.5 LOD units) was considered evidence for a recombinant, since such a decrease indicates a loss in sharing. With imperfect information content, the LOD may decrease over several cM and across several markers. The innermost (high) position before the change in LOD indicates the innermost position of the recombinant, and the outermost (low) position after the LOD change, the outermost position. To remain conservative, we considered only the outermost recombinant positions. The positions of all recombinants inferred across all 20 localization pedigrees were overlaid to identify the consensus region. We identified a 3-recombinant consensus region (region bound by three recombinants on each side) between DXS1193 and DXS7493, a 4.1 cM region of 2,868,449 bp at Xq28. This region contains approximately 34 genes. Within this, the 2-recombinant consensus region is only 1.1 cM (416,515 bp) and contains only two genes. We are currently pursuing mutation screening in haplotype carriers in these two candidate genes.

Valid Haplotype Association Analyses Accounting for Phase Uncertainty. *N.J. Camp, J. Wong, A. Thomas*
Biomedical Informatics, University of Utah, Salt Lake City, UT.

Consideration of haplotypes of candidate genes is the basis for an effective association study of complex diseases. A problem, however, arises because composite genotypes are the observed data. Haplotypes are not directly observed; they are estimated from genotype data. Generally, haplotypes are maximum likelihood estimates (MLE) established using, for example, an expectation-maximization (EM) algorithm. If such estimated haplotypes are considered and analyzed as if they were directly observed data, without considering the phase uncertainly, inflation of the type I errors may occur and tests become anti-conservative. One solution is to use a likelihood approach to consider all possible haplotypes with their corresponding probabilities. Here we discuss an alternative approach using Monte Carlo testing, and introduce hapMC, a JAVA program, that performs such analyses. The key to the Monte Carlo procedure is to appropriately match the observed statistic and the simulated null statistics that form the null, such that a valid test of the correct size is maintained. This is achieved as follows: MLE haplotypes are established for the observed data (cases and controls separately), and the statistic of interest calculated, ignoring the phase uncertainty. The composite genotype data are permuted across all individuals, MLE haplotypes are established for the permuted data (cases and controls separately), the statistic of interest calculated, again ignoring the phase uncertainly, and stored as a null statistic. The permutation procedure is repeated multiple times and a null distribution estimated, from which the observed statistic is assessed for significance. An alternate procedure for estimation of the null distribution is, instead, to use haplotype frequencies estimated from the observed data to generate the null composite genotype data. This second procedure allows the user to estimate MLE haplotypes across cases and controls together. Our program, hapMC, performs the necessary Monte Carlo procedure and EM estimates of haplotypes to provide valid haplotype tests for various standard association statistics, appropriately accounting for phase uncertainty.

Genetic polymorphism of CTLA-4 gene and cervical squamous cell carcinoma among Taiwanese women. *T.Y. Chang[1], Y.C. Yang[1, 2, 4], Y.J. Lee[1, 3, 5], T.H. Su[2, 4], C.K. Chen[1], H.F. Liu[1], C.C. Chu[1], M. Lin[1]* 1) Department of Medical Research, Mackay Memorial Hospital, Taipei, Taiwan; 2) Department of Gynecology and Obstetrics, Mackay Memorial Hospital, Taipei, Taiwan; 3) Department of Pediatrics, Mackay Memorial Hospital, 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. Cytotoxic T-lymphocyte antigen-4 (CTLA-4) is a molecule expressed mainly on activated T cells and is important for the down-regulation of T-cell activation. The aim of this study is to investigate if polymorphisms of the CTLA-4 gene are associated with HPV-induced cervical cancer in the Taiwanese population. The -318 C/T, +49 A/G, and CT60 polymorphisms were genotyped in 165 cervical squamous cell carcinoma (CSCC) patients and 366 age/sex matched healthy controls by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The presence and genotypes of HPV in CSCC patients were determined by E6, E7-based nested PCR. We found that the frequencies of -318 C/T genotype (25.9% vs. 17.2%; OR = 1.68; 95% CI = 1.06-2.67; P = 0.027) and T allele (12.8% vs. 5.7%; OR = 2.44; 95% CI = 1.24-4.81; P = 0.009) were significantly increased in CSCC patients as compared to the control population. No significant associations were found for the +49 A/G and CT 60 A/G polymorphisms. Our results suggest that -318 C/T variant in the promoter region of the CTLA-4 gene takes part in the development of CSCC in the Taiwanese population.

Father-to-daughter transmission of Cornelia de Lange syndrome caused by a mutation in the 5 untranslated region of the NIPBL gene. *G. Borck, M. Zarhrate, C. Cluzeau, E. Bal, J.-P. Bonnefont, A. Munnich, V. Cormier-Daire, L. Colleaux* INSERM U781, Hôpital Necker-Enfants Malades, Paris, France.

Cornelia de Lange syndrome (CdLS) is a developmental disorder characterised by typical facial dysmorphism, growth and mental retardation, microcephaly, and various malformations. Mutations in the NIPBL gene have been identified in ~40% of reported cases, suggesting either genetic heterogeneity or that some NIPBL mutations are not detected by current screening strategies. We screened a cohort of 21 patients with no previously identified NIPBL anomaly for mutations in the 5 untranslated region (5UTR) and the proximal promoter of the NIPBL gene. We identified a heterozygous deletion-insertion mutation in exon 1, 321 nucleotides upstream of the translation initiation codon (c.-321_-320delCCinsA) in one affected girl and her mildly affected father. This mutation altered highly conserved nucleotides, was not found in 400 control alleles, arose de novo in the father and co-segregated with the disease in the family. Using real-time quantitative PCR, we showed that NIPBL mRNA expression was lowered in patients lymphocytes compared to control samples. Finally, we showed that, when subcloned into a luciferase reporter vector, the mutation leads to a significant reduction of reporter gene activity. Our results demonstrate that mutations in the 5 non-coding region of the NIPBL gene can be involved in the pathogenesis of CdLS. Mutations affecting this region of the gene might be associated with a milder phenotype.

Geographic Distribution of Polymorphisms in Folate Metabolism Genes in Mexico. *L.A. Alfaro, I. Silva-Zolezzi, A. Contreras, R. Goya, G. Jimenez-Sanchez* National Institute of Genomic Medicine, Mexico.

Hyperhomocystinemia and folate deficiency are risk factors for birth defects, complications during pregnancy, cardiovascular diseases, psychiatric disorders and cancer. Polymorphisms in genes encoding folate-dependent homocysteine pathway enzymes and other proteins related to folate metabolism have been associated to these conditions. Given the interaction between homocysteine and folate metabolism, particular combinations of genetic variants may underlie susceptibility to a diversity of serious clinical conditions. Except for the *MTHFR* 677C>T allele, most of the associations observed in different populations remain unconfirmed or controversial. To improve the design of association studies with common diseases in the Mexican population, we analyzed geographic distribution of allele frequencies of *MTHFR* 677C>T and 1298A>C, *MTRR* 66A>G, *MTHFD1* 1958A>G, *SLC19A1* 80A>G, and *TCN2* 776C>G, in ~1200 Mexican Mestizos from six different states: Guerrero, Guanajuato, Sonora, Veracruz, Yucatan and Zacatecas. Results show the following average allelic and genotypic frequencies associated to the phenotypes in the studied population, as well as the observed range between states: 1) *MTHFR* 677C>T, T=0.484 (0.432-0.575) and TT=0.229 (0.189-0.342); 2) *MTHFR* 1298A>C, C=0.142 (0.085-0.256) and CC=0.027 (0.006-0.073); 3) *MTRR* 66A>G, G=0.765 (0.663-0.858) and GG=0.597 (0.461-0.749); 4) *MTHFD1* 1958G>A, A=0.577 (0.468-0.629) and AA=0.333 (0.207-0.395); 5) *SLC19A1* 80A>G, A=0.530 (0.426-0.520) and AA=0.244 (0.168-0.275); and, 6) *TCN2* 776C>G, G=0.338 (0.289-0.351) and GG=0.115 (0.082-0.157). For the *MTHFR* our results are similar to reported frequencies in the Mexican population: for 677C>T, T (0.43-0.58), TT (0.170-0.357), and for 1298A>C, C (0.147-0.280), CC (0.023-0.155). In addition, our results show significant differences between several states, indicating that geographic distribution analysis of these allele frequencies will be of relevance to better design association studies for these genes in Mexican Mestizo population.

Mismatch repair detection technology as a tool for high throughput somatic mutation screening. *S. Bentivegna, A. Seymour* Pfizer Global Research and Development, Groton, CT.

The process of tumorigenesis occurs through the acquisition of a series of specific genetic alterations. Somatic point mutations and small insertions and deletions represent an important subgroup of these alterations but are often difficult to detect in the tumor genome via traditional screening technologies due to varying levels of infiltrating normal tissue present in the sample. Affymetrix has developed a mismatch repair detection (MRD) technology that utilizes a bacterial mismatch repair system in vivo to detect somatic mutations with a high degree of parallel processing. In this study, we evaluate the sensitivity of the MRD platform to detect somatic mutations in cancer cell line and colorectal and breast tumor DNA samples.

The coding regions of 30 kinase genes were screened in a panel of DNA samples derived from 54 colorectal tumors, 38 breast tumors and 23 cancer cell lines. Additionally, DNA derived from matched normal tissue for each tumor sample was screened in order to determine the germline variation present in each sample. A total of 311 somatic mutations were detected via MRD, all confirmed by dideoxy DNA sequencing. Here, we present the data detailing the frequency of these mutations, their locations within the genes, the effects on the open reading frames and the class of sample in which they occur. Moreover, we discuss the consistency of our data with findings that have been well documented in the scientific literature, including an increased incidence of somatic mutation in the BRAF and KRAS genes in colorectal cancer.

Evaluation of Ancestry and Linkage Disequilibrium Sharing in Admixed Population in Mexico. *J.K. Estrada, A. Hidalgo-Miranda, I. Silva-Zolezzi, G. Jimenez-Sanchez* National Institute of Genomic Medicine, Mexico.

More than 80% of the Mexican population is considered Mestizo, resulting from the admixture of ethnic groups with Spaniards. To generate an initial estimate of ancestral contribution (AC) of populations from Europe, Africa and Asia to the Mexican Mestizos, we genotyped 104 samples from the states of Sonora (n=20), Yucatan (n=17), Guerrero (n=21), Zacatecas (n=19), Veracruz (n=18) and Guanajuato (n=8) using the 100K Affymetrix SNP array, and used data from the International HapMap Project as the parental population information. From 3,055 ancestry informative SNPs reported by Smith et al. and Choudhry et al., we identified 105 present in the 100K array and used them to calculate AC from each population to our sample. To infer AC we used Structure software under the admixture model. Based on this analysis, the average AC in our samples is 58.96% European, 10.03% African and 31.05% Asian. Sonora shows the highest European contribution (70.63%) and Guerrero the lowest (51.98%) where we also observe the highest Asian contribution (37.17%). African contribution ranges from 7.8% in Sonora to 11.13% in Veracruz. Based on these data, we grouped our population according to European AC (<50%, 50-60%, 60-70% and >70%). We used the Carlson algorithm to derive European tagSNPs from the 100K marker set. To explore Linkage Disequlibrium Sharing (LDS) between Mestizos and Europeans, we calculated the proportion of tagSNP-marker pairs that maintained an $r^2$0.8 in each evaluated population. In general, comparison of LDS between European and Asian population is ~73%, whereas comparison with African population is ~40%. Mestizos from Guerrero show the lowest LDS (74%), whereas those from Sonora show the highest (77%). Similar results are seen in the group of lower (<50%) and higher (>70%) European ancestry. Our results suggest that the Mexican Mestizo population shows ancestry-based stratification that will requiere the appropriate corrections to avoid spurius results in association studies. Our results show that admixed populations have unique patterns of LD depending on levels of ancestral contribution.

Stimulation of respiratory chain (RC) complexes in RC-deficient patient cells: a pharmacological approach. *J. Bastin[1], F. Aubey[1], A. Munnich[2], F. Djouadi[1]* 1) CNRS UPR9078, Faculté Necker, Paris, France; 2) INSERM U781, Hôpital Necker, Paris, France.

We tested the ability of drugs acting as agonist of PPARs (peroxisome proliferator activated receceptors) to stimulate the expression of RC complexes in cell lines from control or from patients with various RC defects. In control fibroblasts, cell treatment by 400M bezafibrate for 2 days resulted in an increase in the levels of complex II (+30%), III (+50%), and IV (+50%), determined by spectrophotometry, or western-blot analysis. Quantitative RT-PCR studies showed that this was accompanied by parallel increases in RC complex mRNA. The effects of bezafibrate were then tested in cells carrying mutations in nuclear genes encoding complex II (SDH-FP), complex III (BCS1) or complex IV (COX 10, SURF 1). This confirmed the stimulatory effects of bezafibrate and, in particular, revealed that this drug could enhance the gene expression and protein levels of deficient RC complexes. Additional data were obtained in muscle cells from patients presenting a myopathic form of COX deficiency with unknown molecular basis. Indeed, myoblast exposure to bezafibrate allowed to fully correct the initial COX enzyme defect (-60% compared to control) that was found in untreated cells of these patients, and this drug effect was confirmed in myotubes differentiated from myoblasts. Western-blot analysis showed that bezafibrate increased 2-fold the expression of nuclear (COX2) and of mitochondrial-encoded (COX4) protein subunits. Induction of COX activity after drug treatment was also demonstrated by histochemical staining of patient myoblasts. In parallel, gene expression levels of COX4 (+31%) and COX 2 (+39%) as well as mRNA of muscle specific subunits (COX7a and COX8a, +40%) were found significantly increased in response to the drug. Altogether, these data indicate that bezafibrate can trigger an increase in the level of deficient RC complex in patient cells, and this likely involves a stimulation of gene expression of the mutated protein. This study also suggests a possibly new approach for the correction of RC deficiency, for which therapeutic approaches remain extremely limited.

Mouse models: using mutant phenotypes to investigate human disease. *S.M. Bello, D.L. Burkart, M.A. Cassell, L.L. Washburn, B. Richards-Smith, A. Anagnostopoulos, R. Babiuk, H. Onda, M. Tomczuk, I. Lu, H. Dene, C. Smith, J.T. Eppig* The Jackson Laboratory, Bar Harbor, ME.

Mammalian models of human disease are critical to increasing our understanding of disease mechanisms and discovering potential new therapies. The use of the mouse to create such models is facilitated by the wealth of genetic tools available, including high-resolution genetic maps, myriad inbred strains, a sequenced genome, and well-developed transgenic techniques, as well as the accessibility of all life stages of the mouse to investigation. The Mouse Genome Informatics Database (MGI, http://www.informatics.jax.org/) provides integrated access to genetic and phenotypic data for mouse models of human disease.

In MGI, models of a disease are annotated to the Online Mendelian Inheritance in Man (OMIM) term for that disease. In addition a detailed phenotypic description of the model is entered using the Mammalian Phenotype ontology, a vocabulary of phenotypic traits. Use of these vocabularies allows researchers to easily access the data using many different approaches. For example, one can ask "What mutation or combination of mutations produce mouse models of Insulin-Dependent Diabetes Mellitus?" or "What mutations modify the severity of Insulin-Dependent Diabetes Mellitus in mouse models of the disease?" Or one can search for specific phenotypes, such as hyperglycemia and polyphagia, to identify models that reproduce select symptoms of the disease.

MGI currently has nearly 1600 genotypes annotated to OMIM terms and over 10% of OMIM terms have one or more associated mouse models.

Supported by NIH grant HG00330.

Mutational Screening of *CDO* using dHPLC in Holoprosencepahly (HPE) Patients. *S. Domene[1], M. Ouspenskaia[1], E. Roessler[1], R. Krauss[2], M. Muenke[1]* 1) NHGRI, NIH, Bethesda, MD; 2) Mt. Sinai School of Medicine, NY.

Holoprosencepaly (HPE) is the most common structural anomaly of human brain development, with a prevalence of 1 in 250 conceptuses and 1 in 16000 at birth. Mutations in at least eight different genes account for only 25 percent of the total cases. Among these HPE genes, several encode components of the Sonic Hedgehog (SHH) pathway: the secreted ligand SHH, its receptor, PTCH, and the transcription factors GLI2 and ZIC2.

CDO is the founding member of a distinct subfamily of the Ig superfamily (IgSF) that has recently been described to bind Shh, and likely modulate its action in the forebrain during early embriogenesis. Patterning of the midline of the ventral floorplate and midface is particularly sensitive to Shh signaling strength; this might explain why Shh depends on Cdo for augmentation of this important midline signal. In addition, the originally described Cdo knock-out mouse exhibits the hallmark facial defects associated with microforms of HPE, a single central incisor, which led to our study of this gene as a potential candidate gene for HPE. Cdo is not essential for Shh signaling in general, as other Shh-dependent body structures, such as the limb, develop normally in $Cdo^{-/-}$ animals. DHPLC analysis for all 19 coding regions and intron-exon boundaries of human CDO (followed by sequencing of abnormal chromatograms) in 285 HPE patients and 96 normal controls was performed. A total of 38 polymorphisms (detected equally in patients and controls) and 6 missense mutations (that appear to be disease-specific) were identified. It will be interesting to study the functional consequences of these missense mutations to see if they might underlie some cases of HPE alone, or in combination with other genes involved in midline development.

Phenotypic heterogeneity of N370S homozygotes with type 1 Gaucher disease: An analysis of 798 patients from the ICGG Gaucher Registry. *C. Fairley[1], A. Zimran[2], M. Cizmarik[3], J. Yee[3], N. Weinreb[4], S. Packman[1]* 1) UCSF, CA; 2) Shaare-Zedek Med Ctr, Israel; 3) Genzyme Corp, MA; 4) Univ Res Found for Lysosomal Storage Disorders, FL.

Purpose: The N370S/N370S genotype is thought to confer a mild phenotype presenting in adulthood in patients with type 1 Gaucher disease. We aimed to describe the heterogeneity and severity of the presenting phenotype of N370S homozygotes. **Methods:** The clinical characteristics at or near the time of diagnosis of all N370S homozygotes with available data enrolled in the International Collaborative Gaucher Group (ICGG) Gaucher Registry were analyzed. The same characteristics for all N370S compound heterozygotes with available data were analyzed for comparison. **Results:** 798 N370S homozygotes were identified; 32% were diagnosed before age 20y. 1,278 N370S compound heterozygotes were identified; 64% were diagnosed before the age 20y. At diagnosis, N370S homozygotes versus N370S compound heterozygotes had the following clinical characteristics: irreversible skeletal lesions (infarctions or AVN) 17% (34/198) vs 26% (76/290); decreased lumbar spine BMD (Z-score -2.5) 11% (8/70) vs 8% (4/49); anemia (age and sex-adjusted) 18% (59/327) vs 29% (145/494); thrombocytopenia (Plt <60k/mm^3) 9% (29/327) vs 16% (73/453); hepatomegaly (>1.25x nl) 44% (83/190) vs 72% (141/195); and severe splenomegaly (>15x nl) 11% (22/193) vs 39% (77/195). **Conclusions:** N370S homozygosity does not appear to invariably confer a mild, adult-onset phenotype. N370S homozygotes may present with severe clinical manifestations or be diagnosed before the age of 20y. The proportions of severely affected N370S homozygotes may be overestimates as asymptomatic patients may not be diagnosed nor be enrolled in the Registry. N370S compound heterozygotes are more likely than N370S homozygotes to present with a severe phenotype and more likely to be diagnosed before the age of 20y. The phenotypic heterogeneity of N370S homozygotes should be considered in genetic counseling and clinical decision-making.

Identification of entire LMX1B gene deletions in nail patella syndrome: final evidence for haploinsufficiency as the main pathogenic mechanism underlying dominant inheritance in man. *E.M.H.F. Bongers[1], I.J. de Wijs[1], E. Levtchenko[2], C. Marcelis[1], L.H. Hoefsloot[1], N.V.A.M. Knoers[1]* 1) Dept of Human Genetics, Radboud University Medical Centre Nijmegen, Nijmegen, The Netherlands; 2) Dept of Paediatric Nephrology, Radboud University Medical Centre Nijmegen, Nijmegen, The Netherlands.

Nail patella syndrome (NPS) is an autosomal dominant disorder characterized by nail and skeletal malformations, nephropathy, and glaucoma caused by mutations in the transcription factor LMX1B. Glomerular podocytes from Lmx1b-/- mice reveal aberrant foot processes and slit diaphragm formation. It is as yet unclear why heterozygous LMX1B mutations cause NPS nephropathy in humans, whereas Lmx1b+/- mice develop no renal symptoms. The hypothesis that haploinsufficiency is the main mechanism underlying dominant inheritance in human NPS is based on the fact that the same phenotypic variability is observed in individuals with LMX1B missense, nonsense, frameshift or splice-site mutations and that the range and severity of symptoms varies both within and between families. This assumption is supported by the lack of any dominant-negative effect observed by in vitro-experiments studying missense and truncation LMX1B mutations. By MLPA analysis with specific probes for the different exons 1-8 of LMX1B, we found a deletion of the entire gene in two unrelated individuals and a deletion of exons 3-8 in another patient from eight classic NPS families (3/8; 38%) in which no mutation could be detected by sequencing LMX1B. This first identification of entire LMX1B deletions strongly confirms the hypothesis that haploinsufficiency is the principal pathogenic mechanism of NPS. The fact that NPS nepropathy is observed in individuals with heterozygous deficiency of LMX1B and resembles a milder variant of kidney disease in Lmx1b-/- mice, whereas Lmx1b+/- mice are phenotypically normal, supports the assumption that a difference in dosage sensivity for this gene emerge in the two species.

Analysis of enzyme replacement therapy dose-response relationships in patients with type 1 Gaucher disease. *G. Grabowski[1], J. Charrow[2], C.E.M. Hollak[3], K. Kacena[4], P. Mistry[5], S. vom Dahl[6], A. Zimran[7]* 1) Cinn Childrens Hosp, OH; 2) Northwest Univ Sch Med. IL; 3) AMC, Netherlands; 4) Genzyme Corp, MA; 5) Yale Univ Sch Med, CT; 6) St Franziskus-Hosp, Germany; 7) Shaare-Zedek Med Ctr, Israel.

Objective: To analyze if enzyme replacement therapy (ERT) with imiglucerase demonstrates dose-response relationships in patients with type 1 Gaucher disease (GD) within the range of doses used in routine clinical practice and across disease parameters.**Methods:**The analysis included all patients with type 1 GD with intact spleens enrolled in the ICGG Gaucher Registry, diagnosed in 1980 or later, and for whom 12 mo of follow-up data were available as either ERT-naïve or ERT-treated. ERT dose was defined as the average dose over the first 3 yr of treatment. Propensity score matching was used to control for differences in baseline disease severity between groups. Hemoglobin, platelet count, liver and spleen volumes were assessed. For each parameter, a stepwise analysis examined ERT response (ERT vs. no ERT), followed by analysis of dose-response relationships. Non-linear mixed effects models were constructed for follow-up (0-60 mo). The rate (t50) and extent (Emax) of ERT treatment effect for each parameter were compared across groups.**Results:**975 patients met the inclusion criteria (795 ERT-treated, 180 ERT-naïve). The most frequent dosing regimens were 15, 30, 60 U/kg/2wk. Propensity scoring matching resulted in four statistically similar groups of 78 patients each (no ERT; ERT at 15, 30, 60 U/kg/2wk). Statistically significant dose-response relationships were found across groups for each parameter analyzed: hemoglobin, platelet count, liver and spleen volumes, in regard to rate and extent of improvement over 60 mo.**Conclusions:**ERT with imiglucerase results in statistically significant dose-dependent improvement in hematological parameters and organomegaly in patients with type 1 GD. Propensity scoring matching and non-linear mixed effects models can be used to assess outcomes based on observational data from an international rare disease registry. Further analysis of clinically significant disease parameters is ongoing.

Screening of deletions and duplications in 1500 genomic loci in a single assay. *J. Fan[1], S.J. White[2], M. Bibikova[1], L. Zhou[1], J. Chen[1], E. Wickham-Garcia[1], M.E. Kalf[2], M. Kriek[2], G.J.B. van Ommen[2], M.H. Breuning[2], L. Guo[3], S.-H. Lu[3], Q. Zhan[3], W. Jiang[3], O. Chan[4], J. Wang-Rodriguez[4], D.L. Barker[1], J.T. den Dunnen[2]* 1) Illumina, Inc, San Diego, CA, USA; 2) Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, Nederland; 3) Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China; 4) The VA Medical Center, UCSD, San Diego, CA, USA.

Copy number variations (CNV), i.e. deletions and duplications of genomic DNA, are known to be involved in many genetic diseases. Recent genome-wide analyses revealed extensive CNV in the human genome, the vast majority of which have not been correlated directly with disease. We have developed a highly multiplexed genotyping assay (GoldenGate assay) coupled with a universal BeadArray readout for CNV testing of hundreds to thousands of genomic regions in hundreds to thousands of samples. Specifically, we designed an array for parallel analysis of 1,500 genomic loci, which allows detection of all known trisomies, sub-telomeric rearrangements and micro-deletion syndromes as well as providing a low-resolution whole genome CNV scan. We first assayed genomic DNA isolated from patient blood samples. Analysis of X- and Y-chromosome probes showed excellent quantitative discrimination between males and females. We applied the assay to a variety of known variations, from whole-chromosome trisomies to subtle DMD-gene rearrangements (deletions, duplications, triplications), and were able to detect all known rearrangements reliably. Analysis of samples from 170 patients with mental retardation of unknown etiology revealed, based on the criteria selected, 20-30 new CNV's, which are currently being analyzed in more detail. We also analyzed 43 pairs of gDNA extracted from formalin-fixed cancerous and adjacent normal tissues of esophageal cancer patients. Highly reproducible genomic deletions and amplifications were detected in these archived tissue samples. Our data indicate that screening a selected set of 1500 loci using a bead-based assay is a rapid, sensitive and cost-effective method for detecting copy number changes in up to 96 samples simultaneously.

Assessing Exclusionary Power of a Paternity Test Involving a Pair of Alleged Grandparents. *D. Einum[1], R. Staub[2], M. Scarpetta[3]* 1) Sorenson Genomics, 2495 South West Temple Ave., Salt Lake City, UT 84115; 2) Orchid Cellmark, 13988 Diplomat Drive, Suite 100, Farmers Branch, TX 75234; 3) Orchid Cellmark, 2947 Eyde Parkway, Suite 110, East Lansing, MI 48823.

The power of a genetic test battery to exclude a pair of individuals as grandparents is an important consideration for parentage testing laboratories. However, a reliable methodology to calculate such a statistic using short tandem repeat (STR) genetic markers has not been presented. Two formulae describing the Random Grandparents Not Excluded (RGPNE) statistic at a single genetic locus were derived: RGPNE = $a(4-6a+4a^2-a^3)$ when the paternal obligate allele (POA) is defined and RGPNE = $2[(a+b)(2-a-b)][1-(a+b)(2-a-b)] + [(a+b)(2-a-b)]$ when the POA is ambiguous. A minimum number of genetic markers required to yield cumulative RGPNE values ≤ 0.01 was calculated using weighted average allele frequencies of the CODIS STR loci. RGPNE data for actual grandparentage cases are also presented to empirically examine the exclusionary power of routine casework. A comparison of RGPNE and Random Man Not Excluded (RMNE) values demonstrates the increased difficulty involved in excluding two individuals as grandparents compared to excluding a single alleged parent. A minimum of 12 STR markers is necessary to achieve RGPNE values ≤ 0.01 when the mother is tested; >25 markers are required without the mother. Cumulative RGPNE values for each of 22 non-exclusionary grandparentage cases were ≤ 0.01 but were significantly weaker when calculated without data from the mother. Calculation of the RGPNE provides a simple means to help minimize the potential of false inclusions in grandparentage analyses. This study also underscores the importance of testing the mother when examining the parents of an unavailable alleged father (AF).

Molecular studies and phenotype correlation in Costello syndrome. *K.W. Gripp[1], L. Nicholson[1], K.M.B. Vinette[2], A.E. Lin[3], J.D. Hoffman[4], D.L. Stabley[2], K. Sol-Church[2]* 1) Genetics, A. I. duPont Hosp, Wilmington, DE; 2) Biomed. Research, A.I.duPont Hosp, Wilmington, DE; 3) Genetics& Teratology, MGH, Boston, MA; 4) Genetics, Tufts-NEMC, Boston, MA.

Costello syndrome (CS) is a rare tumor predisposition syndrome having phenotypic overlap with cardio-facio-cutaneous (CFC) syndrome. The discoveries of *HRAS* mutations in CS (Aoki 2005) and *BRAF, KRAS, MEK1* or *MEK2* mutations in CFC (Niihori 2006; Rodriguez-Viciana 2006; Schubbert 2006), all involving the RAS-ERK-MAPK cascade, provide insight into the biological mechanism resulting in phenotypic similarities.
Of 60 pts enrolled in our CS study, we detected an *HRAS* mutation in 44 (73%)(G12S noted in 40). Two pts had a mutation resulting in G12A; and one each showed a G12C or G13C change. We hypothesized that all *HRAS* mutations occurred in the paternal germline. Informative families had paternal origin in 14 and maternal in 2, a result neither consistent with our hypothesis nor with an equal chance for maternal and paternal origin (P=0.0018). One unusual pt in whom standard analysis failed to show a mutation was found to have somatic mosaicism. Allelic quantitation revealed presence of the G12S mutation in 25-33% of buccal cell DNA, but 1% of blood DNA. Further studies in the 16 *HRAS* mutation negative pts showed 5 *BRAF* and 1 *MEK1* mutations, consistent with a CFC diagnosis.

We conclude that an *HRAS* mutation confirms a clinical CS diagnosis; conversely, the lack of an *HRAS* mutation likely excludes CS and further studies may reveal a mutation consistent with CFC. Clinical evaluation and critical interpretation of molecular results remain essential, as demonstrated by the pt with somatic *HRAS* mutation mosaicism. Most, but not all, *HRAS* mutations occur in the paternal germline, and there is no apparent phenotypic difference between maternally and paternally derived cases. While the genotype phenotype delineation is hampered by the fact that 40/44 (91%) share the G12S change, we found no difference in the characteristic anomalies of the few pts with other *HRAS* mutations.

The Role of A-Tail Heterogeneity in Alu Retroposition Efficiency. *M.S. Comeaux, P. Deininger* Tulane Cancer Center, Tulane University HSC, New Orleans, LA.

Over 50% of the human genome is composed of repetitive DNA. The most numerous mobile element in the genome is the approximately 300 bp Alu, which is found roughly 1.1 million times, accounting for about 11% of the genome. Alu elements contribute to human disease directly, by inserting new copies of itself into gene-rich regions that alter gene expression, and indirectly, due to unequal homologous recombination causing duplication or deletion of genetic material. Alu elements are transcribed by RNA polymerase III, and the transcripts are reverse-transcribed and inserted into new genomic locations after hijacking the ORF2 protein of L1 elements. Only the youngest of these Alu elements are currently active in the human genome. It does not appear that transcription is the primary factor controlling Alu activity. It has been shown that the length of the A-tail is one of the key differences between the older and younger Alu elements and that its length plays a role in the ability of an Alu element to retropose in the genome. However, the observed difference is much too small to explain the lack of amplification of the older Alu elements. Another key difference between the older Alus and the younger ones is that the older ones have more disruption in the A-tail than their younger counterparts. In this study, several different Alu elements were generated that have variable levels of disruption in the A-tail region. By utilizing a retroposition assay adapted from Dewannieux et al., we have compared the retroposition ability of Alu elements with variable levels of A-tail disruption. We observe that modest levels of sequence interruption have little impact on the amplification rate of Alu elements. However, larger levels of heterogeneity result in a significant decrease in its ability to retropose. This difference, however, is still insufficient to explain the almost total lack of amplification of the older Alu elements. Thus, we are currently characterizing other typical differences between old and young Alu elements to identify the features that define a highly active Alu element from the vast bulk of inactive elements.

Two stage whole genome linkage analysis of a phonological working memory component phenotype of dyslexia: Identification of a locus on chromosome 4p. *Z. Brkanac, N.H. Chapman, R.P. Igo, J.B. Thomson, M. Matsushita, T. Holzman, V.W. Berninger, E.M. Wijsman, W.H. Raskind* University of Washington, Seattle, WA.

Dyslexia is a common and complex genetic disorder. To elucidate the genetic architecture of dyslexia, we are using an approach that couples careful phenotypic evaluations with univariate component based linkage analyses. Impaired working memory is a common characteristic of dyslexia. We have shown through aggregation and segregation analyses that working memory phenotypes have a genetic basis in our sample. We assessed phonological working memory with the Non Word Repetition (NWR) task of the Comprehensive Test of Phonological Awareness. Families were collected through probands with Verbal IQ (VIQ) 90, and performance on at least one of ten measures of reading or writing below the population mean and 1 SD below VIQ. Two genome scans with 10 cM resolution were performed through the Marshfield Center for Human Genetics. The first sample consisted of 438 people from 51 families selected for power to detect linkage for NWR. The second sample consisted of 693 people from 93 families chosen without regard for NWR performance. Linkage analyses were performed using Variance Component (VC) and Markov-chain Monte Carlo (MCMC) joint segregation and multipoint linkage methods, followed by parametric analyses in regions of interest. In the first genome scan numerous linkage signals met our preset criteria for a region of interest: VC dominance lod score 1.0 and MCMC intensity ratio (IR) 5.0. Follow up parametric lod scores > 2.0 were obtained on chromosomes (chr) 4p, 7q, 12p and 17q. Signals on chr 4p, 7q and 12p were also seen in the second scan. Combined analysis of both data sets significantly increased the VC dominance lod score to 2.1 and MCMC IR to 20 on chromosome 4. Our study has identified a novel locus on chr 4p for NWR in families with dyslexia. This region contains the gene cluster of GABA receptor subunits that are involved in the mediation of fast synaptic inhibition. In depth analysis to further refine the location of interest and to evaluate the candidate genes is ongoing.

A new autosomal dominant distal myopathy: linkage to chromosome 3q and candidate gene analysis. *A. Gad[1], D. Chen[2], M. Matsushita[1], M. Kumar[1], L. Chen[1], J. Wolff[1,3], H. Lipe[3], T. Bird[1,2,3,4], W. Raskind[1,4]* 1) Depts of Medicine and; 2) Neurology, University of Washington, Seattle, WA; 3) GRECC and; 4) MIRECC, VA PSHCS, Seattle, WA.

We studied a four-generation American family with an adult onset myopathy, characterized by progressive muscle weakness and atrophy, distal greater than proximal, and hypoactive reflexes. EMGs suggested a myopathy with occasional myotonia-like activity. Muscle biopsy revealed a chronic, non-inflammatory necrotizing myopathy. Unexpectedly, given the absence of dementia or brain atrophy, diffuse -synuclein positive Lewy bodies were seen in the frontal cortex, cingulate gyrus, amygdala, substantia nigra, pons and midbrain of an affected person who died at age 88. DNA was obtained from 14 individuals. A DNA test for type-1 myotonic dystrophy was negative and targeted analysis excluded linkage to Welander myopathy (2p), Udd (2q31-33), Nonaka myopathy (9p1-q1), MPD3 (8p22-q11, 12q13-q22), Markesbery-Griggs (10q22.2-q23.3), and Laing myopathy (14q11). Assuming a disease frequency of 0.0001 and 90% penetrance, power analysis suggested that a maximum lod score of 3.21 at a recombination fraction () of 0.00 could be obtained with the available samples. Using the same penetrance estimate, a whole genome scan at 10 cM level found strongly suggestive evidence of linkage to chromosome 3 with a two-point lod score of 2.36 at =0.00. Other loci providing positive lod scores were ruled out with additional markers and haplotype analysis. Finer scale mapping provided maximum two-point and multipoint lod scores of 2.94 at =0.00 for D3S1614. By haplotype construction, a 5 cM critical region between D3S1264 and D3S3725 in 3q26.1-3 was defined. All affected individuals and two unaffected people, one of whom is younger than the average age of onset, carried the disease-associated haplotype. The 40 genes mapped to this region have been prioritized as candidates based on expression patterns and their known functions. A new polymorphism was detected in *PI12/SERPINI1* but no coding region sequence alterations were found in *GPR160*, *MYNN* and *CLDN11*. Analyses to detect gross genomic or splicing abnormalities are ongoing.

Mutator genes permissive for genomic rearrangements at *Alu* repeats. *K.M. Chisholm[1], P.L. Welcsh[2], M.-C. King[1,2]* 1) Department of Genome Sciences, University of Washington, Seattle, WA; 2) Department of Medicine, Division of Medical Genetics, University of Washington, Seattle, WA.

Tumors display a high degree of genetic heterogeneity suggesting that genomic instability drives carcinogenesis. Genomic instability is under genetic control and the genes responsible are likely critical to tumor development. At the BRCA1 tumor suppressor locus, large genomic deletions frequently occur as inherited mutations in families and as somatic loss of heterozygosity in breast tumors. These genomic deletions appear to be the result of aberrant homologous recombination between *Alu* repetitive elements, which are densely packed at the BRCA1 locus. My hypothesis is that loss of function of mutator genes that suppress homologous recombination would increase the frequency of genomic rearrangements, and thus may contribute to the development of breast cancer. My goal is to identify such mutator gene(s) using BRCA1 as a model. I have created a construct incorporating two identical *AluSp* sequences flanking URA3. These *Alu* repeats have been identified as the site of rearrangement in many inherited BRCA1 mutations. This construct has been inserted into a yeast transformation plasmid, and then screened for deletion of the URA3 marker in 99.2% of the Saccharomyces Genome Project collection of (4672) yeast strains comprised of all viable single gene knockouts. Of these strains, 11.8% revealed an increased rate of rearrangement. Upon further evaluation, 12 strains appeared to be candidate mutators, with mutation rates 1.5 to 10.61 fold over wildtype. These strains include known mutators such as *tsa1* and *elg1*, as well as DNA helicases and proteins involved in meiotic recombination. Human homologs have been identified for seven genes. Currently, these genes are being screened for mutations in high-risk breast cancer families who are wildtype for all known breast cancer predisposing genes and in sporadic breast tumors. These genes are also being evaluated as modifiers of BRCA1 in families with known BRCA1 mutations. Though the primary aim of this research is to identify mutator genes involved in breast carcinogenesis, these same genes may be involved in other cancers as well.

Experience with parental sample submission in abnormal array CGH cases: Is free parental testing helpful? *J. Coppinger, B.A. Bejjani, L.G. Shaffer* Signature Genomic Laboratories, LLC, Spokane, WA.

Parental testing is routinely recommended for the evaluation of cytogenetic abnormalities of uncertain clinical significance, or abnormalities that may have occurred due to a cytogenetic rearrangement in a parent. The percentage of parental samples received is usually suboptimal in these circumstances, and literature evaluating the genetic counseling process and impact on submission of parental samples is limited. In the past few years, comparative genomic hybridization has emerged as a promising cytogenetics application that improves the diagnostic potential for microscopic and submicrocopic chromosome abnormalities. Yet, analysis of parental samples remains an essential step for complete clinical interpretation of unbalanced translocations, subtelomeric alterations, de novo deletions/duplications, suspected polymorphisms, and results of unclear significance detected by array CGH. We report our experience with the receipt of parental samples for testing in abnormal cases in our first 3600 consecutive clinically diagnostic array CGH cases. A total of 303 (8.4%) abnormal cases were identified, and of these, 166 (54.8%) required parental samples for complete clinical interpretation. Parental samples were received in 88 (53%) of cases. To assess whether cost of testing may be a barrier to submission of samples, parental testing was offered at no charge beginning in January, 2006. Analysis of parental samples received 1 month out and 3-4 months out identified an increase from 38% to 62%, thereby confirming that cost is likely one barrier to parental testing. Other potential barriers may include clinicians interpretations of abnormal test results, sub-optimal communication of the need for parental samples to patients or parents, family dynamics or psychosocial barriers, or other possibilities. Identification and elimination of barriers to parental testing will provide more complete interpretations of abnormal test results, improving the potential for accurate diagnosis, genetic counseling, and medical management of patients.

Variations in RANK Gene are Associated with Adult Height in Caucasians. *Y. Chen[1,3*], D.H. Xiong[2,3*], T.L. Yang[4], F. Yang[1,3], H. Jiang[1,3], F. Zhang[3,4], H. Shen[2], P. Xiao[2,3], H.W. Deng[1,2,4], * Equal contribution* 1) Laboratory of Molecular and Statistical Genetics, Hunan Normal University, Changsha, China; 2) Departments of a) Orthopedic Surgery and b) Basic Medical Sciences, University of Missouri-Kansas City, Kansas City, USA; 3) Osteoporosis Research Center and Department of Biomedical Sciences, Creighton University, Omaha, USA; 4) The Key Laboratory of Biomedical Information Engineering of Ministry of Education and Institute of Molecular Genetics, Xi'an Jiaotong University, Xi'an, China.

Height is a complex trait significantly influenced by genetic factors, with the heritability ranging from 48% to 98%. Previous genetic studies on height have yielded a number of important genomic regions and candidate genes that may account for the variation of height in human populations. However, more height genes still wait for identification. Recent studies have revealed that RANK (tumor necrosis factor receptor superfamily, member 11a) is a vital factor for chondroclastic/osteoclastic differentiation and activity that influence the morphology of growth plates as well as the linear bone growth. Despite its importance to bone physiology, little effort has ever been done to find out whether the RANK polymorphisms are associated with adult height variation in the normal populations. Herein, we performed a family based association test (FBAT) in 1873 white subjects from 405 nuclear families. Eighteen single nucleotide polymorphisms (SNPs) and 7 blocks in the RANK gene were studied in terms of their association with the variation of final adult height. One SNP, called rs6567274, was detected to be significant (P = 0.0032) even after multiple-testing correction. In corroboration with single-locus analysis, a major haplotype in block 5 bearing the variant T of rs6567274 was significantly associated with higher stature (P = 0.0073). Our findings firstly suggested that the genetic variants of RANK might contribute to the adult height variation. Further researches on RANK need to be launched to replicate the present results and further unravel the molecular mechanism underlying the significant associations discovered.

Dense SNP marker analysis identifies regions of linkage for rheumatoid arthritis. *C.I. Amos[1], W.V. Chen[1], X. Liu[2], D. Mosher[3], W. Maksymowych[4], D. Zhu[1], S. Shete[1], K. Siminovitch[2]* 1) Epidemiology, U.T. M.D. Anderson Cancer Center, Houston, TX; 2) Department of Medicine, University of Toronto and Mount Sinai Hospital, Toronto, Canada; 3) Dalhousie University, Halifax, Nova Scotia, Canada; 4) University of Alberta, Edmonton, Alberta, Canada.

Dense linkage analysis using SNPs increases information for linkage studies, but also can increase the probability of obtaining false positive linkage evidence when SNPs are falsely assumed to be in linkage equilibrium. We analyzed data from Affymetrix 100K analysis of 79 sib pairs affected with Rheumatoid Arthritis who did not have parental samples. Results of linkage analysis that assumed linkage equilibrium showed several regions with LOD scores exceeding 3. To check the validity of these findings, we compared results from two approaches to adjust for linkage disequilbrium (LD). First, we adopted an 'interleaving' method of Baccanu (Genetic Epidemiology 29:195-203, 2005) in which the data were divided into 10 disjoint sets of markers. Then, we performed analysis using SNPLINK (Bioinformatics 21:3060-1, 2005) to remove any remaining markers that showed excessive LD ($R^2 > 0.7$). Results from these 10 runs were then averaged and the empirical p-value was obtained at each point. Alternatively, we used Merlin (Am J Hum Genet. 77:754-67, 2005), which allows for LD by 'clusting' tightly linked markers. Results of these analyses led to diminished Z-scores compared with not allowing for LD, but analysis using Merlin appeared excessively conservative as it led to a far greater reduction in the number of markers that were retained for analysis. For example, on chromosome 6 we found that 22% of markers were dropped by the interleaving approach while 88% of markers were dropped by the clustering approach. Z-scores that were obtained on chromosome 6p in the HLA region were 2.14 by interleaving and 0.72 by clustering. Interleaving identified regions on chromosomes 1 and 8 with maximal Z-scores of 2.32 at 82.5 Mb and 2.48 at 19.42 Mb. These results indicate the promise that is provided by the interleaving approach, though integrating the data from the multiple scans presents challenges in data handling.

Genotype-phenotype correlation in mitochondrial optic neuropathies. *P. Amati-Bonneau, M. Ferré, C. Verny, M. Barth, V. Guillet, A. Chevrollier, Y. Malthièry, D. Bonneau, P. Reynier* Dept Biochem & Molecular Biol, INSERM U694,CHU Angers,France.

Mitochondrial optic atrophies, due to the degenerescence of retinal ganglion cells and optic nerve fibers, include Autosomal dominant optic atrophy (ADOA), Leber's hereditary optic neuropathy (LHON) and autosomal dominant optic atrophy and cataract (ADOAC). ADOA generally starts in childhood and is characterized by a progressive decrease in visual acuity and optic atrophy.Mutations in the optic atrophy 1 (OPA1) gene are implicated in about 40% of the cases of ADOA. OPA1 encodes a dynamin-related GTPase, located in the mitochondrial inter-membrane space. LHON, which is caused by specific mutations in mitochondrial DNA, is characterized by severe optic atrophy responsible for acute or subacute visual loss, usually starting between the ages of 18 and 35. In addition to optic atrophy, some patients present neurological signs of central nervous system involvement. ADOAC, a rare phenotype of optic atrophy and cataract, is due to mutations in a mitochondrial protein nuclear encoded (OPA3). We report here a molecular study of 600 patients with optic atrophy referred to our center for molecular diagnosis purpose during the period 2001-2005. Of these, 60% were familial and 40% sporadic cases or more often with unknown mode of inheritance.The mutation responsible for the disease was identified in 290 patients (49%). Mitochondrial DNA mutations (LHON) were found in 90 patients (15%), OPA1 mutations in 186 patients (31%) and OPA3 mutations in 14 patients (2%). Several ADOA patients were affected by additional neurological signs such as neurosensorial deafness or peripheral axonal neuropathy. A specific genotype-phenotype correlation was observed in patients harboring the R445H OPA1 mutation and optic atrophy associated to deafness. About 10% of sporadic patients carried pathogenic mutation in the OPA1 gene.Mitochondrial DNA and OPA1/3 gene analyses allow diagnosing about 50% of the patients. Our results suggest that patients with hereditary optic neuropathies could greatly benefit from neurological investigations and that sporadic optic atrophy need to be explored at the molecular level.

Another case with Uterine aplasia, ovarian dysgenesis, amenorrhea and impuberism: a variant from Mayer-Rokitansky-Kuster- Hauser syndrome or a coincidental association? *M. Colombani[1], E. Sapin[2], D. Cau[3], N. Lentz[4], C. Thauvin-Robinet[1], F. Huet[5], L. Faivre[1]* 1) Centre de Genetique, Hopital dEnfants, Dijon, France; 2) Service de Chirurgie Pediatrique, Hopital dEnfants, Dijon, France; 3) Service de Pediatrie et Neonatalogie, H Chalon sur Saone, France; 4) Service de Gynécologie, Chalon sur Saone, France; 5) Departement de pédiatrie, Hopital d'Enfants Dijon, France.

Mayer-Rokitansky-Hauser syndrome (MRKH) is characterized by the association of congenital absence of the vagina, rudimentary uterus, normal tubes and ovaries, normal female secondary sexual characteristics, and normal endocrine and cytogenetic evaluations. Four atypical cases have been described in the literature, associating absence of uterus to gonadal dysgenesis and normal female karyotype. Authors discussed that such cases represent a rare variant of MRKH or a coincidental association. Here we describe a 15½-year-old girl presenting with primary amenorrhea and impuberism. She was the third child of consanguineous parents originated from Senegal. Family history was negative. Her twin brother and her 19-year-old sister had a normal puberty with menarche at age of 14. The proband had a history of autoimmune thyroiditis in childhood. At time of examination, her height was 168 cm, her weight was 58 kg with a normal body mass index. The secondary sexual characteristics assessment showed absence of breast development, pubic and axillary hair (Tanner stage 1) are noted. Prepubertal external genitalia and a vagina ending in a blind pouch were found at the gynecological exam. There was no sign of masculinisation and she had a normal psychomotor development. Endocrine evaluation revealed a hypergonadotropic hypogonadism. Standard cytogenetic examination revealed a normal female karyotype, 46 XX. Antimullerian hormon was absent. Cardiac and renal ultrasounds are normal. Delayed bone age was also noted (13½ for a chronological age of 15½ years). Pelvic ultrasound showed a complete absence of uterus and an absence of gonads. Coelioscopic study revealed two ovarian bandelets in favor of gonadal dysgenesis and normal fallopian tubes. This is the fifth case with the association of absence of uterus and gonadal dysgenesis in a female with a 46,XX karyotype, which could be in favor of a genuine entity.

Modulation of genetic susceptibility by behavioral factors and glucose regulation status in the general population exemplified by the impact of the *APOA5* -1131T>C variant on serum lipids. *G. Andersen[1], T. Sparsø[1], S.I. Castella[1], A. Albrechtsen[1], C. Glümer[1,2], K. Borch-Johnsen[1,2,3], T. Jørgensen[2], T. Hansen[1], O. Pedersen[1,3]* 1) Steno Diabetes Center, Gentofte, Denmark; 2) Research Centre for Prevention and Health, Glostrup University Hospital, Glostrup, Denmark; 3) Faculty of Health Science, University of Aarhus, Aarhus.

Previous studies suggest that the C-allele of the *APOA5* -1131T>C polymorphism confers an increase in serum triglyceride concentrations. The aim of the present study was at the population level to estimate the effect of the *APOA5* -1131T>C polymorphism on fasting serum lipids and to elucidate the potential modulating impact of health behavior and glucose regulation status. The *APOA5* -1131T>C polymorphism (rs662799) was genotyped in a population-based sample of 5,873 treatment-naïve Danes (2,929 men, 2,944 women; age 46 8 years; BMI 26.3 4.6 kg/m^2) using chip-based MALDI-TOF mass spectrometry. The *APOA5* -1131T>C polymorphism was associated with dyslipidaemia in a case-control study of 1,439 patients with dyslipidaemia and 4,432 normolipidaemic subjects, $P = 4x10^{-6}$ for minor allele frequency (8.2 % [7.2-9.2 %] vs. 5.7 % [5.2-6.2 %]) and $P = 1x10^{-5}$ for genotype distribution. Correspondingly, in the total population of 5,873 participants the C-allele was associated with increased fasting serum levels of triglyceride ($P = 1x10^{-15}$) and total cholesterol ($P = 0.0003$) and decreased fasting serum HDL-cholesterol ($P = 0.004$). Significant epistasis on fasting serum triglyceride concentrations was observed between the *APOA5* -1131T>C polymorphism and smoking ($P = 0.0003$), daily alcohol intake ($P = 0.005$), and dietary monounsaturated fatty acid intake ($P = 8x10^{-6}$). The effect of the polymorphism on fasting serum triglyceride was more profound in people with impaired glucose regulation than for glucose-tolerant subjects ($P = 5x10^{-9}$). In conclusion, at the level of the general population of middle-aged people the common C-allele of the *APOA5* -1131T>C polymorphism greatly influences the fasting serum lipid levels. Importantly, the impact of this gene variant on fasting serum triglycerides is strongly modulated by behavioral factors and hyperglycaemia.

SCA17 transgenic mice show a severe neurodegenerative phenotype. *P. Bauer, Y. Golub, C. Bauer, T. Ott, T. Hennek, O. Riess* Dept Medical Genetics, Univ Tubingen, Tubingen, Germany.

SCA17 is a progressive neurodegenerative disease leading to cerebellar ataxia and dementia. Several accessorial symptoms such as Parkinsonism, dystonia, and psychiatric disturbances commonly aggravate to disease course. Genetically, a CAG/CAA expansion in the TATA binding protein (TBP) is expanded in SCA17 patients, leading to an expanded polyglutamine chain in this ubiquitously expressed transcription factor. We have generated transgenic mice overexpressing a 64 CAG/CAA repeats containing human TBP (Q64TBP) gene under the control of the truncated human prion protein promoter (PrP). Transgene expression throughout different brain regions (cortex, basal ganglia, cerebellum, and brain stem) was clearly demonstrable for both: Q64TBP transcripts and the respective transgenic protein, as analyzed by in-situ hybridization and Western Blot. Five months old transgenic animals displayed a severe motor deficit, objectified in the Accelerod paradigm and had a mean survival of less than 8 months. We present detailed morphologic and phenotypic data of the first rodent model of SCA17 allowing to study the pathogenesis of progressive human disease.

Higher prevalence of Hirschsprung disease in China explained by a common *RET* mutation. *M. Garcia-Barcelo[1], J. Amiel[2], G. Antinolo[3], S. Borrego[3], G. Burzynski[4], I. Ceccherini[5], E. Emison[6], C. Eng[7], R. Fernandez[3], P. Griseri[5], R. Hofstra[4], C. Kashuk[6], F. Lantieri[5], S. Lyonnet[2], X. Miao[1], P. Tam[1], A. Tullio-Pelet[2], K. West[6], A. Chakravarti[6]* 1) Dept Surgery, Univ Hong Kong, Hong Kong, China; 2) Dept Génétique et Unité INSERM U-393, Paris, France; 3) UC Genética y Reproducción, HH UU Virgen del Rocio, Sevilla, Spain; 4) Dept Medical Genetics, Univ Groningen, The Netherlands; 5) Lab di Genetica Molecolare, Ist Gaslini, Genova, Italy; 6) Inst Genetic Medicine, Johns Hopkins Univ Baltimore, USA; 7) Dept Molecular Genetics, Ohio State Univ, Columbus, USA.

RET is the major gene implicated in Hirschsprung disease (HSCR), congenital megacolon. TDT analysis of 13 *RET* SNPs on 876 HSCR families from three continents showed that all SNPs tested were associated with HSCR. The largest contribution to risk was made by an enhancer mutation in intron 1. This mutation is predominantly associated with male patients with the least severe form of HSCR. The overall frequencies of the HSCR-associated SNPs and haplotypes were significantly higher in Chinese, in both patients and general population. The haplotype composition also differed between populations: two main HSCR-haplotypes (long and short), sharing the 5 *RET* region were identified in Caucasians while only one (long) in Chinese. SNPs and haplotypes were tested in an expanded Chinese case-control sample. By estimating the association of each single SNP conditional on the haplotype background, five markers (including the enhancer mutation) wrere highly correlated with HSCR. These 5 markers were distributed across the shared 5 region coinciding with a single origin for a HSCR susceptibility locus. The most frequent haplotype in the Chinese patients (long) was also the most prevalent in the general population. Over 55% of the patients were homozygous for the long HSCR-associated *RET* haplotype and 72% homozygous for all those haplotypes comprising the most HSCR correlated markers. This detailed profile of the *RET* gene in the Chinese population provides an insight for the higher incidence of the disease in this population.

P-Values For Haplotype Association May Be Very Different Depending On The Analysis Method. *E. Fransen, M. Thys, E. Van Eyken, L. Van Laer, G. Van Camp* Dept Medical Genetics, University of Antwerp, Antwerp, Belgium.

Testing a candidate gene for association with a some disease often involves haplotypes-based association testing in addition to singleSNP association testing. It has been suggested that haplotype analysis could be more powerful than single SNP testing. When working with independent (non-family) samples, these analyses need to include haplotype inference. Many methods for haplotype inference and haplotype association testing are available, and its not always clear what the most appropriate method is. Four SNPs in a candidate gene for otosclerosis (binary trait) were typed and individually tested for association using the Armitage test. Then we compared various methods for haplotype-based association studies. This included the programs WHAP, FAMHAP, HTR (Haplotype Trend Regression) or PHASE2.0. In each of these programs, the haplotype inference and the association test is performed simultaneously. In addition, a two-step procedure was followed, where first the most likely haplotype of each subject was inferred using either SNPHAP, PHASE2.0, FAMHAP or HTR , along with its likelihood. Subsequently, these inferred haplotypes were tested for association using either a weighted c^2 test using the likelihood of the inferred haplotype as a weight factor in the analysis. We observed very different p-values depending on the method. The same dataset can show a highly significant association with one method, whereas a different method shows no or only a marginal association.

Significant Linkage to Airway Responsiveness on Chromosome 12q24 in Families of Children with Asthma in Costa Rica. *J.C. Celedon[1,2,3], M.E. Soto-Quiros[4], L. Avila[4], S.L. Lake[1,3], C. Liang[1], E. Fournier[4], M. Spesny[4], C.P. Hersh[1,3], J.S. Sylvia[1], T.J. Hudson[5], A. Verner[5], B.J. Klanderman[1,3], N.B. Freimer[6], E.K. Silverman[1,3], S.T. Weiss[1,3]* 1) Channing Lab, Brigham & Women's Hosp, Boston, MA; 2) Division of Pulmonary and Critical Care Medicine, Beth Israel Deaconess Medical Center, Boston, Massachusetts; 3) Department of Medicine, Harvard Medical School, Boston, Massachusetts; 4) Division of Pediatric Pulmonology, Hospital Nacional de Niños, San José, Costa Rica; 5) McGill University and Genome Quebec Innovation Centre, Montreal, Canada; 6) Department of Psychiatry, University of California at Los Angeles, Los Angeles, California.

We enrolled 8 pedigrees ascertained through Costa Rican schoolchildren with asthma(physician-diagnosed asthma, >=2 respiratory symptoms or asthma attacks in the previous year, airway hyper-responsiveness or bronchodilator responsiveness) and >=6 great-grandparents born in the Central Valley of Costa Rica. A genome scan with short-tandem repeat (STR) markers spaced every ~8 cM was performed, and genome-wide linkage analyses of asthma (n=638) and airway responsiveness (n=488) were conducted. Nonparametric two-point linkage analysis of asthma was peformed by the NPL-PAIR allele-sharing statistic, and variance component models were used for the multipoint linkage analysis of airway responsiveness (dose-response slope to methacholine). All analyses were repeated after exclusion of the phenotypic data of ever-smokers. Two-point linkage analysis for asthma showed some evidence of linkage to chr. 12q, particularly in nonsmokers ($P < 0.01$). Multipoint linkage analysis for airway responsiveness revealed suggestive evidence of linkage to chromosome 12q24 (LOD=2.33 at 146 cM) in nonsmokers. After genotyping 18 additional STR markers on chr. 12q, there was significant evidence of linkage to airway responsiveness on chromosome 12q24 (LOD=3.79 at 144 cM) in nonsmokers, with a relatively narrow 1.5-LOD unit support interval for the observed linkage peak (142 to 147 cM). Our results suggest that chromosome 12q24 contains a locus (or loci) that influence a critical intermediate phenotype of asthma (airway responsiveness) in Costa Ricans.

Genetic heterogeneity in Stüve-Wiedemann Syndrome. *N. Dagoneau[1], L.I. Al Gazali[2], A. David[3], E. Flori[4], A. Green[5], B.C.J Hamel[6], M. Le Merrer[1], F. Prieur[7], A. Raas-Rothschild[8], J. Vigneron[9], A. Munnich[1], V. Cormier-Daire[1]*
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Stüve-Wiedemann syndrome (SWS) is a severe autosomal recessive condition characterized by bowing of the long bones, with cortical thickening, flared metaphyses with coarsened trabecular pattern, respiratory distress and hyperthermic episodes responsible for early lethality. Studying a series of 19 SWS families, we have mapped the disease gene to chromosome 5p13 and identified null mutations in the LIFR gene, responsible for an impairment of the JAK/STAT signalling pathway in patient cells. Following this initial study, we have collected the samples of 17 additional SWS families. Among them, we identified LIFR mutations in 10 families including six novel mutations responsible for either a premature stop codon (in exon 3, 8, 11, 15) or an aminoacid change in the Ig-like and FNIII domains (L292P, A622V). In the other 7 families, the absence of LIFR mutation prompted us to perform a linkage analysis in the consanguineous families (4/7) and to exclude the 5p13 locus as the disease locus. In addition, we have tested the activation of the JAK/STAT pathway in the fibroblasts from 4 patients with no LIFR mutation and observed a normal activation of this pathway in response to LIF excluding the LIFR as the disease gene. Finally, we reviewed the manifestations of the SWS patients with no LIFR mutation and failed to identify any distinctive feature. Taken together, our data show that SWS is a clinically homogeneous but genetically heterogeneous condition. Ongoing studies will help to further understand the pathogenesis of SWS.

A point mutation in the 3 untranslated region of *CNTN4* is associated with spinocerebellar ataxia type 16. *Y. Fukumaki[1], S. Miura[1,2], H. Shibata[1], H. Furuya[3], Y. Ohyagi[2], M. Osoegawa[2], Y. Miyoshi[2], H. Matsunaga[1], N. Takeuchi[1], A. Shibata[1], N. Matsumoto[1], A. Iwaki[1], T. Taniwaki[2], H. Kikuchi[2], J. Kira[2]* 1) Research Center for Genetic Information, Medical Inst Bioregulation, Kyushu University, Fukuoka, Japan; 2) Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; 3) Department of Neurology, Neuro-Muscular Center, National Omuta Hospital, Omuta, Japan.

Spinocerebellar ataxia type 16 (SCA16) is an autosomal dominant form of pure cerebellar syndromes and has been mapped to chromosome 8q22.1-24.1. We reanalyzed the linkage of the original pedigree using two additional subjects and revealed that SCA16 links not to 8q22.1-24.1 but to 3p26.2-pter (maximum LOD score = 5.177) partly overlapping with the region of spinocerebellar ataxia type 15 (SCA15) which is also an autosomal dominant form of pure cerebellar syndromes. We screened all exons of seven genes located in the critical region and identified only one point mutation (C4256T) in the 3 untranslated region (3-UTR) of the contactin 4 gene (*CNTN4*) on chromosome 3p26.2-p26.3, which cosegregated with the disease. This mutation was not detected in 520 control subjects. Therefore, we concluded that *CNTN4* is the responsible gene of SCA16. As we were unable to prove that the single nucleotide substitution in the 3-UTR of *CNTN4* is an immediate cause of SCA16, there is still a possibility that the T-allele is tightly linked with the true SCA16 causative allele nearby. We also discuss the relation between SCA15 and SCA16 in terms of clinical and genetic aspects.

The rare form of Pseudohypoparathyroidism type Ic (PHP-Ic) is caused by mutations in the C-terminal of the GNAS gene. *L. de Sanctis[1], B. Ceoloni[1], F. Cresi[1], M. Street[2], M. Salerno[3], S. Grosso[4], W. Ahrens[5], O. Hiort[5]* 1) Dept. Pediatric Sciences, Univ Torino, Torino, Italy; 2) Dept. of Pediatrics, University of Parma, Parma, Italy; 3) Dept. of Pediatrics, Federico II University, Naples, Italy; 4) Dept. of Pediatric, University of Siena, Siena, Italy; 5) Klinik für Kinder- und Jugendmedizin, Universitätsklinikum Schleswig-Holstein, Lübeck, Germany.

The Pseudohypoparathyroidism (PHP) is a heterogeneous group of disorders characterized by PTH resistance and divided in Ia, Ib, Ic and II type on the basis of clinical, hormonal, biological features. PHP-Ia and Ic patients shared multihormone resistances (to PTH, TSH and FSH/LH) and the Albright Hereditary Osteodystrophy (AHO) phenotype. An impaired in vitro Gs-alpha protein activity, caused by loss-of-function mutations spread throughout the coding region of the GNAS gene, has been described in the PHP-Ia condition. The in vitro Gs-alpha protein activity is normal in the PHP-Ic type; recently, one GNAS mutation in the C-terminal of the gene, encoding for the receptor-binding domain, has been indicated as responsible for this rare form of PHP. We report 3 subjects with multihormone resistances and AHO phenotype in which the molecular analysis by PCR amplification and direct sequencing of entire coding region of GNAS gene allowed to detect the causal mutation in the last exon of the gene (E392X in 2 patients and E392K in the third). The in vitro Gs-alpha protein activity, performed in each subject, was normal (108.1% for the patient with E392K, 102.1% for the patients with the E392K mutation), thus indicating the PHP-Ic subtype. This study reports 2 new mutations in the GNAS gene causing the rare form of PHP-Ic. It confirms that a defect in the receptor-mediated Gs-alpha activation can be responsible for this disorder, accordingly to the pathogenetic mechanism proposed for the first mutation described. A nucleotide change at the receptor binding domain could thus explain the clinical features related to an impaired Gs-alpha activity, notwithstanding normal in vitro functional assays, that explore the retained adenylate cyclase activity of the protein.

Evaluation and validation of Preimplantation Genetic Diagnosis (PGD) by PCR analysis: comparison of the blastomere and corresponding embryo genotype. *J. Dreesen[1,3], M. Drüsedau[1], H. Smeets[1,3], C. De Die-Smulders[1], J. Dumoulin[2], J. Evers[2], J. Geraedts[1,3], J. Herbergs[1,3]* 1) Depts. Clinical Genetics; 2) Obstetrics & Gynaecology, Academic Hospital Maastricht; 3) GROW, Maastricht University, Maastricht, The Netherlands.

PGD can be an alternative for prenatal diagnosis for couples at high risk of a monogenic disorder. Unaffected embryos, genotyped by analysis of biopsied blastomeres, are selected for embryo transfer (ET). The aim of the present study is to validate the PGD-PCR procedure, and determine the diagnostic value. PCR analysis of embryos not selected for ET and unsuitable for cryopreservation after PGD were used as golden standard. According to embryo morphology quality scores, embryos on day 4 post fertilization (day ET) were divided into class 1-4, with class 4 being the lowest embryo morphology score. The genotype from the biopsied blastomere and the corresponding embryo were compared. To establish the validity of PGD-PCR procedure, sensitivity(Se), specificity(Sp), and Likelihood Ratio (LR) were calculated for the total, class 4 excluded and class 4 embryo group. For the diagnostic value, Positive- (PPV) and Negative Predictive Value (NPV) were calculated. In our centre 80 women underwent PGD-PCR, resulting in 793 embryo genotypes, 241 unaffected embryos were used for ET. From 447 embryos the blastomere genotype has been compared with the reanalysed embryo genotype. PGD-PCR blastomere outcome, scored as affected or aberrant in 234/241 positive embryos (Se; 97,1%), and scored unaffected in 181/206 negative embryos (Sp: 87,9%). Out of the 7 false negative embryos, 6 were graded as class 4. The Se in the class 4 embryo group was 90,2% (55/61) and Sp 93,2% (41/44). Exclusion of class 4 embryos resulted in a Se of 99,4% (179/180), a Sp of 86,4% (140/162) and a LR positive test of 7.3 and LR negative test of 0.006. The PPV of an abnormal PGD-PCR is 89.1%, the NPV of a normal PGD-PCR is 99.3% in this group. PGD-PCR procedure is validated as a diagnostically reliable method for selecting unaffected embryos for ET. Accuracy of PGD-PCR analysis improves by rejecting class 4 embryos for ET.

PCSK9, from gene to protein and plasma. *M. Abifadel, B. Jessica, A. Marques, M. Devillers, D. Erlich, J.P. Rabès, C. Boileau, M. Varret* INSERM U781, INSERM U781 hôpital Necker, France, Paris cedex 15, France.

Autosomal Dominant Hypercholesterolemia (ADH) is one of the most frequent human inherited disorders. Until 2003, mutations in two major genes, LDLR and APOB, had been clearly implicated in the disease. We were the first to identify a third gene involved in ADH by positional cloning in French non LDLR/non APOB families. The PCSK9 gene (Proprotein Convertase Subtilisin Kexin 9) encodes an enzyme Narc-1 (Neural Apoptosis Regulated Convertase). Several hypercholesterolemic mutations of PCSK9 have been reported: S127R, F216L, D374Y, R218S and R357H and three variations have been associated with LDLR mutations in patient with severe hypercholesterolemia. Two non sense variations Y142X and C679X (frequency of 2% in black Americans) were associated with a reduction of LDL-cholesterol levels and of CHD. The R46L variation (frequency of 3.2% in white Americans) is associated with a reduction of LDL-cholesterol of 15% and 47% of CHD. We studied the frequency of 2 variations in 600 Caucasian (400 hypercholesterolemic and 200 normolipemic) subjects. R46L was found only in the control population with a frequency of 2%. A443T was found in a woman aged 50 who presented with mild hypercholesterolemia and no mutation in LDLR and APOB genes. This variation was not found in 340 French Caucasians controls. This variation turns out to be a rare polymorphism in whites, more frequent in blacks, associated with lower plasma levels of LDL-C but which has been found in both low and high LDL-C subjects in the Dallas Heart Study. We have also studied PCSK9 protein in the plasma of two S127R carrier patients by western blot analysis after resolution by SDS-PAGE (4-12%). The profile found for these plasmas was not different from the control. The S127R mutation doesnt seem to modify the molecular weight of the secreted PCSK9 forms. But modifications of its interaction with other proteins or of the amount of PCSK9 secreted cannot be ruled out and are under investigation. PCSK9 is an attractive therapeutic target for LDL-C lowering but further investigations are required to understand its precise role in cholesterol homeostasis and to identify its substrates and inhibitors.

GeneSniffer, A Gene Prioritisation Tool: New Developments and Application to Type 2 Diabetes Genetics. *K. Elliott[1], W. Rayner[1], E. Zeggini[1], S. Wiltshire[1], JT. Bell[1], M. McCarthy[2], Int Type 2. Diabetes 1q Cons[1]* 1) WTCHG, Oxford, UK; 2) OCDEM, Oxford, UK.

Researchers pursuing large-scale genetic studies face enormous challenges in interpreting their association findings in the context of local genomic annotation and the extensive biological information available from numerous online databases. We have previously presented a datamining tool, GeneSniffer, developed to aid the integration of large scale genetic data with diverse sources of biological information. For each of the genes in a region the program interrogates information from NCBIs Gene, OMIM, PubMed, UniGene, HomoloGene, BLAST and Jacksons MGI databases, using a list of weighted disease-specific keywords, and a disease relative score is generated. We used GeneSniffer to generate Type 2 Diabetes(T2D) candidacy scores for each of the 428 genes in the replicated 1q T2D linkage region. The 1q Consortium has undertaken high-density mapping of between 3536 and 3922 SNPs (ave 1SNP/5kb) in 4472 individuals from 8 populations. SNPs in the vicinity of 6 genes showed significant association with T2D. A Wilcoxon rank test was used to test the correlation between high GeneSniffer scores and proximity to associated SNPs for the 428 genes in the 1q region. A significant p-value of 0.0095 indicated that GeneSniffer identified candidate genes with a significant chance of harbouring disease causing polymorphisms. The scope of GeneSniffer has been extended to screen gene products from the NCBIs Gene database, for curated interactions, sourced primarily from BIND and HPRD. We have applied this approach to the Warren 2 Consortium T2D pedigree resource. GeneSniffer revealed a network of genes, known to interact at the protein level, that is consistent with the preliminary evidence for epistasis at the linkage level in these data. We are currently extending the search for more complex network patterns and are formally assessing the statistical significance of our findings. GeneSniffer can be applied to any disease, and will be used to assist the ongoing gene identification efforts of the Type 1 Diabetes, T2D, 1q and Wellcome Trust Case Control International Genetics Consortia.

Genetic Partitioning of Polycystic Ovary Syndorme (PCOS) families provides evidence for two susceptibility loci within the TGFbeta signaling pathway. *C. Ackerman, M. Urbanek* Medicine/Endocrinology, Northwestern University, Chicago, IL.

PCOS is the most common cause of anovulatory infertility among reproductive age women in western societies and is also associated with metabolic abnormalities including obesity, cardiovascular disease, and a 7x increased risk of developing type 2 diabetes mellitus. While there is a strong familial component to PCOS, the mode of inheritance of PCOS is unclear and its etiology remains unknown. To date the strongest evidence for a PCOS susceptibility locus ($p<0.0006$) is D19S884, a dinucleotide repeat polymorphism, mapping to an intron of the fibrillin-3 gene (FBN3) in which allele 8 (A8) has been shown to be associated with PCOS in multiple cohorts. The follistatin gene (FST) is a second PCOS susceptibility locus that showed significant evidence for linkage with PCOS in preliminary studies ($p=0.0003$) although these findings were not replicated in follow-up study. Interestingly both fibrillins and follistatin are thought to affect TGFbeta signaling by sequestering and thereby biologically inactivating TGFbeta ligand(s). Therefore, these genes may act in a complementary manner to alter the signaling pathway. We used genetic partitioning of multiplex PCOS families to investigate this question by dividing 78 multiplex PCOS families into D19S884 A8+ ($n=25$) and D19S884 A8- ($n=53$) subsets and evaluating evidence for linkage with PCOS at FBN3 and FST in each group. In the complete dataset FBN3 had 59% identity by descent (IBD) ($p=0.027$) and FST 58% IBD ($p=0.053$). However, for FBN3 the A8+ families had 77% IBD ($p=0.0007$) while the A8- families had no evidence for association with PCOS (IBD=53%, $p=0.53$). The converse was true for FST; A8+ families have no evidence for sharing (IBD=48%, $p=0.84$) while A8- families have significant evidence for linkage (IBD=62%, $p=0.014$). These analyses support the hypothesis that the TGFbeta signaling pathway contribute to the etiology of PCOS and that at least two genes in this pathway, FBN3 and FST, contribute to PCOS. Additionally, genetic partitioning can improve the power to detect linkage in small sample sizes by reducing genetic heterogeneity.

Mitochondrial coupling defect in fibroblasts from patients with Mnf2-related Charcot-Marie-Tooth type 2A. *D. Bonneau, D. Loiseau, C. Verny, A. Chevrollier, V. Guillet, MA. Pou, Y. Malthièry, P. Amati-Bonneau, P. Reynier*
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Charcot-Marie-Tooth(CMT) disease is one of the most frequent inherited neurological disorder affecting about 1/2,500 individuals. It refers to an heterogeneous group of progressive peripheral neuropathies leading to length-dependent loss of sensation, weakness, muscle atrophy, loss of deep tendon reflexes and foot deformities. Various additional symptoms such as optic atrophy or spastic paraparesis can be associated to the different forms of the disease. Age of onset, expressivity and severity of the disease are variable. CMT are classically classified in predominantly demyelinating (CMT type 1) or axonal (CMT type 2). CMT2 has been subdivided into eight groups by linkage studies and five genes involved in these forms have been identified so far. CMT2A (MIM 118210) was mapped to chromosome 1p35-36. Two genes have been associated in this locus, KIF1B (kinesin family member 1B) and Mfn2 (Mitofusin 2). The Mfn2 gene encodes a dynamin GTPase, located in the outer mitochondrial membrane, that is an essential component of the mitochondrial fusion machinery. Mutations of Mfn2 were recently found to be responsible of most cases of Charcot-Marie-Tooth (CMT) disease type 2A. We investigated mitochondrial energetic metabolism and structure in skin fibroblasts of 4 patients presenting novel missense mutations in Mfn2 in comparison to controls. We found a significant mitochondrial coupling defect responsible for a 30% reduction of the mitochondrial membrane potential (m). The morphology of mitochondrial network was not altered. This result suggests that Mfn2-related Charcot-Marie-Tooth patients have a dramatic decrease in the efficiency of oxidative phosphorylation that might be contribute to the axonal degeneration.

Natural History of Autosomal Recessive Polycystic Kidney Disease/Congenital Hepatic Fibrosis (ARPKD/CHF).

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ARPKD/CHF is a developmental disorder of the kidneys and liver caused by mutations in the *PKHD1* gene. The kidney cysts in ARPKD are non-obstructive dilatations of the collecting ducts. CHF, an invariable part of ARPKD, results from malformation of the developing ductal plate. Approximately 30-50 percent of ARPKD patients present perinatally with enlarged kidneys. Although severely affected neonates die in infancy, survival has dramatically increased because of wide availability of prenatal ultrasound and improved neonatal care. In most patients both kidney and liver disease are progressive, with variable rates of deterioration. More than half of the patients require renal transplantation before 20 years of age. Portal hypertension, the major clinical problem associated with CHF, causes hypersplenism and esophageal varices. A subset of patients also exhibit cystic dilatation of the medium sized and larger intrahepatic bile ducts (Carolis syndrome). Hypertension, renal insufficiency, bleeding from esophageal varices, and recurrent cholangitis are the major sources of morbidity; only symptomatic therapy is available. The natural history of ARPKD has not been elucidated in a detailed, longitudinal fashion. To date, we have evaluated 55 patients through an ongoing natural history study of ARPKD/CHF (www.clinicaltrials.gov, trial NCT00068224). Here we present data on 35 typical ARPKD/CHF patients, based on clinical and/or molecular data. Significant findings include normal urinary protein, glucose and calcium excretion, largely intact proximal tubule function, mildly impaired distal tubule function, normal plasma norepinephrine, mildly elevated serum ammonia, and small cystic dilatations of the peripheral intrahepatic bile ducts in the face of normally sized central ducts. We continue to produce comprehensive longitudinal data on ARPKD/CHF to provide the groundwork for more focused studies and future therapeutic interventions.

The CIDR GWA LIMS -- A Laboratory Process Tracking System for the Illumina HumanHap300 and Human-1(100K) BeadChip Products. *M. Barnhart, J. Goldstein, B. Craig, C. Bark, R. King, K. Hetrick, K.F. Doheny, L. Watkins* IGM/CIDR, Johns Hopkins Univ/CIDR, Baltimore, MD.

The Center for Inherited Disease Research (CIDR) is a centralized facility established to provide genotyping and statistical genetics services for investigators seeking to identify genes that contribute to human disease. In January 2006, CIDRs technology development team began genotyping ~2,600 samples from a Finland-United States Investigation of NIDDM Genetics (FUSION) case-control pilot project in order to evaluate the performance characteristics of the Illumina HumanHap300 beadchip product. Informatic support was unavailable for this Infinium product at that time. Although Illumina was developing a LIMS, CIDR required an immediate solution in order to ensure the highest level of data quality possible. Having created a custom data entry system for the Infinium 100K (Human-1) product, CIDR used the existing codebase, whose development involved approximately 500 hours of programming time over a two-month period, to create a similar LIMS for the HumanHap300 product. Modifying the 100K LIMS to accommodate lab processes unique to the HumanHap300 product involved approximately 30 additional hours of programming time over a one-month period. Both the original and the derived LIMS consist of a Java Swing graphical user interface (GUI) backed by a MySQL relational database management system (RDBMS) that tracks each sample through all stages of processing, from DNA plate through association to a beadchip, while recording reagent lot numbers, reaction temperatures, and other variables essential to quality control. Rigorous data validation ensures that reagents scanned are valid for each phase and that each plate or beadchip scanned is ready for processing in the phase being recorded. When processing for a set of samples is complete, users can generate an import file for the Illumina BeadStudio software as well as a variety of quality-control reports for the production laboratory. Source code is available upon request.

Portal Hypertension Associated with Autosomal Dominant Polycystic Kidney Disease (ADPKD). *E. Font-Montgomery[1], I. Ocak[2], P. Choyke[2], L. Guay-Woodford[3], T. Heller[4], R. Kleta[1], P. Mohan[5], Z. Quezado[6], M. Gunay-Aygun[1], W. Gahl[1]* 1) MGB, NHGRI, NIH, Bethesda, MD; 2) NCI; 3) University of Alabama, Birmingham, AL; 4) NIDDK; 5) CNMC, Washington,DC; 6) NIH Clinical Center.

The characteristics of liver disease differ in autosomal dominant (AD) compared with autosomal recessive (AR) polycystic kidney disease (PKD). All ARPKD patients have congenital hepatic fibrosis (CHF), often with portal hypertension. A subset of patients exhibit cystic dilatations of the medium sized and larger intrahepatic bile ducts (Carolis Syndrome), caused by ductal plate malformations affecting the central biliary system. In contrast, the liver disease of ADPKD is relatively benign. Cysts develop from the peripheral liver tissue (von Meyenburg complexes), and are disconnected from the biliary system. Portal hypertension rarely occurs. Under the NHGRI natural history protocol on ARPKD/CHF (www.clinicaltrials.gov, trial NCT00068224), we evaluated 2 ADPKD families with CHF and portal hypertension. In one family, the 52-year-old father was on dialysis. His 33-year-old daughter presented with hepatosplenomegaly at 6 months and was diagnosed with CHF by liver biopsy. She required esophageal variceal banding and renal cyst decompressions in young adulthood. Her daughter (16) and son (14) had kidney cysts without liver involvement. In the second family, the 9-year-old index case presented at age 4 with esophageal variceal bleeding. Several relatives in 3 previous generations had ADPKD without portal hypertension. Her asymptomatic 14 year old brother had kidney cysts, splenomegaly and esophageal varices and her 12-year-old brother had only renal involvement. Sequencing of the *PKD1*, *PKD2* and related genes and potential modifiers might shed light on the reasons for intrafamilial variability of the liver phenotype in these families.

A genome-wide scan for genes involved in vesicoureteral reflux. *D.E. Barton[1,2], H. Kelly[1,3], A. Yoneda[1,3], J.M. Darlow[1,3], D. Shields[3], C. Molony[4], A.J. Green[1,2], P. Puri[3]* 1) National Centre for Medical Genetics and Department of Medical Genetics, University College Dublin, Ireland; 2) Conway Institute for Biomolecular and Biomedical Research, University College Dublin, Ireland; 3) Children's Research Centre, Our Ladys Children's Hospital, Crumlin, Dublin, Ireland; 4) Rosetta Inpharmatics, Seattle, Washington.

Vesicoureteral reflux (VUR, OMIM 193000), the reverse flow of urine from the bladder towards the kidney, is found in 1-2% of children. It is a common cause of end-stage renal disease and severe hypertension in children. VUR is caused by a shortening of the segment of the ureter that runs through the submucosal layer of the bladder wall. Milder grades may resolve with age but more severe grades require medical or surgical intervention. Genetic studies indicate that VUR is heterogeneous; inheritance appears to be autosomal dominant in many families. Blood was collected from 167 Irish families with more than 1 child affected with VUR. We are using an affected-sib-pair approach to search for VUR genes. We studied the numbers of sib-pairs to see if there was any distortion of the expected 50:50 distribution of same-sex versus opposite-sex pairs. Of 104 independent sib-pairs, same-sex pairs outnumbered opposite-sex pairs by almost 2:1 (^{2}=7.01, p = 0.008). This novel finding suggests either a pseudoautosomal location for a gene involved in VUR or another sex-specific mode of inheritance or expression of the disorder. We carried out a genome scan using 4753 SNP markers on 620 samples from 133 families. Multipoint linkage analysis of the autosomal data indicated 11 regions with p<0.01, on 1q23-25, 2q37, 4p15, 6q24-25, 6q27, 7q36, 10q26, 13q33, 16q24, 20p12 and 21q22. We then removed families of cases with duplex ureters (n=20), reasoning that these may be a different aetiological subset, and reanalyzed. Some peaks diminished and some increased, giving 4 highly significant signals, at 2q27 (p=0, NPL 4.35), 6q25 (p=0.00006, NPL 3.21), 7q36 (p=0.003, NPL 2.50) and 10q26 (p=0.00012, NPL 2.94). Candidate genes for VUR are now being investigated in these regions. Genes at some of the peaks that diminished may be associated with duplex ureteric budding.

Lack of response to high dose ERT in a patient with Type III Gaucher Disease and a mesenteric mass consisting of Gaucher Cells. *D. Freedenberg[1], M. Dudek[1], G. Tiller[3], A.. Bircher[1], W. Rhead[2]* 1) Dept Peds, Div Med Gen, Vanderbilt Univ School of Medicine, Nashville, TX; 2) Dept of Peds Medical College of Wisconsin Miwaukee, WI; 3) Kaiser Medical, California.

We report a 13 year old hispanic female with type III Gaucher disease and a mesenteric mass consisting of Gaucher cells that is unrepsonsive to high dose ERT. The child was diagnosed with Type III Gaucher disease at the age of 2 years after an evaluation for organomegally. Her molecular studies noted her to be homozygous for L44P mutation. ERT with Cerezyme at standard dosage of 60-90 units/kg biweekly was started at age 2. She appeared to have a decrease in splenic volume, although at 8 years was still noted to have a spleen that was 3.5 times normal, and hepatic volume that was 1.2 normal. She did not appear to have symptomatic bone involvement and was noted to have cognitive delays. Hematologic parameters had normalized. At age 11 years she developed symtoms of abdominal obstruction. At laporotomy she was noted to have a large multi cystic mass with a portion of the mass encasing the mesenteric vasculature. She had multiple areas of studding along the omentum and the mesenteric nodules apppeared to be lymph nodes. The cyst was decompressed but the mass was unable to be resected. Bx of the omental nodules, peritoneal fluid lavage, cystic wall, and seeding nodules, all noted histiocytic infiltration. Electron microscopy confirming the presence of Gaucher cells. She has now received 28 months of high dose ERT at 90u/kg of Cerezyme weekly. Monitoring CT of the abdomen noted no change in size of the mass but increased clacification of the mass that is now characterized as a large soft tissue mass encasing the SMA and infiltrating the greater omentum with associated bowel wall thickening. Her biomarkers note the ACE in the normal range, chitotriosidase is undetectable and Trap remains elevated, although decreasing. Our patient illustrates that there appear to be visceral compartments that are inacessable to ERT and there may be unexpected complications of Gaucher disease, even in patients on ERT. She will be started on substrate reduction therapy in addition to standard dosing of ERT.

Investigation of the putative axonal guidance gene *ROBO4* in attention-deficit/hyperactivity disorder. *K.M. Dorval[1], K.G. Wigg[1], J. Crosbie[2], R. Tannock[2], J.L. Kennedy[3], A. Ickowicz[2], T. Pathare[2], M. Malone[2], R. Schachar[2], C.L. Barr[1,2]* 1) Dept Cell & Molecular Biol, Toronto Western Research Inst, Toronto, ON, Canada; 2) The Hospital for Sick Children, Toronto, ON; 3) Centre for Addiction and Mental Health, Toronto, ON.

Purpose: To determine the association of variants in the *ROBO4* magic roundabout gene with attention-deficit/hyperactivity disorder. **Methods:** Recently, an inversion observed at chromosome 13q31.1;31.3 in a proband presenting with ADHD and TS led to the investigation of the *Slit* and *Trk-like* family member 1 gene (*SLITRK1*). Further analysis revealed two rare *SLITRK1* variants in probands with TS and ADHD or obsessive compulsive symptoms. These variants functionally decreased protein expression and dendritic outgrowth. We sought to investigate other genes involved in the *SLIT/ROBO* signaling pathway in ADHD. Four single nucleotide polymorphisms (SNPs) were genotyped in the *ROBO4* gene in 232 nuclear families identified through ADHD probands. Transmission of alleles from heterozygous parents to affected offspring was examined using the transmission/ disequilibrium test (TDT). Quantitative trait analysis was performed using the FBAT program. **Results:** One of the four SNPS located in intron 2 (rs4078483) demonstrated a trend for association with ADHD as a categorical trait (2=2.960, p=0.085). The remaining SNPs (rs4635093, rs6590109, rs12823) did not reveal significantly biased transmission (p 0.320). Quantitative trait analyses did not reveal any significant association of the *ROBO4* variants with the ADHD symptom dimensions of inattention or hyperactivity/impulsivity (p 0.330). **Conclusions:** Variants in the *ROBO4* gene were not significantly associated with ADHD. Based on the implication of the *SLITRK1* gene in ADHD and TS and the *ROBO1* gene in developmental dyslexia, further analysis of the role of putative axonal guidance genes in the pathogenesis of neuropsychiatric disorders is warranted.

Genetic heterogeneity in hereditary capillary malformation. *M. Amyere[1], I. Eerola[1], N. Revencu[1], L. Boon[1, 2], M. Vikkula[1]* 1) Lab Human Molecular Genetics, Christian de Duve Institute & UCL, Brussels, Belgium; 2) Centre for Vascular Anomalies, Division of Plastic Surgery, Cliniques universitaires St-Luc & UCL, Brussels, Belgium.

Capillary malformation or 'port-wine stain' is a common vascular malformation affecting cutaneous capillary vessels in 0.3% of newborns. Sometimes it occurs in combination with other vascular anomalies, such as in Sturge-Weber syndrome, Klippel-Trenaunay syndrome, and Parkes Weber syndrome (Boon et al., 2005). Capillary malformations are usually not inherited. We recently recognized atypical capillary malformations to be the hall-mark of a hereditary condition that we named capillary malformation-arteriovenous malformation (CM-AVM). Its defining features are atypical cutaneous multifocal capillary malformations often in association with high-flow lesions. This phenotype is caused by mutations in the RASA1 gene (Eerola et al., 2003). Herein, we report a Japanese family with inherited classical CM. No mutation was found in RASA1 using DHPLC (denaturing high-performance liquid chromatography) and direct sequencing in affected patients. Linkage analysis with markers in RASA1 locus also excluded the RASA1 gene. These results support the etiological distinction between CM-AVM and classic CM, and suggest a genetic basis in some cases for classic CM. (http://www.icp.ucl.ac.be/vikkula)(vikkula@bchm.ucl.ac.be).

A new locus for keloids with hypertrophic scarring. *L. Boon*[1], *M. Amyere*[2], *M. Vikkula*[2] 1) Centre for Vascular Anomalies, Cliniques universitaires St-Luc & UCL, Brussels, Belgium; 2) Laboratory of Human Molecular Genetics, Christian de Duve Institute & UCL, Brussels, Belgium.

Keloids consist of pathologic fibrosis which occurs in the skin after trauma and which grow beyond the boundaries of the injury. These cutaneous lesions are formed by excessive deposition of extracellular matrix, mainly collagen. Keloids occur in people of all racial backgrounds; however, individuals of African descent are more susceptible. Linkage of two keloid African-American and Japanese families has been reported to loci on 2q23 and 7p11 (Marneros et al., 2004). We have performed linkage analysis in a Belgian family to test if it is also linked to one of these loci. The affected individuals of this family showed formation of small keloids and hypertrophic scars. Linkage analysis was performed using dense microsatellite maps inside the linked regions. The LINKAGE and GENEHUNTER packages were used for parametric (LOD) and non-parametric (NPL) analysis. They excluded both 2q23 and 7p11. Genome scan using the GeneChip Human Mapping 10K SNP Array shows linkage to a locus of 13 Mb with maximum Lod scores 2.5. This finding provides further evidence for locus heterogeneity genes that regulates formation of keloids with hypertrophic scars. (http://www.icp.ucl.ac.be/vikkula)(vikkula@bchm.ucl.ac.be).

Plasma obestatin and ghrelin levels in subjects with Prader-Willi syndrome. *M.G. Butler, D.C. Bittel* Children's Mercy Hospital and University of Missouri-Kansas City, Kansas City, MO.

Prader-Willi syndrome (PWS) is a complex obesity disorder characterized by feeding difficulties and failure to thrive during infancy but excessive food intake, rapid weight gain and obesity during early childhood. Food intake is regulated by the hypothalamus but directly influenced by gastrointestinal peptides responding to the nutritional status and body composition of an individual. Ghrelin is a 28-amino acid peptide derived by post-translational processes from preproghrelin and secreted by the stomach. Ghrelin increases appetite, food intake and body weight while leptin (secreted by adipose tissue) and peptide YY (released by the intestine) inhibit food intake. Ghrelin is reportedly elevated in PWS infants, children and adults and binds to a growth hormone secretagogue receptor in the hypothalamus. Obestatin, a recently identified 23-amino acid peptide also derived post-translationally from preproghrelin, works in opposition to ghrelin by decreasing appetite. Obestatin does not readily cross the blood-brain barrier but is highly conserved across species with sequence homologies of 87% between rodents and humans. Serum obestatin concentrations in rodents are more than 300 pg/ml but no published data exists in humans. Hence, peptide YY, leptin, ghrelin and now obestatin are primary candidates to investigate in PWS which affect eating behavior. We report fasting plasma obestatin and ghrelin levels in 16 subjects with PWS (mean age = 16.0 $\pm$ 13.3 years; age range 1-44 years) along with age and gender matched controls. Significantly higher obestatin levels were seen in the 16 PWS subjects (398 $\pm$ 102 pg/ml) compared with 16 controls (325 $\pm$ 109 pg/ml; matched t-test, $p = 0.04$), as well as 5 young (3 years old) PWS subjects (460 $\pm$ 49 pg/ml) compared with 5 young controls (369 $\pm$ 96 pg/ml; matched t-test, $p = 0.03$). However, no difference in ghrelin levels were seen in the two subject groups. No significant correlation was observed for either peptide when compared with body mass index but a significant negative correlation was seen for ghrelin and age in PWS subjects. Our report represents the first study of obestatin measures in humans.

Experimental identification of regulatory Conserved Non-Coding sequences (CNCs) using the chicken genome.

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Comparative genome analysis across mammals has revealed a large number of highly conserved non-coding sequences (CNCs). We had studied CNCs from 21q and found that their evolutionary features strongly suggested functional importance. However, the function of the majority of these sequences is unknown. We hypothesised a possible role of a subset of CNCs in transcriptional regulation. *In vitro* luciferase and DNaseI hypersensitive assays were used to evaluate this putative regulatory function; the results of both assays in more than 110 sequences revealed that only 15% of CNCs have properties of transcriptional regulators. This suggests that there may be insufficient evolutionary distance between humans and others mammals to easily distinguish conserved regulatory sequences. The chicken contains a more divergent genome for comparative analysis; we thus hypothesised that human-chicken genome comparisons could provide improved phylogenetic resolution for identification of regulatory elements. Alignment of 21q CNCs with the chicken genome revealed that ~5-6% of these are conserved down to birds (100bp, 70% homology). The homogenous proliferating neuroepithelium of the early developing chick retina offers the opportunity to evaluate the transcriptional potential of CNCs in precursors (undifferentiated) cells. We thus electroporated chick retinas (embryonic day E3 & E6) with GFP-reporter vectors to determine the regulatory potential of a set of those CNCs. Analysis of 15 CNC sequences and non-conserved single copy control sequences suggests that the majority of human-chicken CNCs (80-85%) strongly activate the GFP expression in explanted retina. Interestingly, we observed that some CNCs behave as enhancers in early retina (E3) but not at later developmental stages (E6). Our results suggest that the chicken genome could be used to identify transcriptional regulators among the mammalian CNCs, and that many human-chicken CNCs act at early stages of development.

UMOD-related familial juvenile hyperuricemic nephropathy : mutation detection rate and mutational spectrum.

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A mutation in the UMOD gene has emerged as the major cause of familial juvenile hyperuricemic nephropathy (FJHN). However, the mutation rate detection as well as the spectrum of mutations remains to be evaluated in a large cohort of families. We report on these findings in 50 families (31 from France, 11 from Belgium, 2 from Italy, 2 from Morocco, 1 from England, 1 from Australia, 1 from Germany and 1 from Netherlands) with FJHN. In all kindreds, at least one affected individual had a history of either progressive renal insufficiency related to a histological or clinical picture of chronic interstitial nephritis or hyperuricemia (serum uric acid level > 6 mg/dl) preceding renal failure or disproportionate to the rate of glomerular filtration. In addition, a history of either gout in childhood or in young adulthood or chronic renal failure (CRF) or both was present in at least one first-degree relative among 6, 17 and 27 of families respectively. Two propositus had preserved renal function (2/50, 4%) at a mean age of 53 (range : 35-71), 19 had CRF (19/50, 38%) at a mean age of 40 (range : 2-72) and 29 had reached end-stage renal failure (ESRF) (29/50, 58%) at a mean age of 43.1 (range : 28-64). Renal imaging available in 23 propositus, all with CRF or ESR, revealed the presence of cysts in 14 of them (19/23, 82%). Twenty-one patients had mutation in UMOD gene (21/50, 42%). Of the 21 changes (19 missense, one in-frame deletion and one splicing mutation), all are unique with the exception of a cysteine substitution (p.C315Y) also seen in 2 unrelated patients. These mutations are distributed throughout the exon 4 (18/21, 86%) and 5 (3/21, 14%). This mutational study shows a mutation detection rate of 42 % in a large cohort of FJHN families and confirms that missense mutation involving the 5 region corresponding to codons 32 to 315 of Tamm Horsfall protein is the major mechanism for UMOD defect (19/21, 90%).

Locus homogeneity in Glomuvenous Malformation. *P. Brouillard[1], L.M. Boon[1, 2], O. Enjolras[3], J.B. Mulliken[4], M. Vikkula[1]* 1) Lab Human Molecular Genetics, Christian de Duve Institute & UCL, Brussels, Belgium; 2) Centre for Vascular Anomalies, Cliniques universitaires St-Luc, Brussels, Belgium; 3) Pediatric Vascular Clinic, Hôpital d'Enfants A. Trousseau, Paris, France; 4) Vascular Anomalies Center, Children's Hospital, Harvard Medical School, Boston, MA, USA.

Glomuvenous malformation (GVM) is a localized cutaneous vascular lesion histologically characterized by the presence of maldifferentiated smooth muscle-like glomus cells around distended vein-like channels. GVM is caused by mutations in Glomulin (GLMN) (Brouillard et al., 2002). Most mutations lead to premature termination codons, probably resulting in loss-of-function. Glomulin is specifically expressed in vascular smooth muscle cells (vSMCs), but its exact function remains unknown (McIntyre et al., 2004). The TGF and HGF signaling pathways may be involved. Hypothesis of paradominant inheritance was supported by the identification of a double-hit mutation in one lesion, resulting in complete localized loss of glomulin. Moreover, we showed that glomus cells in GVM do not express glomulin nor another late vSMC marker, smoothelin-b, whereas earlier differentiation markers are detected (McIntyre et al., submitted). Thus, GLMN plays a role in the differentiation of vSMCs, which have deviated in their differentiation process due to lack of glomulin expression. To date, we have identified altogether 30 different mutations in 86 families, one of them being present in 40% of them. As a mutation was identified in each family with clear GVM diagnosis, it suggests that no locus heterogeneity exists for GVM. (brouillard@bchm.ucl.ac.be).

Mutation analysis of the three FHM genes in a set of 29 sporadic hemiplegic migraine patients. *B. De Vries[1], K.R.J. Vanmolkot[1], A.H. Stam[2], J.B. Koenderink[3], E. Babini[4], G.M. Terwindt[2], M. Pusch[4], A.M.J.M. Van den Maagdenberg[1,2], R.R. Frants[1], M.D. Ferrari[2]* 1) Department of Human Genetics, Leiden University Medical Centre, Leiden, The Netherlands; 2) Department of Neurology, Leiden University Medical Centre, Leiden, The Netherlands; 3) Department of Pharmacology and Toxicology, Nijmegen, The Netherlands; 4) Insititutio di Biofisica, Genova, Italy.

Patients with sporadic (SHM) and familial forms of hemiplegic migraine (FHM) have similar clinical characteristics, including attacks of migraine with aura associated with hemiparesis. It is unknown whether FHM and SHM share a genetic etiology. Here, we report on sequence analysis of the three FHM genes CACNA1A, ATP1A2, and SCN1A in 29 well-characterized SHM patients. Patients were considered sporadic when none of the first-degree relatives had hemiplegic migraine. We identified a total of seven missense mutations, of which 2 were found in the CACNA1A gene, four in the ATP1A2 gene and one in the SCN1A gene. Different studies were performed to determine the functional consequences of these mutations. In conclusion, we identified mutations in 7 out of 29 SHM patients; four mutations were located in the ATP1A2 gene. Therefore, our findings suggest that the ATP1A2 gene is a good candidate for genetic testing in SHM cases. In the majority of our SHM cases however no mutations were found, indicating that other genetic factors might be involved as well.

Severe palpebral hamartoma and aplasia cutis congenita in a child with Schimmelpenning-Feuerstein-Mims syndrome. *M. Gerard[1], D. Bremond[2,3], V. Desilets[4], M. Elmaleh[5], M.L. Jaquemont[1], C. Baumann[1], A. Verloes[1,3]* 1) Medical genetics, Hôpital Robert Debré, Paris, France; 2) Ophthalmology, Hôpital Robert Debré, Paris, France; 3) INSERM U6764, Hôpital Robert Debré, Paris, France; 4) Pediatrics, Hôpital Ste Justine, Montréal, Canada; 5) Radiology, Hôpital Robert Debré, Paris, France.

Schimmelpenning-Feuerstein-Mims syndrome (OMIM 165630), or epidermal nevus syndrome, associates ocular anomalies, cerebral defects, and systematized linear sebaceous nevus following the lines of Baschko. All cases are sporadic. The mechanism suggested by Happle is a dominant lethal gene arising as a somatic mutation in the early embryo, and surviving by mosaicism (Happle and al., 1986). Ocular anomalies are frequent, such as extension of nevus to lid, coloboma of lid or/and iris and retina, epibulbar lipodermoid, corneal opacities, optic nerve hypoplasia, oculomototor dysfunction, nystagmus, or cortical blindness. We followed a 3 months-old girl born to non-consanguineous parents, with a linear sebaceous nevus, localised on the back, with verruquous development on a pigmented band of the arm, and association with severe ophthalmologic malformations, bilateral superior ablepharon (agenesis of superior lid), peri-ocular calcified hamaortomas with colobomatous microphthalmia. Inferior lids were normal. A cutaneous aplasia of the scalp was associated. Facial scanner identified hamartomas of superior lids with macro-calcifications of the ocular globe fused with the orbit roofs. Abnormal ossification or calcification joined the intern part of the lid with a frontal insertion. Such an ablepharon with calcified hamartoma of lids have been rarely described in SMS, and no aplasia cutis. The epidermal nevus is non specific, and can be seen in SPS, Proteus syndrome, CHILD, nevus comedonicus, and pigmented hairy epidermal nevus (Happle, 1995). Ablepharon is a rare dysmorphological pattern, found in ablepharon-macrosomia syndrome (OMIM 200110), and Barber-Say syndrome (OMIM 209885). It will be of interest to study the genes responsible for osseous formation in the hamartomas of the lids, on tissue samples post reparative surgery.

A copula approach to missing data in genetic association studies. *G.M. D'Angelo*[1], *E. Feingold*[1, 2] 1) Biostatistics, University of Pittsburgh GSPH, Pittsburgh, PA; 2) Human Genetics, University of Pittsburgh GSPH,Pittsburgh, PA.

A limited amount of research has focused on association studies with missing genotype data. Typically, missing data is assumed to be missing completely at random (MCAR) and complete case analysis is the method of choice resulting in biased and inefficient results. When fitting models with genotypes at several markers as simultaneous predictors, the complete case approach can lead to significant reduction in sample size. To address the problem where the SNPs are missing at random (MAR), we suggest using a copula approach to improve the coefficient estimates and their standard errors in a logistic regression model. An advantage of employing the copula is that the joint distribution of the outcome and covariate is specified, and since the covariates are missing their distributions must be specified. An added benefit to the copula approach is that it can handle nonmonotonic data. We compare the copula approach to multiple imputation and complete case analysis.

Association of Polymorphisms in S-nitroso-glutathione Reductase with Asthma. *S. Choudhry[1], L. Liu[2], L.G. Que[3], C. Eng[1], S. Nazario[4], J. Casal[4], A. Torres[4], I. Gomez[4], J.K. Fagan[5], J. Salas[6], R. Chapela[6], J.G. Ford[7], P.C. Avila[8], W. Rodriguez-Cintron[4], E.G. Burchard[1]* 1) Department of Medicine, University of California, San Francisco; 2) Department of Microbiology & Immunology, University of California, San Francisco; 3) Center for Comparative Biology of Vulnerable Populations, Duke University; 4) San Juan VAMC, School of Medicine, University of Puerto Rico; 5) The Harlem Lung Center, Harlem Hospital, New York; 6) Instituto Nacional de Enfermedades Respiratorias, Mexico City; 7) Johns Hopkins Bloomberg School of Public Health, Baltimore; 8) The Feinberg School of Medicine, Northwestern University.

S-nitroso-glutathione (GSNO), a bronchodilator present in airway lining fluid (ALF) of healthy people, is depleted from ALF of asthmatic patients. Depletion of GSNO may contribute to impaired airway relaxation in asthmatics. Genetic deletion of GSNO reductase (GSNOR), the enzyme important for S-nitrosothiols metabolism in vivo, protected mice from experimental asthma. To determine whether polymorphisms in the GSNOR gene are associated with asthma, we carried out family-based transmission disequilibrium tests (TDT) in Puerto Rican and Mexican trios with asthma. Puerto Ricans and Mexicans have the highest and the lowest asthma prevalence, respectively, in the US. We identified 39 SNPs in the GSNOR gene by sequencing 72 Puerto Ricans, Mexicans and African Americans with asthma. Many SNPs identified in the gene were in strong linkage disequilibrium. Five SNPs, one in promoter region and four in 3 UTR were selected for further genotyping in 386 Puerto Rican and 300 Mexican asthmatic trios. TDT analysis showed that several GSNOR SNPs, including the SNP in the promoter region (p=0.05) and 3 SNPs (p=0.03 to 0.008) in the 3UTR, were significantly associated with predisposition to asthma in the Puerto Rican cohort. Significant association of GSNOR and asthma in Puerto Ricans was further supported by haplotype analysis (p=0.02). No association of the GSNOR SNPs with asthma was found in Mexicans. Our data suggest a genetic association of GSNOR and asthma in Puerto Ricans, and further implicate GSNO metabolism in asthma pathogenesis.

A descriptive study of international genetic counselor education: program features and student characteristics.

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There is an increasing international demand for genetic counselors, and as a result new training programs are being developed yearly. Today, training programs exist in at least 15 countries around the world. Most studies of genetic counseling education have focused on programs in the U.S. and less is known about the greater international genetic counseling community. This study describes the current state of international genetic counseling programs and explores the characteristics of students attending these programs. Program directors completed a survey of their programs structure and admissions characteristics. Students participated in a survey assessing their experience and personal characteristics prior to entering their program. Twenty-five programs in 14 countries completed the first survey. Data were also collected from 126 students training in 11 countries. These data show that programs vary in number of yearly applicants and students in attendance, and in the duration of the training curriculum. Students were born in 30 countries on 6 continents, yet they are demographically similar and have similar backgrounds. Most students had prior experience in biological science, research, or healthcare, and more than 75% were motivated to enter the career based on a desire to help others, an interest in genetics, and the desire for intellectual stimulation at work. International students were an average of 6 years older and had more advanced study and work experience than students in the U.S. (Lega, et al. 2005). Overall, 90.7% of students were confident in their career choice, although over 50% considered their opportunities for employment to be limited. This study uncovered variations in program structure and student characteristics of internationally. The common backgrounds and motivations found among students, despite their numerous nationalities and countries of training, may reflect an underlying genetic counseling philosophy that attracts certain individuals to the career.

Combined Linkage and Association Mapping of Quantitative Trait Loci with Missing Genotype Data. *R. Fan[1], L. Liu[1], J. Jung[2], M. Zhong[1]* 1) Dept Statistics, Texas A&M Univ, College Station, TX; 2) Department of Human Genetics, University of Pittsburgh, Graduate School of Public Health, Pittsburgh, PA 15261.

In this study, the impact of missing genotypes is investigated for high resolution combined linkage and association mapping of quantitative trait loci (QTL). Two regression models, ``genotype effect model" and ``additive effect model", are proposed to model the association between the markers and the trait locus. The expected number of genotypes or alleles is used as weight to model the effect of the genotypes or alleles. By analytical formulae, we show that the ``genotype effect model" can be used to model the additive and dominance effects simultaneously; the ``additive effect model" only takes care of additive effect. Based on the two models, F-test statistics are proposed to test association between the QTL and markers. The non-centrality parameter approximations of F-test statistics are derived to make power calculation and comparison, which show that the power of the F-tests is reduced due to the missingness. By simulation study, we show that the two models have reasonable type I error rates for a dataset of moderate sample size. The method are applied to analyze the angiotensin-1 converting enzyme (ACE) data.

CRISPLD2 and Nonsyndromic Cleft Lip with or without Cleft Palate. *B.T. Chiquet[1,2], S.H. Blanton[3], S. Stal[4], J.B. Mulliken[5], J.T. Hecht[1]* 1) University of Texas Medical School at Houston; 2) University of Texas Dental Branch at Houston; 3) Duke University Medical Center, Durham, NC; 4) Texas Children's Hospital, Houston; 5) Children's Hospital, Boston, MA.

Nonsyndromic cleft lip with or without cleft palate (NSCLP) is a common birth defect with a birth prevalence of 1/700 live births. NSCLP is a complex disorder postulated to be caused by multiple genes and environmental factors. Candidate gene testing has identified a number of genes that contribute to NSCLP. Previously, we identified a significant association with an allele in D16S3037 (p=0.000063), a STR marker at chromosome 16q24.1, and NSCLP. The cysteine-rich secretory protein LCCL domain containing 2 (CRISPLD2) gene is immediately 5 of D16S3037. CRISPLD2 was previously reported to be expressed in the placenta, brain and soft tissue, but the biologic function has not been identified. The proximity of CRISPLD2 to D16S3037 made it a prime candidate gene for NSCLP. SNP genotyping and *in situ* hybridization of CRISPLD2 was performed. Sixty-three multiplex NSCLP families and 287 simplex parent-child NSCLP trios were genotyped using TaqMan SNP genotyping assays for 15 SNPs flanking and spanning CRISPLD2. Fourteen of 15 SNPs were in HWE. There were significant allele frequency differences between Hispanic and non-Hispanic white populations for 8 of 15 SNPs. As a consequence, data analysis was performed separately for each population. In the white population, there was significant association between SNP rs1546124 and NSCLP (p=0.00167). rs1546124 is in exon 2, which is located 51 base pairs 5' of the CRISPLD2 protein start codon. Three additional nonsynonymous SNPs in the coding region of the CRISPLD2 protein have been identified and are currently being genotyped to test for association with NSCLP. To test if CRISPLD2 is expressed during craniofacial development, *in situ* hybridization was performed using a probe amplified from a RIKEN mouse cDNA clone of CRISPLD2. Preliminary results suggest that CRISPLD2 is expressed in the naso-/oropharynx area during mouse embryogenesis as well as other tissues (ie, liver). These results suggest that variation in CRISPLD2 may be involved in the etiology of NSCLP.

Trisomies 13 and 18: population rates, characteristics, and prenatal diagnosis, metropolitan Atlanta, 1994-2003.

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BACKGROUND: In recent years, prenatal diagnosis and elective pregnancy termination have affected the reported birth prevalence of trisomies 13 and 18. METHODS: We examined the prevalence and characteristics of these conditions using 1994-2003 data from a population-based surveillance system, the Metropolitan Atlanta Congenital Defects Program (MACDP). In 1968, MACDP began ascertaining structural defects and chromosome abnormalities among infants and fetuses born at 20 weeks gestation to residents of metropolitan Atlanta through hospital record reviews. In 1994, MACDP abstractors began to visit the offices of perinatologists to ascertain pregnancies diagnosed with defects prenatally. RESULTS: Inclusion of prenatal diagnoses increased the population prevalence (number of cases per number of live births) of trisomy 13 by 44.4% from 1:11,470 to 1:6,372 (n = 72) and of trisomy 18 by 44.7% from 1:4,498 to 1:2,493 (n = 184). Among affected pregnancies ascertained only from perinatal offices, 85.6% were electively terminated. Among all affected pregnancies, an amniocentesis was reported for 71.0% of those with trisomy 13 and 72.9% with trisomy 18. The reported rates of amniocentesis remained stable throughout the 10-year period. As expected, maternal age 35 years was a risk factor for both conditions. However, while 67.1% (n = 55) of the trisomy 18 case mothers were 35 years, only 46.9% (n = 15) of the trisomy 13 case mothers were 35 years. Among live births, 60.4% (n = 32) with trisomy 18 and 48.3% (n = 14) with trisomy 13 were female. In contrast, among electively terminated pregnancies (generally between 15 and 21 weeks gestation), only 48.3% (n = 43) of the fetuses with trisomy 18 and 42.4% (n = 14) of those with trisomy 13 were female. CONCLUSIONS: Inclusion of prenatal diagnoses from perinatal offices markedly increased our ascertainment of both trisomy 13 and trisomy 18 and affected characteristics such as sex ratios. Inclusion of pregnancies that are prenatally diagnosed is critical for accurate surveillance and population-based analyses of these conditions.

The design and performance of a genome-wide association study to identify genetic predispositions to rare adverse drug reactions. *M.G. Ehm[1], M.L. Jones[2], J. Almenoff[1], V. Ameen[1], S.-A. Bacanu[1], M.R. Barnes[1], T. Bhinder[1], S.R. Browning[3], K. Davies[1], S. Gordon[1], S. Haneline[1], K.S. King[1], E.H. Lai[1], M.R. Nelson[1], S. Ray[1], A.D. Roses[1], S. Subramanian[1], D.P. Yarnall[1], I. Johnson[1], L.C. McCarthy[1]* 1) GlaxoSmithKline, Res Triangle Park, NC; 2) North Carolina State University, Raleigh, NC; 3) University of Auckland, Auckland, New Zealand.

Adverse drug reactions (ADRs) can pose a serious threat to the health of patients receiving a drug as well as threaten the viability of an otherwise efficacious drug that is in development or even approved for marketing, if the potential benefits do not outweigh the risks. In many cases, genetics is believed to play an important role in determining ones risk of experiencing an ADR. We present the case of one drug which is associated with two relatively rare (~0.1%) and different ADRs. Due to the low frequency of these ADRs, only 9 cases of each ADR were ascertained using post-marketing data with informed consent for genetic research. We ascertained an additional 225 drug-treated patients that did not experience these ADRs as controls. After carrying out a pathway-focused candidate gene study, we completed a genome-wide association study using three available genetic marker panels: the Affymetrix 100K genechip, a custom 266,719 SNP panel from Perlegen, and the Affymetrix 500K Early Access genechip. Power to identify associations as well as the information captured by this comprehensive genome scan will be summarized. A small number of highly significant results were validated using alternative genotyping methods showing that as expected not all highly significant SNPs will be repeatable. A variety of analysis methods and plots were used, which ultimately identified a number of biologically relevant regions that may have future drug development and diagnostic value. We report the challenges and opportunities faced in conducting such a whole-genome screen and implications for future research on the genetics of ADRs.

Asthma and genes encoding components of the vitamin D pathway. *Y. Bosse[1], C. Laprise[2, 3], M. Lemire[1], J.H. White[4], T.J. Hudson[1]* 1) McGill University and Genome Quebec Innovation Centre, Montreal, PQ, Canada; 2) Community Genomic Medicine Centre, University of Montreal, Chicoutimi University Hospital, Chicoutimi, PQ, Canada; 3) Université du Québec à Chicoutimi, PQ, Canada; 4) Department of Physiology, McGill University, Montreal, PQ, Canada.

Genetic variants within the vitamin D receptor (VDR) gene were associated with asthma and atopy. We hypothesize that polymorphisms in other genes of the vitamin D endocrine system are associated with atopic and asthmatic phenotypes. Twelve candidate genes were chosen for this study. Genes encoded components of the vitamin D metabolism pathway (CYP27A1, CYP27B1, CYP2R1, CYP24A1, GC) or were known to be transcriptionally regulated by vitamin D (IL10, IL1RL1, CD28, CD86, IL8, TSLP, SKIIP). For each gene, we selected the maximally informative set of common SNPs (tagSNPs) using the CEPH HapMap dataset. These were genotyped in a French-Canadian family sample ascertained through asthmatic probands (422 nuclear families, 1277 persons) and evaluated using the FBAT program. For asthma, twelve SNPs located within five genes reached a p value threshold below 5%. CYP24A1, responsible for the inactivation of the active form of vitamin D, and IL10 showed the strongest and the most consistent results. Six out of the eight genotyped SNPs in the CYP24A1 gene were associated with asthma, atopy or IgE levels (range of p values = 0.049 to 0.002). For IL10, five SNPs out of the eight were associated with asthma (range of p values = 0.03 to 0.012). SNPs in the CYP2R1, IL1RL1 and CD86 genes also suggested association with asthma related-phenotypes (p 0.05). In conclusion, a number of genes involved in the vitamin D endocrine system demonstrated modest evidence of association with asthma-related phenotypes. Replication of positive findings is currently underway in two independent samples.

Identification of genes required for normal forebrain development using ENU mutagenesis. *D. Beier, A. Tilt, Y. Yun, R. Stottman* Div Genetics, Brigham & Women's Hosp/Harvard Medical School, Boston, MA.

The forebrain is the largest portion of the human brain and is responsible for higher order cognitive functions. Despite its central importance, many of the genes required for cortical development remain unknown. To address this, we have begun an ENU mutagenesis screen in the mouse. We use a 3-generation 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 severe defects in organogenesis that would be lethal in a newborn animal. These should resemble birth defects seen in the human newborn population and provide animal models of clinical significance. Indeed, the utility of this approach has been validated by its application for the discovery of a causal gene for congenital diaphragmatic hernia (Ackerman et al., PLoS Genetics, 2005). Our approach is designed to be unbiased, which should serve to uncover novel or poorly annotated genes with a role in nervous system development, as well as implicate genes in brain development which have been previously characterized in other physiological settings. Our neurodevelopmental screen has three components: a) a morphologic examination complemented by histological analysis, b) use of a RARE-lacZ reporter allele to highlight distinct brain structures, and c) use of *Lis1* as a sensitizing mutation. While the screen is still in progress, we have already identified multiple lines of interest. Some of the mutations affect multiple tissues in the embryo, while others appear to be specific to the brain. The most intriguing phenotype shows severe cortical agenesis as well as defects in the appendicular skeleton. Other mutations show significant neuronal heterotopias or changes in brain size. Several phenotypes appear normal upon gross examination and are only detectable upon histological analysis. One line highlights the utility of a reporter allele as the phenotype is only revealed by the perturbation of its expression pattern in the cortex. Several mutants have been mapped using a whole-genome SNP genotyping panel (Moran et al., Genome Research 2006) and positional cloning is in progress.

The Keratodermas: Dilemmas in Diagnosis and Treatment. *R. Blackston[1], N. Brady[1], S. Dills[1], W. McLean[2]* 1) Div Med Gen, Dept Pediatrics, Emory Univ Sch Medicine, Atlanta, GA; 2) Ninewells Hospital and Medical School, Dundee, Scotland, United Kingdom.

The keratodermas are a heterogenous group of disorders of keratin accumulation usually of hands, feet, and pressure areas. Classification and nomeuclature are long, confusing. There are multiple etiologies; they can be chronically recurrent, and often debilitating. Advances in genetic DNA studies in recent years have brought greater clarity to the genetic mutations and variability of these disorders. The course of keratoderma can be chronically recurrent, painful, and debilitating with no specific therapeutics aside from supportive care such as soaking and debridement. The following case presentation manifests the difficulties of diagnosis, therapeutic interventions, and painful disability. R.R. was first seen at age 8 years, with onset peeling and accumulations of paraffin cakes on the soles of her feet at age 4 years. The waxy cakes thickened, were painful, and were treated with periodic debridement. The pain precluded weight bearing and often required narcotic pain medicines. She was described as having hyperkeratosis or possible psoriasis. Biopsies showed hyperkeratotic accumulations but did not clarify a specific diagnosis. At the first visit to our clinic, we noted thick paraffin like layers on the soles of the feet, thickened, sand-paper like feel of skin, and thick hair. She was bright, but in pain and required a wheel chair. Family history was negative for similarly affected relatives. A diagnosis of pachyonychia congenita was entertained. We referred to the pachyonychia project where she was able to get DNA studies for this rare dominant condition. Four keratin genes K 6A, K 6B, K16, K17, and also connexin 30 were performed in Dr. McLeans lab in Scotland, with all studies normal. Specific diagnosis remains unknown, though DNA studies of other keratodermas are being considered. This case indeed evidences the dilemmas faced by patients with these challenging disorders. Hopefully further DNA advances will elucidate the etiology, genetic patterns and new therapies.

Evaluation of Five Parameters Relating to Genetics Education in the International MD Program at Universidad Autónoma de Guadalajara, School of Medicine: Are There Implications for Enhanced USA-Mexico Collaboration in Genetics Education and Research? *J.S. Eperjesi[1,2], N.N. Batta[1,2], R.J. Samouh[1,2], M.A. Gutierrez-Franco[1,2], F.G. Martinez-Sandoval[1,2]* 1) Department of Medical Genetics, Universidad Autónoma de Guadalajara, Guadalajara, Jalisco, Mexico; 2) International MD Program, Universidad Autónoma de Guadalajara, Guadalajara, Jalisco, Mexico.

Substantive efforts are being made at Universidad Autónoma de Guadalajara School of Medicine (UAGSOM), the only medical school in Mexico with an international MD program (IP) and significant numbers of American students, to incorporate core competencies developed in recent years by the AAMC and ASHG into the medical genetics (MG) curricula. In this study, we investigated American student perceptions relating to MG education at UAGSOM in order to gain insights for 1)the advancement of genetics teaching by UAG faculty to American students and 2)the possibilities of exchange of knowledge, skills and attitudes in MG between USA and Mexico. We developed a student-centered survey for first semester US students enrolled in the IP who had taken the formal didactic course in MG from March-May 2006. It consisted of 5 questions to which students were asked to assign quantitative ratings as responses (i.e. 1=lowest; 5=highest); questions pertained to the utility of the MG course vis-a-vis clinical assignments in the Mexican community setting, and the importance of international collaboration between Mexico and USA for research, education and public awareness of genetics. All first semester US students (n=72) responded to the survey. Although other classes might be different, simple analysis by scantron technology warrants consideration as follows: 1) Mexican faculty is doing positive work in teaching MG to US students in a manner relevant for treatment of Mexican and international patients; 2)there is strong support among the students we surveyed for student-presentations as a learning tool for MG; 3)US students strongly support bilateral cooperation between Mexico and USA in the area of MG education, collaborative research and public awareness. This is an area we hope to pursue through fortifying relationships between experts in both countries.

Novel Unbiased Cloning Vectors, Methods, and Applications. *R. Godiska[1], N. Ravin[2], S. Vande Zande[1], V. Gilbert[1], D. Mead[1], C. Wu[1]* 1) Lucigen Corp., Middleton, WI; 2) Centre BioEngineering RAS, Moscow, Russia.

The quality of genomic libraries depends greatly on the cloning vectors and methods used for construction. Conventional cloning vectors create numerous clone gaps, due to inherent bias common to most plasmid, fosmid, and BAC vectors, including: 1) vector-driven expression of deleterious sequences, 2) insert-driven transcription into the vector that interferes with vector stability, 3) instability of secondary structures formed by tandem repeats and palindromic sequences, 4) supercoiling, and 5) unreliable antibiotic selection. The pSMART series of transcription free cloning vectors were developed to alleviate these problems. These vectors lack an indicator gene and associated promoter, have termination signals on either side of the insertion site, and are kanamycin or chloramphenicol resistant. The pSMART vectors show much higher stability of inserts containing AT-rich sequences, direct and inverted repeats, coding regions, or other deleterious DNAs. A novel E. coli cloning vector, pJAZZ, is stably maintained as a linear plasmid and incorporates transcription-free cloning capabilities. This linear plasmid shows unprecedented ability to maintain large AT-rich DNAs and di-, tri-, and tetra-nucleotide repeats. In standard BAC libraries, additional cloning bias and gaps are due to the preparation of DNA fragments. These libraries are based on partial restriction digestion of high molecular weight (HMW) genomic DNA. Because restriction sites are not evenly distributed across a genome, such BAC libraries are substantially biased, with numerous gaps. Generating as much as 20X genomic coverage in a partial-digest BAC library can not eliminate this bias. Whereas this problem may be avoided by randomly shearing HMW genomic DNA for BAC cloning, fragments larger than 100 kb are so fragile that no randomly sheared BAC library with large inserts has been reported. Recently we have successfully developed techniques to construct unbiased, randomly-sheared BAC libraries with large inserts (e.g., over 100 kb). With these novel techniques and tools, we are cloning previously unclonable DNA and working toward filling existing gaps in the human genome.

Texas institute for genomic medicine (TIGM): Knocking out all murine genes in embryonic stem cells. *R. Finnell[1], D. Markesich[2], G. Hansen[2], C. Friddle[2], A. Abuin[2]* 1) Texas Institute for Genomic Medicine, Houston, TX; 2) Lexicon Genetics, INC, The Woodlands, TX.

With support from the state of Texas, TIGMs mission is to create the worlds largest collection of mouse embryonic stem cells that have been engineered for the study of gene function, allowing researchers to identify genes that could lead to advancements in human healthcare. At the present time, TIGMs genetic resources include the Lexicon Genetics OmniBank I gene trap library of 275,000 ES cell clones in the 129 strain containing approximately 14,000 genes or 64% of the mouse genome. In addition, Lexicon Genetics has indicated a willingness to make available to TIGM, over time, mutated ES cell clones of up to an additional 3000 distinct genes, generated using gene targeting in 129 ES cells, pursuant to Lexicons existing contract with the National Institute of Environmental and Health Sciences (NIEHS) or similar arrangements contemplated under the NIHS repatriation initiative. There is an additional OmniBank II gene trap library under exclusive construction for TIGM that, when completed in early 2008, will contain 350,000 ES cell clones in the C57BL/6J strain containing approximately 16,000 genes or 73% of the mouse genome. TIGM has taken delivery of over 90,000 ES cell clones consisting of ISTs that have been mapped to the genome. This combined resource of genetically modified embryonic stem cells in two murine strains represents a unique resource that is intended to be widely distributed at reasonable costs to the academic and biotechnology research communities, to accelerate the pace of medical discoveries, and to foster the development of the biotechnology industry in Texas and worldwide.

Toward the development of an adolescent-focused framework for genetic counseling. *M.L. Galvin[1,2], C. Shuman[1,2], R. Babul-Hirji[1,2], D. Chitayat[1,2,3], M.J. Esplen[4], M. Kaufman[1,2]* 1) Hospital for Sick Children, Toronto, ON, Canada; 2) University of Toronto, Toronto, ON, Canada; 3) Mount Sinai Hospital, Toronto, ON, Canada; 4) Toronto General Hospital, Toronto, ON, Canada.

Adolescents undergo important emotional and cognitive developments, including increased autonomy and individuality. These developmental changes present unique challenges to medical professionals who work with this patient population. As the field of genetic counseling grows, genetic counselors are increasingly called upon to provide counseling to adolescents. Few studies have been published, however, regarding best practice guidelines for genetics health care providers to use when working with adolescent clients and their parents. The objective of the present study was to explore the challenges faced by genetic counselors when counseling adolescents. A focus group, consisting of eight genetic counselors who have experience working with adolescents, was facilitated in order to explore and describe the experiences of working with this population. The focus group was audio taped and transcribed. The transcriptions were coded for emergent themes using manual analysis. Focus group participants identified three domains that were particularly challenging: (i) balancing adolescent counseling and informational needs with parental needs, (ii) encouraging adolescent involvement in their own health care, and (iii) incorporating the developmental level of the adolescent in counseling and discussion of psychosocial issues. Genetic counselors also discussed what further information would assist them in addressing the identified challenges. Participants were interested in learning about developmental approaches to counseling adolescents and techniques for providing information to adolescent clients. The findings from this study were combined with information from the current adolescent medicine and psychology literature to develop a preliminary framework which offers specific techniques, resources, theories, and conceptualizations that genetic counselors can draw upon when working with adolescents. The ultimate goal of this framework is to enhance the genetic counseling encounter with adolescent clients.

A second-generation combined linkage-physical map of the human genome. *F. Chen[1], C. He[1], X. Kong[1], W. Chen[2], M. Hansen[4], F.L. Hyland[3], G.C. Kennedy[2], S. Murray[4], Y. Turpaz[2], F.M. De La Vega[3], J. Ziegle[3], T.C. Matise[1]* 1) Genetics Dept, Rutgers Univ., Piscataway, NJ; 2) Affymetrix, Inc., Santa Clara, CA; 3) Applied Biosystems, Foster City, CA; 4) Illumina, Inc., San Diego, CA.

Accurate and comprehensive linkage maps are critical for the success of positional cloning projects and other genetic studies. We have constructed a second generation high-resolution genetic map that includes a much larger set of polymorphic markers (N=28,219) than the first version and is consistent with the latest genome assembly (NCBI Build 36). To build the new combined linkage-physical map, a set of SNP genotype data including 5,172 SNPs from the Affymetrix Human Mapping 10K Array, 3,506 SNPs from the Applied Biosystems SNPlex Human Linkage Mapping Set 4k, and 4,782 SNPs from the Illumina Linkage IV Panel were collected and integrated into our existing data set (N=14,759). In the initial mapping stage, only markers with known physical positions were used and were added to the map only if the results of linkage analysis were consistent with physical position. Markers with no physical position were added in later steps using only linkage information. The data were also cleaned by removal of genotypes that are likely to be erroneous, identified as those that lead to close double-recombination events. Then markers whose linkage positions did not originally match physical position were re-evaluated against the more complete map. Finally, Those markers which still had discordant positions were assigned to map intervals determined by linkage analysis. This map represents the most comprehensive linkage map ever constructed. The average map resolution is 0.17 cM (127kb) between neighboring markers, with 58 percent of neighboring markers showing no recombination events. This map is a valuable tool for obtaining map distances for linkage studies to identify genetic loci. It is disseminated via our MAP-O-MAT website (http://compgen.rutgers.edu/mapomat).

Analysis of Genetic Polymorphisms Associated to Cardiovascular Disease in Mexican Mestizo Population. *E. Balam-Ortiz, J. K. Estrada, A. Inchaustegui, I. Silva-Zolezzi, K. Carrillo, G. Jimenez-Sanchez* National Institute of Genomic Medicine, Mexico.

Cardiovascular diseases (CD) are the leading cause of death in Mexico.1-Adrenergic receptor (*ADRB1*), 2-adrenergic receptor (*ADRB2*) and angiotensin-II receptor type 1 (*AGTR1*), mediate the effects of catecholamines and angiotensin II, that increase heart rate, contractility, renin release and lipolysis. SNPs in these genes influence the risk for CD and the therapeutic response to -adrenergic antagonists. To evaluate geographical distribution of variants in these genes in Mexico, we genotyped 6 functional SNPs in 180 Mestizo volunteers from 5 states (n=880): Zacatecas (Zac), Sonora (Son), Yucatan (Yuc), Veracruz (Ver), and Guerrero (Gro). MAFs showed: 1) *ADRB1* 145A>G (rs1801252) Zac=0.20, Yuc=0.31, Son=0.28, Ver=0.33 and Gro=0.35; 2) *ADRB1* 1165C>G (rs1801253) Zac=0.18, Yuc=0.12, Son=0.14, Ver=0.1 and Gro=0.1; 3) *ADRB2* (rs1042713) Zac=0.42, Yuc=0.41, Son=0.44, Ver=0.37 and Gro=0.49; 4) *ADRB2* (rs17287411) Zac=0.19; Yuc=0.15, Son=0.23; Ver=0.13 and Gro=0.1; 5) *AGTR1* 43732C>T (rs5182) Zac=0.5, Yuc=0.39, Son=0.56, Ver=0.45 and Gro=0.42; and, 6) *AGTR1* 44325A>C (rs5186) Zac=0.36, Yuc=0.25, Ver=0.32, Son=0.29 and Gro=0.29. All variants were in HWE. Genotypic frequencies of *ADRB1* 145A>G were different ($p<0.005$) to those for Caucasians (CEU), Han Chinese (CHB), Japanese (JPT) and Africans (YRI) in dbSNP. Similar results were obtained for *ADRB1* 1165C>G except when compared to JPT. Unphased haplotypes and LD analysis between *ADRB1* 145A>G and 1165C>G showed complete LD in all states but Gro where LD was minimum. Haplotype blocks were compared with populations from the International HapMap Project, showing that all included states share a similar block structure with CEU, except Gro who was similar to YRI. This is consistent with Gro being a state with a higher African ancestral contribution. Our results show that SNPs in *ADRB1, ADRB2* and *AGTR1* in Mexican Mestizos have significant MAF differences between some states and, in some cases with other populations. This suggests genetic stratification in Mexican Mestizo population that may influence results in genetic association studies.

Allelic Frequencies of Polymorphisms Associated to Type 2 Diabetes in Mexican Population. *L. del Bosque-Plata, A. Inchaustegui, K. Carrillo-Sanchez,, G. Jimenez-Sanchez* Investigation, National Institue of Genomic Medicine, Mexico, D.F., Mexico.

Type 2 Diabetes (T2D) affects approximately 5% of the general population, with a prevalence in Mexico of ~10.8%. Increased genetic risk for T2D has been associated to specific polymorphisms in several genes. However, only a few of them have been replicated in different populations. More than 80% of the Mexican population is Mestizo, resulting from the admixture of any of the 62 ethnic groups living in Meso-America with Spaniards. To explore the hypothesis of different allele frequencies in T2D-associated genes in different geographic areas in Mexico, we analyzed SNPs in nine genes previously associated to the disease ($p<0.01$): sulfonylurea receptor (ABCC8), potassium inwardly-rectifying channel (KCNJ11), glucagon receptor (GCGR), peroxisome proliferator-activated receptor gamma (PPARG), hepatocyte nuclear factor 4A (HNF4A), solute carrier family 2, member 1 (SLC2A1), calpain-10 (CAPN10), glucokinase (GCK) and, transcription factor 7-Like 2 (TCF7L2). We included 1104 individuals from 6 states of Mexico: Guanajuato, Guerrero, Sonora, Veracruz, Zacatecas and Yucatan. Initial results include average of minor allele frequencies (MAF), as well as ranges between the lowest and highest frequencies by states:PPARG (rs1801282) G=0.12 (0.06-0.17);CAPN10 (rs297576) G=0.09(0.07-0.13);KCNJ11 (rs5219) T=0.39(0.36-0.44);HNF4A (rs2144908) A=0.49(0.43-0.58) and TCF7L2 (rs185384) A=0.19(0.15-0.26). We compared average MAFs from Mexican Mestizo population with that of other populations reported by the International HapMap Project. Interestingly, the KCNJ11 functional SNP showed a higher MAF in all states of Mexico (Zacatecas 0.45, Sonora 0.39, Veracruz 0.39, Guanajuato 0.38, Guerrero 0.37 and Yucatan 0.37) than that reported for Caucasians (0.34), Yorubans (0.08), Han Chinese (0.24) and Japanese (0.30). These results support the hypothesis that the Mestizo population has differences with other populations and between geographical regions within Mexico. This information will contribute to stratify populations in association studies and will increase our knowledge of genetic predisposition to T2D.

High resolution analysis of cytogenetic aberrations in hepatocellular carcinoma using oligo array CGH. *A. De Witte[1], L. Guo[2], J. Collins[1], Y. Dragan[2]* 1) Agilent Technologies, Inc., Santa Clara, CA; 2) National Center for Toxicological Research, FDA, 3900 NCTR Road, Jefferson, AR.

Hepatocellular carcinoma (HCC) is one of the most commonly occurring tumors in the world. The major etiologies of HCC are chronic hepatitis B virus (HBV) or hepatitis C (HCV) infection, exposure to aflatoxins, chronic liver disease and alcohol abuse. Chromosomal aberrations play an important role in liver cancer development and progression. In this study, we report the analysis of genomic changes in DNA samples collected from 11 HCC patients (aged 46-72; 10 male) comparing the neoplasms with the surrounding tissue from the same individual. Microarrays consisting of ~185,000 in situ synthesized 60-mer oligonucleotide probes optimized for array CGH (Comparative Genomic Hybridization) applications (Agilent Technologies) were used. We compare our results with published aberrations identified by lower resolution techniques such as conventional CGH based on the use of fluorescence in situ hybridization (FISH), spectral karyotyping (SKY) and BAC (bacterial artificial chromosomes) array-based CGH. We observed recurrent DNA copy number changes, including a characteristic 8p deletion and 8q amplification. We mapped several breakpoint regions associated with aberrations to the gene resolution level and we discovered novel small aberrations in several samples. Our results contribute to a more detailed description of genetic aberrations associated with HCC and demonstrate that oligo array CGH enables the mapping of breakpoints at a much higher resolution than traditional techniques.

Differential expression profiling of oral squamous cell carcinoma identifies the involvement of a major signaling pathway. *S. Chakraborty[1], S.M.A. Mohiyuddin[2], K.S. Gopinath[3], A. Kumar[1]* 1) MRDG, Indian Institute of Science, Bangalore, Karnataka, India; 2) RL Jalappa Hospital and Research Centre, Kolar, India; 3) Bangalore Institute of Oncology, Bangalore, India.

Oral squamous cell carcinoma (oral cancer) accounts for almost 40% of all cancers in India. Despite rapid advancement in therapeutic strategies, its low five-year survival period remains unchanged in the past two decades. Thus, finding a biological tumor marker to increase the early diagnosis and treatment monitoring rates is of paramount importance. To elucidate the molecular alterations underlying oral cancer, we employed the DDRT-PCR technique. Our DDRT-PCR profiling showed 51 fragments (cDNAs) that were differentially regulated. Of these, 27 fragments were confirmed as true differentials by reverse Northern analysis. Eight of the genes have been validated by RT-PCR in a panel of 16 matched normal and tumor patient samples and show potential as future therapeutic targets. One of the genes identified by DDRT-PCR has been shown to interact with another tumor suppressor gene which is an important regulator of a major signaling pathway. Investigation of the mechanism of downregulation of this tumor suppressor gene, which we observed in patient samples, led to the detection of loss of heterozygosity at the gene locus. Analysis of some of the key players of this pathway by RT-PCR revealed aberrant gene expression patterns. Hyperphoshorylation of some of the kinases in this pathway in oral tumors also indicated its constitutive activation. Probing the oral transcriptome by DDRT-PCR has identified some interesting genes not associated with oral cancers earlier. Our initial results seem to implicate the involvement of a major signaling pathway that may play a role in triggering the onset or progression of a subset of cancers of the oral cavity for the first time. The results will be presented and discussed (Financially supported by DBT and CSIR, New Delhi).

Karyotyping analysis in mentally retarded children and babies born with dismorphic features from Pediatric North-West India population. *B.S. Chavan, S. Mishra, A. Sehgal, G. Kaur* Genetic Centre,, Goverment Medical College and Hospital, Chandigarh, UT, India.

We have done karyotyping in 20 mentally retarded children, referred from mental retardation centre, GIMRC, Chandigarh. Five cases were diagnosed with trisomy 21, one child with mosaicism. Rest of the children were found having normal karyotyping, indicating other biochemical metabolic defects. Out of 30 cases with dismorphic feature referred by the Pediatric department we have reported ten cases of Downs syndrome, with mongoloid features muscular hypotonea, brachycephaly, protruding tongue, small low set ears upward sloping paplpebral fissures, single palmar crease, flat nasal bridge. It is noteworthy here that one case of Klinefelters syndrome with 47, XXY karyotype is also observed. The affected individual was 18 days old baby with the features of low set ears malformed right sided pinna, high arched palate, abnormal cry, wide spaced nipple, tuft of hair at sacral region, sandal gap in right foot, short neck and ambiguous genitalia and rest of the babies were observed to be with normal karyotype. In addition, we have done karyotype in 35 couples with recurrent spontaneous abortion and karyotype observed in these cases was normal, no gross translocation was observed.

Modeling the mutational effects on calcium binding to the epidermal growth factor (EGF) domain of fibrillin-1.

S-C. Chao[1,5], C-H. Tsai[2], Y-J. Lin[2], J-S. Chen[3], C-H. Lin[2], L. Chuang[4], J-M. Wu[2], S-J. Lin[2], H.S. Sun[1,3] 1) National Cheng Kung University, Institute of Molecular Medicine, Tainan, Taiwan; 2) Department of Pediatrics, National Cheng Kung University Hospital, Tainan, Taiwan; 3) Institute of Basic Medical Sciences, National Cheng Kung University Medical college, Tainan, Taiwan; 4) Department of Obstetrics and Gynecology, National Cheng Kung University Hospital, Tainan, Taiwan; 5) Department of Obstetrics and Gynecology, PingTung Christian Hospital, PingTung, Taiwan.

Fibrillin-1 gene (FBN1) encodes a key structural component of the elastin-associated microfibills of the extracellular matrix in connective tissues. Mutations in the FBN1 gene have been associated with a broad spectrum of disorders (i.e., the type I fibrillinopathies), including Marfan syndrome (MFS) and neonatal MFS (nMFS). Although previous studies of type I fibrillinopathies have shown remarkable clinical heterogeneity of the diseases, the underlying mechanism that causing the heterogeneity remains largely unknown. We have identified a novel heterozygous mutation in the exon25 of the FBN1 gene in a female newborn nMFS case. The G3208C (D1070H) mutation is located on the highly conserved calcium binding epidermal growth factor like domain 12 (cbEGF12). Because the base substitution is predicted to impair calcium binding onto this region, we further modeled the 3D structure of altered proteins to illustrate the conformational effect of this amino-acid substitution. Results of molecular modeling support the hypothesis that the base substitution affect calcium binding onto the cbEGF12 domain and severely damage the fibrillin-1 function. Our data suggest an important aspect to dissect the complicated correlation of genotype-phenotype link in type I fibrillinopathies as well as other phenotypically diverse disorders.

DFNB21 locus, the second cause of autosomal recessive non- syndromic hearing loss in Iranian population. *N. Bazazzadegan[1], N. Meyer[4], K. Kahrizi[1], M. Mohseni[1], P. Imani[1], S. Arzhangi[1], B. Azadeh[3], A. Daneshi[2], M. Farhadi[2], R.J.H. Smith[4], H. Najmabadi[1]* 1) Genetics Research Centre, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran; 2) Research Centre of Ear, Nose, Throat, and Head and Neck Surgery, Iran university of Medical sciences, Tehran, Iran; 3) Isfahan province welfare consultation centre, Isfahan, Iran; 4) Molecular Otolaryngology Research Laboratories, Department of Otolaryngology Head and Neck Surgery, University of Iowa, IA, United States.

Background: Genetic factors are thought to account for approximately one half of cases of childhood hearing loss, the majority of which is non-syndromic and not associated with other abnormalities. The autosomal recessive non-syndromic hearing loss is associated with mutations in a majority of DFNB loci. Mutations in the gene encoding Alpha Tectorin (TECTA) are associated with both dominant and recessive modes of inherited hearing loss. Mutations in this gene previously have been reported in one family in Lebanese population therefore the objective of our study was to determine the prevalence of this gene in our population. Materials and methods:Eighty five families with autosomal recessive non-syndromic hearing loss with two or more affected children originating from different ethnic groups of Iranian population that were negative for GJB2 and GJB6 mutations, that are located on the most prevalent locus (DFNB1) of hearing loss, were screened for DFNB21 locus by linkage analysis . We used D11S1998, D11S4464, and D11S1299 STR markers for this study. Results: Four out of eighty four families were linked to this locus. Two novel mutations have been detected so far. In two families a 266 Del T mutation and a large 9611bp deletion that starts from intron 9 which includes exon 10 in TECTA gene were detected. Mutation detection of the other two families is performing. Conclusion: We concluded after DFNB1, Tecta gene mutations are responsible for the most prevalent cause of autosomal recessive non- syndromic hearing loss in Iranian population.

Investigation of four MCPH loci (MCPH1, MCPH2, MCPH3, MCPH5) based on Homozygosity mapping in 30 families. *S. Esmaeeli Nieh[1], S.S. Abedini[1], K. Kahrizi[1], F. Behjati[1], S. Ghasemi Firoozabadi[1], V. Hadavi[2], R. Vazifehmand[1], D. Habibi[1], M. Falah[1], A. W. Kuss[3], S. Banihashemi[1], H.H. Ropers[3], H. Najmabadi[1, 2]* 1) Genetics Research Center, University of Social Welfare & Rehabilitation sciences,Tehran, Iran; 2) Kariminejad and Najmabadi Pathology and Genetics Center, Tehran, Iran; 3) Max Planck Institute for Molecular Genetics, Berlin, Germany.

Microcephaly is defined as a reduction in head circumference and this clinical finding infers that an individual has a significant diminution in brain volume. Microcephaly can be usefully divided into primary microcephaly, in which the brain fails to grow to the correct size during pregnancy, and secondary microcephaly, in which the brain has the expected size at birth but subsequently fails to grow normally. Seven out of ten Non-Syndromic Autosomal Recessive Mental Retardation (NS-ARMR) loci are associated with microcephaly, including MCPH1-MCPH6 which belong to the family of MCPH (autosomal recessive primary microcephaly),and ARFGEF2. Based on MCPH heterogeneity studies in Pakistani and Indian population MCPH5, MCPH2, MCPH3, MCPH1 are more common MCPH loci. The objective of this study was to investigate four more common MCPH loci in 30 Iranian families. Each family was subjected to complete clinical examinations and karyotype abnormalities. We performed Homozygosity mapping by using STRs (Short Tandem Repeats) markers. In MCPH5 linked families, we amplified entire the ASPM gene In order to determine the mutations in this gene by using DNA-sequencing method. Among 30 families analyzed, 3 families were linked to MCPH5 locus,3/30 (10%), two families had genotype data which were consistent with linkage to the MCPH2 locus, 2/30 (6/7%), the remaining 25 families were not linked to these four loci, 25/30 (83/3%). So far, the results of DNA-sequence analysis of 1 families which were linked to MCPH5 locus, revealed a previously unreported mutation, C>T change at nucleotide position 3055 (c.3055C>T) in exon11 in a homozygous state resulting a mutation at codon position 1019 (R1019X). This novel mutation was associated with novel phenotype.

Evolutionary structural dynamics of macaque chromosome 6 neocentromere. *F. Antonacci[1], N. Archidiacono[1], A. Cellamare[1], P. DAddabbo[1], E.E. Eichler[2], M. Rocchi[1], M. Ventura[1]* 1) Department of Genetics and Microbiology, University of Bari, 70126 Bari, Italy; 2) Department of Genome Sciences, University of Washington School of Medicine, Seattle, Washington 98195, USA.

Evolutionary centromere repositioning is the ectopic seeding of a centromere in a new location along the chromosome coupled with the inactivation of the old centromere. The new centromere rapidly evolves the complexity that characterizes a normal centromere, by recruiting satellite DNA and by generating/recruiting pericentromeric segmental duplications. The dynamics of this rapid evolution is obscure. Our previous studies on the evolutionary history of human chromosome 6 disclosed that both macaque (Macaca mulatta, MMU) and human chromosome 6 centromeres were the result of centromere repositioning events that independently occurred in the Old World monkey ancestor and Hominoidea ancestor, respectively. Here we report on the assembly of ready-to-sequence BAC contigs spanning the centromeric transition of macaque chromosome 6. BAC-end sequencing and FISH analysis of these BACs provided a unique opportunity to compare the organization of the MMU6 evolutionary neocentromere with the fully-sequenced orthologous human region, at 6q24, representing the ancestral organization of the region before centromere seeding. The comparison revealed that the region involved in neocentromere reorganization was relatively small (~250 Kb), and that this short DNA segment was duplicated on both sides of the MMU centromere. These sequences were pushed apart to accommodate a large block of alpha satellite DNA, and the pericentromeric sequences were enriched with stretches of alphoid sequences. The region, in humans, appears to be a gene-desert, and no sequences from other pericentromeric regions were involved in the reshuffling.

The HUGO Gene Nomenclature Committee. *E.A. Bruford[1], M.W. Wright[1], K.M.B. Sneddon[1], T.P. Sneddon[1], M.J. Lush[1], V.K. Khodiyar[1], R.C. Lovering[1], C.C. Talbot Jr.[2], S. Povey[1]* 1) HUGO Gene Nomenclature Committee, University College London, London NW1 2HE, United Kingdom; 2) Johns Hopkins School of Medicine, Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD 21205-2196, USA.

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 nomenclature for all genes in the human genome. Nomenclature is assigned primarily to protein-coding genes, pseudogenes and non-protein-coding (npc) RNAs, and to phenotypes and genomic features based on community requests; a recent area of interest has been the naming of variable copy number genes and npcRNAs. Approved gene symbols are based on names describing structure, function or homology wherever possible, and are stored in a searchable public database (www.gene.ucl.ac.uk/nomenclature) containing manually curated data and links to other databases. Researchers are encouraged to contact the HGNC (nome@galton.ucl.ac.uk) to confirm and request approved nomenclature for specific genes and gene groupings, and a panel of around 100 specialist advisors advise on the nomenclature of over 70 specific gene families. The HGNC have 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. A recent addition to the HGNC website is the HGNC Comparison of Orthology Predictions (HCOP) search tool which allows users to compare the orthology assertions between human, mouse, rat and chicken genes as predicted by various orthology resources. The HGNC has a strong working relationship with other database curation groups, and also actively add Gene Ontology (GO) annotation terms to the GO database. Many databases prominently display HGNC nomenclature; entering HGNC approved symbols into a search tool will 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.

A multiple testing correction method for genetic association studies using correlated single nucleotide polyphisms. *X. Gao* Dept Statistics, North Carolina State Univ, Raleigh, NC.

Multiple testing is a challenging issue in genetic association studies using large numbers of single nucleotide polymorphism (SNP) markers, many of which exhibit linkage disequilibrium (LD). Failure to adjust for multiple testing appropriately may produce excess false positives or overlook true positive signals. The Sidak and Bonferroni methods of adjusting for multiple comparisons are easy to compute, but are well known to be conservative in the presence of LD. On the other hand, permutation-based correction can correctly account for LD among SNPs, but is computationally intensive. Recent attempts to quickly and accurately adjust for multiple testing have lead to advances in both areas. However, given information about the degree of LD, these methods remain unnecessarily conservative or difficult to compute. In this work, we propose a new multiple testing correction method, $CLDM_{eff}$, for association studies using SNP markers. It is shown to be simpler and more accurate than the recently developed methods and is comparable to the permutation-based correction using both simulated and real data. The efficiency and accuracy of the $CLDM_{eff}$ method makes it an attractive choice for multiple testing adjustment when there is high intermarker LD in the SNP dataset.

Quantifying, testing and correcting for population stratification in association studies: a unified approach. *P. Gorroochurn[1], G.A. Heiman[2], S.E. Hodge[1,3], D.A. Greenberg[1,3]* 1) Division of Statistical Genetics, Department of Biostatistics, Columbia University, New York, NY; 2) Dept of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY; 3) Clinical-Genetic Epidemiology Unit, New York State Psychiatric Institute, New York, NY.

The HapMap project has given association studies a tremendous opportunity to uncover the genetic basis of complex diseases. However, persistent issues in such studies remain the proper quantification of, testing of, and correction for population stratification (PS). In this paper, we propose a resolution of all these issues through a single quantity (). Previously, we defined a quantity , which can be estimated in a case-control study by using suitable neutral loci from the controls, and showed how this estimated value can be used to quantify and correct for PS. We now present a novel method to test for PS, based on the estimated value of . A major novel feature of our testing procedure is its ability to test for either (i) strictly any PS (i.e., H_0: =0), or (ii) only PS that is practically important (i.e., H_0: $=_0$, where $_0$ is the minimum value of interest to the investigator). We performed simulations to compare our correction procedure with genomic control (GC). Our simulations show that, when =.05, GC becomes over-conservative when 1.0 (rejecting at an average rate of only 2.5%), and loses considerable power when 2.0. In contrast, our correction procedure maintains good Type I error rates and power for all values of , i.e. across all levels of PS.

Milroy disease: Prenatal diagnosis and phenotypic variability within a family. *T.A. Burrow, M.J. Walker, R.J. Hopkin* Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Milroy disease, congenital hereditary lymphedema, frequently results from mutations in *VEGFR3*, with consequential abnormal embryonic development and function of the lymphatic system. Congenital lower extremity lymphedema is the most common presentation. Severity is usually mild to moderate; however, it is quite variable and more severe manifestations have been reported. We describe a two-generation family including four individuals with Milroy disease who exhibit a very broad spectrum of phenotypic features.

The proposita is a 26-year-old Gavida 4, Para 4 Caucasian whose congenital lower extremity lymphedema resolved spontaneously by one year of age with no further symptoms. Three of her four children were also affected. Her first child was prenatally diagnosed with lower extremity lymphedema and a cystic hygroma; the latter resolved prior to delivery. At birth, he had a webbed neck, mild dysmorphic features, and lower extremity lymphedema. The lymphedema has improved, but still persists. At eight years of age, he was diagnosed with osteosarcoma of the right leg. Her second pregnancy resulted in the neonatal death of a male infant with lymphedema, IUGR, and anhydramnios due to ureteral obstruction, which were diagnosed prenatally and at autopsy. In her third pregnancy, a female was prenatally diagnosed with lymphedema, cystic hygroma, and pericardial and pleural effusions. At birth, a webbed neck and mild pedal edema were observed. The child experienced significant pulmonary disease due to chronic pleural effusions and pulmonary lymphangiectasia, which gradually improved over time. In addition, she exhibited failure to thrive, which improved with intervention.

This family illustrates the broad degree of phenotypic variability and serious complications that can be observed in Milroy disease. Fetal ultrasonography is useful in prenatal diagnosis of Milroy disease in at-risk families, and in this family, reveals the dynamic nature of the disorder between the prenatal and postnatal periods.

Simple Correction for Population Stratification Using a Novel Confounder Score. *M.P. Epstein[1], A.S. Allen[2], G.A. Satten[3]* 1) Dept Human Genetics, Emory Univ, Atlanta, GA; 2) Dept of Biostatistics & Bioinformatics, Duke Univ, Durham, NC; 3) Centers for Disease Control and Prevention, Atlanta, GA.

Case-control studies of disease-gene association must often correct for potential bias resulting from the effects of population stratification. We propose a novel correction based loosely on the confounder-score approach of Miettinen (1976). Using data from all subjects, our approach first models the probability of disease as a function of genotypes at a number of null loci using either logistic regression or, if the number of null loci is large (which may be the case in whole-genome association analyses), a high-dimensional procedure such as partial-least squares. We next use the predicted probability of disease given the null-loci genotypes to assign subjects to different strata and subsequently use the stratum assignment as a covariate in a logistic-regression model to assess association between a locus of interest and disease. The resulting association between a genotype at a locus of interest and disease is valid even if population stratification exists in the sample. We feel the confounder-score approach is computationally simple and has the ability to use an enormous number of (correlated) null loci to infer substructure. Additionally, the approach makes fewer modeling assumptions than existing approaches and can easily be extended to haplotype or other kinds of multi-locus association analyses. We will illustrate our approach using both real and simulated data.

A MULTICENTER, OPEN-LABEL STUDY TO EVALUATE THE SAFETY AND RESPONSE TO AN 8-DAY COURSE OF SAPROPTERIN DIHYDROCHLORIDE(TETRAHYDROBIOPTERIN or 6R-BH4) IN SUBJECTS WITH PHENYLKETONURIA (PKU) WHO HAVE ELEVATED PHENYLALANINE LELELS.

B.K. Burton[1], D. Grange[2], A. Milanowski[3], J. Vockley[4], V. Abadie[5], C. Harding[6], E. Crombez[7], D. Dobbelaere[8], A. Smith[9], A. Dorenbaum[10] 1) Children's Memorial Hospital, Northwestern University Feinberg School of Medicine, Chicago, IL; 2) St. Louis Children's Hospital, Washington University School of Medicine, St. Louis, MO; 3) Instytut Matki i Dziecka, Warszawa, Poland; 4) Children's Hospital of Pittsburgh, Pittsburgh, PA; 5) Hôpital Necker-Enfantes Malades Pediatrie Generale, Paris, France; 6) Oregon Health & Science University, Portland, OR; 7) UCLA Medical Center, Los Angeles, CA; 8) CHRU de Lille - Hôpital Jeanne de Flandres, Lille, France; 9) Pacific Data Designs, San Francisco, CA; 10) BioMarin Pharmaceutical, Inc., Novato, CA.

Sapropterin can lower phenylalanine (Phe) levels in patients with PKU and may provide a novel approach to treatment. To identify patients for enrollment in a Phase 3 study, we conducted a multicenter, open-label trial and evaluated the safety and response to an 8-day course of sapropterin. A total of 490 subjects were enrolled with minimum blood Phe levels of 450uM. 485 subjects completed the 8-day (1 day) course of sapropterin 10mg/kg/day and had blood Phe level results for baseline and day 8. The percent of subjects who experienced a 30% Phe drop from baseline on day 8 was 54.4% (31/57) in subjects with baseline Phe 600uM, 23.6% (37/157) in subjects with baseline Phe 600 to 900 uM, 9.6% (13/135) in subjects with baseline 900 to 1200uM, and 7.4% (10/136) in subjects with baseline Phe 1200uM. Among responders, mean(SD) blood Phe levels were 784.0 (294.8)uM on day 1 and 390.6 (207.9)uM on day 8. The mean (SD) change in blood Phe levels was -393.4 (188.3)uM. The majority of adverse events were mild and all events resolved without complications. None of the events were assessed as clinically significant. Sapropterin was well tolerated and effective in rapidly reducing Phe levels in PKU patients across the entire spectrum of PKU phenotypes. These results are consistent with prior data on PKU response to 6R-BH4.

Identification of novel genes contributing to the severity of phenotype in chromosome 17 rearrangements. *S. Girirajan[1], S.R. Williams[1], J. Garbern[3], D.J. Bunyan[4], E. Hatchwell[5], S.H. Elsea[1, 2]* 1) Department of Human Genetics; 2) Department of Pediatrics, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA; 3) Departments of Neurology and Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI; 4) Wessex Regional Genetics Laboratory, Salisbury District Hospital, Wiltshire, Salisbury, U.K; 5) Cold Spring Harbor Laboratory, Genome Research Center, Woodbury, NY.

Rearrangements due to homologous and non-homologous mechanisms involving region-specific low copy repeats or segmental duplications cause deletions/duplications in disease-associated genomic regions. While a number of clinical cases of multiple congenital anomalies/mental retardation syndromes can be attributed to deletions and duplications pertaining to a single chromosomal region, sometimes the variability and severity of the phenotype warrants further molecular studies. To delineate the chromosomal extent of the deletion/duplication and also to identify other possible genomic rearrangements that contribute to the severity of phenotype, whole genome array-comparative genomic hybridization (aCGH) was performed on a cohort of previously diagnosed cases of 17p11.2 rearrangements. The results obtained were confirmed by fluorescent *in situ* hybridization (FISH) using locus-specific probes, while some breakpoints were confirmed at a higher resolution by multiplex ligation-dependent probe amplification (MLPA). Two genes, amiloride sensitive cation channel 1 (*ACCN1*) and glutamate receptor, ionotropic, kainate 3 (*GRIK3*) were shown to have de novo copy number changes in two patients with rearrangements on chromosome 17, respectively. *ACCN1* has been implicated in retinal degeneration in mouse models. Further, our patient with deletion breakpoints encompassing *ACCN1* has significant retinal changes suggesting a causative role for this gene in retinal pathology in this patient. The severity and complexity of the phenotype in these individuals prompted these studies and indicate the importance of considering aCGH to identify genes in other genomic regions that may contribute to the phenotypic variability and severity in known chromosomal disorders.

An association study of ninety-three candidate genes in bipolar disorder. *D. Absher[1], J. Li[1], R.C. Thompson[2], M. Burmeister[2], L.J. Scott[2], Y. Li[2], F. Meng[2], W. Guan[2], M.P. Vawter[4], P. Choudary[3], H. Tomita[4], S.J. Evans[2], W.E. Bunney[4], E.G. Jones[3], J.D. Barchas[5], A. Schatzberg[1], H. Akil[2], S.J. Watson[2], M. Boehnke[2], R.M. Myers[1]* 1) Stanford Human Genome Center, Palo Alto, CA; 2) University of Michigan, Ann Arbor, MI; 3) University of California Davis, Davis, CA; 4) University of California Irvine, Irvine, CA; 5) Cornell University, New York, NY.

Bipolar disorder is a psychiatric disease with a prevalence of ~1% in all human populations. It displays strong familial aggregation and is thought to result from a complex genetic etiology involving multiple genes. While linkage studies have identified some loci of interest, few of these have been confirmed in subsequent studies. We have undertaken a candidate gene approach that involves genotyping 476 bipolar cases and 470 controls for 1,536 SNPs located in 93 genes. The bipolar cases are from the NIMH Human Genetics Initiatives collection and the controls are ethnically matched NIMH control samples that have completed a psychiatric screen. We selected genes for this study based on their association with bipolar disease in previous studies, as well as their aberrant expression in our microarray experiments with human brain mRNA, and their implication in animal models with similar phenotypes. For each of the 93 genes, we selected SNPs from HapMap II to cover all LD bins (r2 > 0.8) containing SNPs with a MAF >= 5%. All genotyping is being performed with Illuminas GoldenGate platform. We will present our preliminary findings.

Autosomal dominant Vocal Cord and Pharyngeal Weakness with Distal Myopathy (VCPDM, MPD2, 5q31): Refinement of the candidate interval and identification of a second VCPDM family. *S.M. Garvey[1], J. Senderek[2], I. Tournev[3], C. Stendel[2], A. Urtizberea[4], V. Guergueltcheva[3], V. Mihailova[3], H. Feit[5], J. Weis[2], H. Lochmüller[6], J.S. Beckmann[7], E. Seboun[8], M.A. Hauser[1], C.E. Jackson[9]* 1) Center for Human Genetics, Duke University Medical Center, Durham, North Carolina, USA; 2) Aachen University of Technology, Aachen, Germany; 3) Department of Neurology, Sofia Medical University, Sofia, Bulgaria; 4) AFM et Généthon, Evry, France; 5) Department of Neurology, Henry Ford Hospital, Detroit, Michigan, USA; 6) Department of Neurology, Friedrich-Baur-Institute, Ludwig-Maximilians-University, Munich, Germany; 7) Department of Medical Genetics, University of Lausanne and Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; 8) Division de Génétique et de Microbiologie, Université Pierre et Marie Curie, Paris, France; 9) Department of Medicine, Scott & White Memorial Hospital, Temple, Texas, USA.

Vocal cord and pharyngeal weakness with distal myopathy (VCPDM) is a late-onset, progressive, autosomal dominant neuromuscular condition. VCPDM had been mapped to an 11.8 Mb interval on chromosome 5q31 in a large family from the US. We were able to identify two newly affected patients within the original pedigree. Linkage analysis using densely spaced STR markers from the candidate interval disclosed a critical recombination event in both patients. This reduced the critical region to 5.3 Mb between newly established STR markers AC108764 and AC089765. This interval still contained 62 genes. The most promising candidate gene was *myotilin* (*MYOT*) as *MYOT* mutations cause a similarly progressive and adult-onset muscle disease. However, defects in *myotilin* were widely excluded by various experimental approaches. We studied additional families with a distal myopathy and with or without accompanying vocal cord paralysis by linkage analysis, identifying a large Bulgarian family that also showed linkage to the VCPDM region. The families do not share a common disease haplotype indicating that they probably carry different mutations in the so far unknown VCPDM gene. Mutation screening of candidate genes is currently underway.

Polymorphisms in the integrin super gene family associated with obesity and type 2 diabetes in Japanese American and Japanese population. *T. Awaya[1], Y. Yokosaki[1], F. Higashikawa[1], K. Yamane[2], N. Kohno[2], A. Eboshida[1]* 1) Department of Public Health, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan; 2) Department of Molecular and Internal Medicine, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan.

Integrins are heterodimeric cell surface glycoproteins that mediate cell-cell and cell-matrix adhesion. Although several polyphorphisms in the integrin gene family are reported to be associated with cardiovascular risks, involvement of genetic variation in this family in obesity or type 2 diabetes is undetermined. In the present study we analyzed 9 SNPs in the integrin gene family, L(Thr402Arg), M(Thr400Met), V(Ile405Val), 2(C804T), 4(Gln844Arg), 9(Gln30Glu), 1(A1176C), 2(C1323T) and 3(Leu33Pro), for obesity and type 2 diabetes. Genomic DNA was isolated from 651 peripheral blood samples, 274 Japanese American in Hawaii(68.713.4 y.o.) and 377 Japanese in Hiroshima(55.312.2 y.o.), and subjected to PCR-RFLP or TaqMan analysis for the polymorphisms. In these 2 populations, where genetic back grounds were homogeneous, it has been reported that Japanese Americans were highly exposed to an American lifestyle, a relatively high fat and simple carbohydrate diet with low physical activity compared to native Japanese. Obesity was defined as body mass index 25 kg/m2 and type 2 diabetes according to WHO criteria, and association was analyzed by logistic analysis adjusted for age and sex. There was no difference in allele frequencies among the 9 SNPs between the 2 populations. We found that 1) C1323T in 2 subunit was associated with obesity in Japanese American population (OR 3.00, 95%CI=1.2 to 7.7, p=0.03, TT vs. CT, CC) but not in Japanese population and 2) Gln844Arg in 4 subunit with type 2 diabetes in Japanese population (OR 5.56, 95%CI=1.1 to 23.3, p=0.04, Gln/Gln vs. Arg/Gln, Arg/Arg) but not in American Japanese population. These results suggest that C1323T polymorphism in 2 subunit appears to be associated with obesity that mainly affected by environmental factors, whereas Gln844Arg in 4 subunit might reflects susceptibility of type 2 diabetes that is genetically regulated and environmentally insensitive.

Under- recognized early manifestation of Prader Willi syndrome. *Y.S. Choy[1], L.H. Ngu[1], W.T. Keng[1], S.K. Tan[3], Ruziana[2], L.C. Bok[3], M. Aminah[2], M.Z. Nirzila[4]* 1) Genetics and Metabolism, Kuala Lumpur Hospital, Kuala Lumpur, Malaysia; 2) Cytogenetic Unit, Kuala Lumpur Hospital; 3) Division of Molecular Pathology Institute of Medical Research, Malaysia; 4) Respiraotry Unit, Pediatric Institute, Kuala Lumpur Hospital.

The well recognized presentation of Prader Willi syndrome (PWS)is characterized by infantile hypotonia , inactivity,failure to thrive and developmental delay followed by childhood obesity. Mortality and morbidity in older individuals were associated with cardio-respiratory insufficiency, diabetes mellitus and obstructive sleep apnoea. The availability of molecular test had enabled us to make early diagnosis in these individuals. In our cohort of 75 patients with PWS, 46 of them (61%) were diagnosed in early infancy and 15 of them (20%) were diagnosed in neonatal period as early as 2nd day. In those diagnosed early, 28 (37%) had recurrent admissions for acute life threatening events (ALTE) in infancy. ALTE was found to be significantly associated with aspiration pneumonia($p<0.001$), sleep disordered breathing($p<0.001$), dilated cardiomyopathy ($p<0.001$)and pericardial effusion ($p<0.01$). Sixteen of them required nasogastric feeding but 10 of them still had respiratory insufficiency,pH study proved significant gastroesophageal reflux disease in them. Six had dilated cardiomyopathy and 3 succumbed. Four had recurrent pericardial or pleural effusion associated with hypothyroidism. Seven of them had proven sleep disordered breathing, which was significantly associated with dilated cardiomyopathy and ALTE. If left untreated it has poor prognostic outcome but home oxygen therapy and vigorous airway management resulted in general clinical improvement. In the second group diagnosed after infancy, they had less feeding problems and none had pneumonia. They presented with neurodevelopmental delay associated with short stature and obesity. Stringent dietary control and vigorous rehabilitative program significantly affected their final outcome. In conclusion, PWS can be usefully classified into 2 major clinical types for management purposes, the severe infantile and the milder childhood type, both having all molecular classes of PWS.

Powerful and efficient whole genome haplotypic analysis using Beagle. *B.L. Browning, S.R. Browning* The University of Auckland, New Zealand.

We show that our variable-length Markov chain (VLMC) haplotypic analysis method [1] can be implemented efficiently to perform haplotypic analysis of whole genome association studies (hundreds of thousands of markers and thousands of samples), and we show that the VLMC method gives increased power to detect low frequency risk-conferring variants relative to single marker tests.

The VLMC model has several advantages over standard haplotypic analysis: there is no need to identify haplotype blocks or choose a haplotype window size, and the model automatically adapts to correlated markers, yielding good power regardless of whether tagging markers are used. The VLMC model automatically clusters similar haplotypes to balance the number of haplotypic tests and information extraction.

Using simulated data that closely approximate empirical data, we show that the VLMC haplotype clusters are more highly correlated than single markers with low frequency disease alleles. Combining single marker and haplotypic tests gave markedly increased power to detect low frequency risk-conferring variants while maintaining power to detect high frequency risk-conferring variants.

We have implemented the VLMC algorithm in a new software package called Beagle. Running time for Beagle is linear in the number of markers and between linear and quadratic in the number of samples. Haplotypic analysis of a phased, real data set of 6600 markers covering 40 Mb genotyped on 1750 samples completed in 3.5 minutes using a single processor. Thus, after phasing data, single marker and haplotypic analysis on 500K markers and 4000 samples can be performed on a single processor in less than 24 hours. Beagle is easy-to-use and can perform allelic and genotypic tests for both single-markers and haplotypes. Beagle can also perform permutation testing to identify markers or haplotype clusters that reach region-wide or genome-wide significance.

[1] Browning SR (2006) Multilocus Association Mapping Using Variable-Length Markov Chains. Am J Hum Genet 78:903-913.

POLAR BODY VS. BLASTOMERE BIOPSY FOR PGD. PB OR NOT PB? *G. Altarescu[1], T. Eldar-Geva[1], B. Brooks[1], Y. Kaplan[1], M. Patt[1], E.J. Margalioth[1], A. Lahad[2], E. Levy-Lahad[1], P. Renbaum[1]* 1) Zohar PGD Lab, and IVF unit, Shaare Zedek Medica Center, Jerusalem, Israel; 2) Department of Family Medicine, Hebrew University, Jerusalem, Israel.

PGD may be performed by biopsy of polar bodies(PB) 1 and 2,or blastomeres from 6-8 cell embryos. PB biopsy allows PGD analysis without removing embryonal cells, and for inconclusive cases, a repeat analysis using a blastomere can be performed. Blastomere-PGD allows direct analysis of both maternal and paternal alleles, but is purportedly associated with higher rates of allele drop-out (ADO). We compared the efficiency of biopsy, molecular diagnosis, ADO, and pregnancy rate (PR) for PB vs. blastomere based PGD. Cases of maternal autosomal dominant (AD), X-linked, and autosomal recessive diseases underwent PB-PGD. Blastomere PGD was done for paternal AD diseases, and when an insufficient number of wild-type oocytes were diagnosed by PB analysis. Samples without results for at least one polymorphic marker on either side of the mutation in addition to the mutation itself (or another internal marker) were considered inconclusive. We analyzed 78 cycles of PGD (901 total biopsies) in 48 couples both by intention to treat and actual treatment.35% of cycles were PB only,50% were blastomere only, and 15% had combined PB and blastomere testing. Biopsy success rates were similar for both PB and blastomeres (95%). Successful molecular diagnosis was significantly higher for PB-PGD(88%), vs blastomere-PGD(76%,p=0.02). ADO rate in 947 reactions was significantly lower in PB (5%) vs. blastomeres (16%,p=0.001). Both by interntion to treat and by actual treatment, there was a trend towards higher PR per embryo transfer in PB-PGD (40%) vs blastomere-PGD (23%). The number of embryos transferred was significantly associated with PR (OR 2.4 per additional embryo, p=0.04),and significantly more embryos were available for transfer in PB-PGD vs. blastomere-PGD(p=0.02). PB-PGD leads to a lower ADO rate and a significantly higher diagnosis rate, compared to blastomere-PGD. Pregnancy rate was higher with PB-PGD, possibly because PB-PGD yielded a significantly higher number of embryos available for transfer.

A 2p12 locus with two co-regulated genes is associated to dyslexia in Finnish and German populations. *H. Anthoni[1], M. Zucchelli[1], H. Matsson[1], J. Schumacher[2], J. Nopola-Hemmi[3], H. Lyytinen[4], G. Schulte-Körne[5], J. Kere[1,6], M.M. Nöthen[7], M. Peyrard-Janvid[1]* 1) Dept of Biosciences and Nutrition, Karolinska Institutet, Huddinge, Sweden; 2) Institute of Human Genetics, University of Bonn, Germany; 3) Dept of Pediatrics, Jorvi Hospital, Finland; 4) Dept of Psychology and Child Research Center, University of Jyväskylä, Finland; 5) Dept of Child and Adolescent Psychiatry and Psychotherapy, University of Marburg, Germany; 6) Dept of Medical Genetics, University of Helsinki, Biomedicum, Finland; 7) Dept of Genomics, Life & Brain Center, University of Bonn, Germany.

Developmental dyslexia is a common, complex disorder characterized by an unexpected difficulty in learning to read despite normal intelligence, senses and education. Genetic loci have been mapped to at least eight different chromosomes, and four candidate genes have been proposed: *DYX1C1*, *DCDC2*, *KIAA0319* and *ROBO1*. We refined our reported 12 Mb dyslexia locus on 2p12 in 19 Finnish kindreds. TDT analysis gave significant p-values for individual markers (p=0.003) and for a four-marker haplotype (p=0.004). The results were replicated on 251 families from Germany, and an overlapping haplotype was identified (p=0.02). To further focus on the 157 kb susceptibility region, marker density was increased to one SNP every 8 kb. Two significant (p=0.005 and p=0.001) overlapping risk haplotypes covering 16 kb were identified in both populations separately and in a joint analysis. Stratification for dyslexia severity on the German sample set increased the OR for the most significant haplotype from 2.2 (p=0.006) to 5.2 (p=0.00005). We characterized thoroughly the three genes in the region, including quantification of gene expression in different parts of brain. Two neighboring genes flanking the associated haplotype were highly co-expressed in fetal and adult brains and in nine separate brain regions. Mutation screening of these two genes in the Finnish families revealed 11 coding changes, with five previously unknown and eight nonsynonymous. We conclude that the two co-regulated transcripts flanking a highly dyslexia-associated haplotype block are excellent candidates for novel dyslexia genes near the *DYX3* locus.

Glutathione-S-Transferase M1, T1, and P1 polymorphisms in childhood asthma from the INMA study: assessing the effects of passive smoking on allergy and wheeze. *F. Castro-Giner[1], M. Àlvarez[1], M. Torrent[2], J. Sunyer[1], X. Estivill[3], R. de Cid[3] on behalf of the INMA Study Group* 1) Centre for Research in Environmental Epidemiology, Municipal Institute of Medical Research, Barcelona, Spain; 2) Area de Salud de Menorca, INSALUD, Menorca, Spain; 3) Gene and Disease Program. Barcelona Node -Spanish Genotyping Center (CeGen), Center for Genomic Regulation (CRG) and University Pompeu Fabra (UPF), Barcelona, Catalonia, Spain.

Asthma is a complex multifactorial disease with a well-defined genetic predisposition and involvement of environmental factors. The glutathione S-transferases (GSTs) are a family of enzymes that protect against oxidative stress by detoxifying various toxic substrates. Several authors suggest a role in asthma pathogenesis for the *GSTT1*, *GSTM1* and *GSTP1* polymorphisms related to modulation of reactive oxygen species (ROS). *GSTM1*, *GSTT1*, and *GSTP1*, have several well-defined genetic polymorphisms named GSTM1-0 and GSTT1-0 in the *GSTM1* and *GSTT1*, respectively, that are null alleles and result in nonfunctional protein, and a common coding polymorphism (105Ile/Val substitution) in the *GSTP1* which has been shown to result in altered catalytic activity. In the present study, GSTT1, GSTM1 and GSTP1 polymorphism are examined related to wheeze, allergy and EST in a children cohort from the Spanish INMA study (Childhood and Environment; http://www.infanciaymedioambiente.org/)(n=373;49%male). We have implemented two semiautomated assays to facilitate the detection of null alleles from *GSTM1*, and *GSTT1*, and the coding variant Ile150Val from the *GSTP1*. Detection of (1) the GSTM1 and GSTT1 null alleles was performed with a modified method from that initially described elsewhere, using a multiplex reaction with a fluorescent-labeled primers assay using the Automated DNA Analyzer ABI XL3100, and (2) analysis of Ile150Val variant in the *GSTP1* with an implemented assay using the Pyrosequencing technology. Frequency of the null alleles were similar to those described in other European populations; 64% for GSTT1-0, 20% for GSTM1-0. Double null genotypes were present in 12% of the individuals analyzed. This work is supported by the FIS:PI04/1705 and Genome Spain.

Induce Mitochondrial mediated apoptosis pathway in Breast cancer tumoral cells lines. *A. Ebrahimi[1,2], M. Houshmand[1], S. Zeinali[2], M. Karimpoor[2], H. Galehdari[3], M. Rostami[1], F. Talebzadeh[1]* 1) Medical genetics, NRCGEB,Tehran, Iran; 2) Biotechnology,Pasture Institue,Tehran, Iran; 3) Genetics Dep. ,Science Faculty, Chamran University, Ahwaz,Iran.

Modulation of apoptosis can be used to treat cancer.The power of apoptosis could be used to alleviate many human diseases. This project focuses on the potential of using apoptosis to treat cancer. Cancer is very common in the developed world such that 1 in 3 people will develop cancer at some point during their lives.apoptosis is a conserved process and so once an effective treatment has been discovered for a particular cancer type, it should be much easier to modify that treatment for other cancer types. Examination of the pathways leading to apoptosis shows many possible points where intervention may be possible.To maintain homeostasis in the human body, an estimated 10 billion cells are made each day just to balance those dying by apoptosis!Death triggering stimuli can take the form of 1)Lack of survival signals e.g. cytokines, growth factors, hormones or 2)Positive inducers of apoptosis (death signals) e.g. receptor ligation, DNA damage (via p53), toxic agents.Different cells exhibit different thresholds to various pro-apoptotic stimuli. For example splenic lymphocytes readily undergo apoptosis when exposed to ionising radiation whereas myocytes are resistant to apoptosis on similar exposure.Two main integration pathways exist and converge to a common execution phase:Death receptor pathway and Mitochondrial pathway.We have designed a double stranded (ds)RNA and The aim of our investigations is to dissect the molecular machinery which mediates gene silencing by antisense RNA and RNAi to trigger mitochondrial mediated apoptosis pathway in tumoral cell lines.

Characterization of expressed anti-parallel transcription units in male germ cells. *W.Y. Chan, S.M. Wu, L. Hovrath, L. Ruszczyk, E. Mikhaylova, O.M. Rennert* Laboratory of Clinical Genomic, National Institute of Child Health and Human Development, NIH, Bethesda, MD.

Transcriptome mapping of developing murine male germ cells using Serial Analysis of Gene Expression (SAGE) revealed the prominent presence of antisense transcripts. Nucleotide sequence analysis of orientation specific RT-PCR products and cloned antisense transcripts showed that the antisense transcripts of four genes, namely,*Uba52*, *Ch10*, *Calm2*, and *Ubb*, were derived from their pseudogenes on different chromosomes. Blasting against the mouse genome showed that these pseudogenes are present in the intron of actively transcribed genes. *Uba52* pseudogene resides in the intron of *Cbx1*; *Calm2* pseudogene is present in the intron of *Prkar2b*; *Ch10* pseudogene is contained in the intron of *Sp3*; and *Ubb* pseudogene resides in the intron of *Catsper2*. Apparently, these pseudogenes were retrotransposed and inserted into the intron of the host genes. The direction of transcription of the functional parent gene is opposite to that of the host gene. Because of the high homology between the functional transcript and its pseudogene, pairing of the pseudogene antisense transcript and the functional transcript would occur. This speculation was supported by the demonstration of the presence of duplex RNA of *Uba52* in cultured cells. For *Uba52*, *Ch10* and *Calm2*, there is no evidence that the antisense strand of the pseudogene is transcribed, and the identified antisense amplicons are likely to be derived from the spliced intron of the host genes. Whether pairing of functional transcript and the pesudogene-containing intron will affect the translation of the functional transcript or the processing of the host gene is unknown. The interaction between the anti-parallel gene pairs, namely, *Uba52*/*Cbx1*, *Calm2*/*Prkar2b*, *Ch10*/*Sp3*, and *Ubb*/*Catsper2* are currently under investigation. These studies demonstrate the complex interrelationship between transcription units and the potential role of pseudogenes, introns, and antisense transcripts as regulators of gene expression.

Survival following treatment with intravenous sodium phenylacetate and sodium benzoate (AMMONUL) for hyperammonemia caused by urea cycle defects. *G.M. Enns[1], S.A. Berry[2], G.T. Berry[3], W.J. Rhead[4], A. Hamosh[5]* 1) Stanford University, Stanford, CA; 2) University of Minnesota, Minneapolis, MN; 3) Jefferson Medical College, Philadelphia, PA; 4) Medical College of Wisconsin, Milwaukee, WI; 5) Johns Hopkins University, Baltimore, MD.

Urea cycle defects (UCDs) are inborn errors of metabolism characterized by hyperammonemic encephalopathy and high morbidity and mortality. The combination of sodium phenylacetate and sodium benzoate in a 10%/10% solution (AMMONUL) is an intravenously administered adjunctive therapy for the treatment of acute hyperammonemic encephalopathy in patients with UCDs. Initial clinical trials demonstrated the potential efficacy of AMMONUL in a small number of UCD patients; ammonia levels typically fell and survival was much improved compared to historical outcomes. We report the results of a 20-year, open-label, uncontrolled study of AMMONUL therapy for acute hyperammonemia caused by UCDs. A total of 1,191 episodes of hyperammonemia in 304 patients with UCDs occurred at 118 hospitals in North America. The mean number of episodes per patient was 3.3 6.16 (range 1 to 79). A total of 1,142 out of 1,191 hyperammonemic episodes in which patients were treated with AMMONUL resulted in survival (96%). Overall, 255 of 304 patients (84%) survived all episodes. Neonates (mortality 27%) and males with ornithine transcarbamylase deficiency (mortality 9%) were more likely to die during an episode ($p<0.001$), compared with older patients and patients with other UCD diagnoses, respectively. Highest mortality was associated with peak ammonia levels of >600 mol/L in neonates (mortality 44%) and >300 mol/L in patients older than 30 days (mortality 6%). Baseline and post-therapy ammonia levels were known for 592 episodes. The median baseline ammonia level was 186 mol/L. The median post-therapy ammonia level was 36 mol/L. The median percent change from baseline was -79%. We conclude that AMMONUL therapy, in conjunction with other treatments, such as intravenous arginine HCl and provision of adequate calories, is effective in normalizing blood ammonia levels. Survival is remarkably improved compared with historical outcomes.

Clinical heterogeneity of familial CHARGE syndrome due to *CHD7* mutation. *A. Delahaye[1], S. Lyonnet[2], S. Wiener-Vacher[3], D. Bremond-Gignac[4], J. Amiel[2], C. Baumann[1], M. Elmaleh-Bergès[5], T. Attié-Bitach[2], A. Verloes[1], D. Sanlaville[2]* 1) Medical genetics department, Robert Debré University hospital, AP-HP, INSERM U676, Paris, France; 2) Medical genetics department, Necker-Enfants Malades University hospital, AP-HP, INSERM U781, Paris, France; 3) ENT department, Robert Debré University hospital, AP-HP, Paris, France; 4) Ophthalmology department, Robert Debré University hospital, AP-HP, Paris, France; 5) Medical imaging department, Robert Debré University hospital, AP-HP, Paris, France.

Background: CHARGE syndrome (OMIM #214800) is a multiple-malformation syndrome with distinctive diagnostic criteria, usually due to *CHD7* (Chromodomain Helicase DNA binding 7) haploinsufficiency. Familial occurrence of CHARGE syndrome is exceptional.

Objective: To describe clinical and genetic analyses in a French family affected by a CHARGE syndrome.

Methods: Clinical study, ophtalmological examinations, vestibular function evaluation and inner ear imaging were carried out. Molecular analysis of the *CHD7* gene was undertaken by direct sequencing.

Results: The two affected brothers had a vestibular dysfunction with hypoplastic semicircular canals, a mental retardation and a facial dysmorphism. None had choanal atresia and only patient 2 presented with coloboma. Patient 1 had multiple congenital abnormalities: bilateral cleft lip and palate, type III oesophageal atresia, complex heart defect and vertebral abnormalities. The mother was only investigated after the discovery of anomalies in her children. A left papillar coloboma and a partial vestibular dysfunction were then detected. Mutation analysis of the *CHD7* gene resulted in the identification of a missense mutation (c.2501CT, Ser834Phe) in the three affected patients.

Conclusion: We report a new missense mutation responsible for a familial mild CHARGE syndrome. Moreover, this observation would shed light on the variable intrafamilial expression suggesting a careful genetic counselling.

Mining the genome diversity of microsatellite markers: genetic signature of population expansion in modern humans. *S. Guha, R. Chakraborty* Center for Genome Information, College of Medicine, University of Cincinnati, Cincinnati, OH.

Microsatellite markers have been the principal types of genomic markers used in studying genetic basis underlying susceptibility to complex diseases, and to understand evolutionary relationships of human populations. As complex disease gene mapping efforts are moving into the paradigm of disease-gene association studies, questions arise as to which populations are most suited for such studies. Prior work implies that small isolated populations and particularly the ones which expanded recently may be more suited to detect disease-gene association due to underlying genetic risk of complex diseases. Using data on 784 microsatellite markers (encompassing 22 autosomal chromosomes) consisting of di-, tri-, tetra-, and pentanucleotide repeat loci from 976 individuals belonging to 36 worldwide populations, we addressed: (a) which population groups show genetic signatures of isolation and/or recent expansion; (b) what is the extent of reduction of genomic diversity of detected isolated populations, and (c) which measures of genetic variation best captures these signatures of population expansion. We found that the heterozygosity and number of segregating alleles show conspicuous signatures of reduced genetic variation in the Native Americans, and in the Oceanians. African populations have the highest diversity. Using the imbalance index estimation we found signatures of recent population size expansion in the populations that show reduced genomic diversity. The Africans also show signatures of population size expansion of most distant past, compared with the other populations. These results are consistent with the Out-of-Africa model of human evolution. Interestingly, variation of genomic diversity by repeat motif size of microsatellite markers in this study is less conspicuous than that has been seen before, perhaps due to the fact in this panel of markers the tetranulceotide loci are most abundant compare to other type of markers. These results have implications with regard to population-specific patterns of genome-wide linkage disequilibria between loci, which have to be investigated in greater details. (Supported by NIH grant GM41399).

Promising susceptibility loci for lipid levels in blood serum detected in extended pedigrees from Samoa. *K. Åberg[1], F. Dai[2], E.D. Keighley[3], G. Sun[4], D. Smelser[4], S. Viali[5], J. Tuitele[6], S.R. Indugula[4], Q. Zhang[4], R. Deka[4], D.E. Weeks[1, 2], S.T. McGarvey[3]* 1) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 2) Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 3) International Health Institute, Brown University, Providence, RI; 4) Center for Genome Information, Department of Environmental Health, University of Cincinnati, Cincinnati, OH; 5) Tupua Tamasese Meaole Hospital, Apia, Samoa; 6) Tafuna Family Health Center, Pago Pago, American Samoa.

Using variance component linkage analysis, developed to take full advantage of extended pedigree structures, we performed genome-wide scans for lipid-related phenotypes in 35 families (3-2,094 individuals/family) from Samoa. For 576 adults (>18 years of age), lipid profiles, including total cholesterol (CHOL), low density lipoprotein (LDL), high density lipoprotein (HDL) and triglyceride (TG), were determined and 376 autosomal microsatellite markers were genotyped. SOLAR/Loki software was used to calculate multipoint LOD scores. Age and gender were used as covariates. On chromosome 12q24.33 we obtained maximum LODs of 3.05 and 1.84 for LDL and CHOL, respectively. For these phenotypes, we detected a second locus on chromosome 6p21.2-p12.1 with LOD=2.15 and 1.72, respectively. A third locus, with LOD=2.08, was detected for CHOL on chromosome 4p14-q12. For TG we detected LOD=1.85 on chromosome 5q35.3 and LOD=1.69 on chromosome 15q12. Telomeric, on chromosome 15q14, we found a promising QTL for HDL with LOD=2.61. Interestingly, cholesterol related traits have previously been reported to 4q12 as well as to 6p12 while TG has been reported to 15q12. Currently, we are extending our investigation to include additional adults (n=677) from American Samoa. The two populations from the Samoa islands have a common population history but, in contrast to Samoa, the environment in American Samoa has been extensively modernized during the last decades. By performing combined linkage investigations with these sample sets, we will increase the power to detect linkage, as well as to explore gene x environment interactions.

An interactive web-based genetics case for medical students. *J. Cowan[1], S. Albright[2], D. Walker[2], L. Demmer[1]* 1) Department of Pediatrics, Tufts School of Medicine, Boston, MA; 2) Tufts University Sciences Knowledgebase, Boston, MA.

As part of a comprehensive effort to expand and integrate genetic education throughout the four year curriculum at Tufts, an interactive web-based case was developed and introduced into the Year 2 medical student genetics course. The exercise reinforced concepts taught in class in addition to emphasizing self-directed learning and applied ethical concepts. The case centered around a couple with a family history of MR and a balanced translocation. Students were asked to complete pedigree analysis, devise testing strategies, perform a literature search, identify unbalanced karyotypes and evaluate FISH results for PGD. Students received immediate feedback upon submission of each answer. Completion of the exercise was required, and concepts emphasized in the case were reflected on the final exam. Time spent on the case, and answers to questions were tracked anonymously.

Results:174 of 175 students worked through the case. 25% of students completed the case during the 2nd week and 72% in the final week of the class. Completion during the 2nd week was associated with a higher number of correct answers, with 78% getting 10 or more of 13 answers correct. 69% of students completed the work in 60 minutes or less. No one question was answered correctly by all students and the majority of questions (10/13) was answered correctly by 79-97% of the class. We learned that our explanations of pedigree construction, unbalanced translocations, and the ethics of informed consent could be improved (correct response rates only 64%, 63% and 66%). Evaluation of write-in answers revealed that 86% of students successfully initiated self-directed learning. 165 students evaluated the exercise and on a scale of 1-5 gave the case an average rating of 4.3 for quality, 4.2 for effectiveness as a learning tool, and 4.4 for ease of navigation.Written student comments were highly favorable.

Conclusion: Development of a web-based, interactive case emphasizing self-directed learning and applied ethics appears to be a welcome and effective educational tool for second year students.

Effect of methylparaoxon on chromosomes from human lymphocytes, in vitro. *I. Aranha, L. Almeida* Inst Biol, PHLC S-525-6, Univ Estado Rio de Janeiro, Rio de Janeiro, Brazil.

The use of pesticides is still the main strategy to fight plagues in agriculture. Organophosphate esters represent a significant proportion of the world production of pesticides. Methylparation is an organophosphate pesticide largely used. In order to exert its biological effect it must be biotransformed into its corresponding oxon-analogous methylparaoxon, in the liver. Previous work in our laboratory, using the chromosome aberrations assay, in human lymphocytes exposed to different concentrations of the drug (0.1, 0.25 and 0.5 ppm) in vitro, has shown that methylparaoxon was responsible for the alterations in structure observed. The objective of the present work was to study the effect of methylparaoxon on chromosomes of human lymphocytes in vitro, using the micronuclei assay. Blood was collected from healthy donors 18 to 30 years old. Peripheral whole blood cells were incubated at 37^{o}C and 5% CO_2 for 72h, in enriched 1640 medium in the presence of methylparaoxon (0.1, 0.25 and 0.5 ppm). Cells from the same donors not exposed to the drug served as control for the experiment. Cytochalasin B (4g/ml) was added to the cultures 44h postinitiation. After fixation, cells were stained with Giemsa Gurr (2%) and analyzed under the optical microscope. In the samples exposed to the pesticide, 12246 binucleated cells were observed at the 0.1 ppm concentration and 53 of them showed micronuclei. We observed 12144 binucleated cells at the 0.25 ppm concentration and 43 of them had micronuclei. We analyzed 12208 binucleated cells at the 0.5 ppm concentration and observed the presence of a micronucleus in 85 of them. In the control group we observed 12682 binucleated cells and 8 of them had micronuclei. The chi-square test showed that the results were significant ($p<0.0001$) and that methylparaoxon was responsible for the micronuclei observed.

Heterogeneity of genetic structure of Hispanic Populations of Continental United States and its impact on understanding their complex disease risks. *B.M. Chakraborty*[1], *R. Chakraborty*[1, 2] 1) Dept. Environmental Health, Univ. Cincinnati College of Medicine, Cincinnati, OH 45267; 2) Center for Genome Information, Univ. Cincinnati College of Medicine, Cincinnati, OH 45267.

The term Hispanics used for some immigrant populations of US is known to have operational as well as methodological problems in relation to complex disease studies. This research reviews this issue in relation to demographic as well as regional heterogeneities of the Hispanic populations of continental USA. The origin of the Hispanics in different regions varies, and so does their genetic structure. For example, of over 40 million US Hispanics, about 66% are of Mexican origin, and they reside mostly in southern, western, and mid-western regions of the country, but the north-eastern Hispanics of US are predominantly of Puerto-Rican, Cuban, Central, or South American origin. Genetic structure of these regional Hispanics populations also reflects this heterogeneity, in terms of proportions of genes of Native American and African ancestry. The age pyramids of these different groups are also different, contributing towards their late-onset disease morbidity and mortality differences. One striking feature, however, is the gene flow in all regional Hispanic populations has a gender bias, with lesser proportions of genes descending from European maternal ancestry. Comparison of admixture estimates based on autosomal, mitochondrial, and Y-chromosomal markers shows heterogeneity of gender bias of admixtures of the different regional populations more conspicuously. The length of residence in US is also varied between these groups. Consequently, differences of acculturation and adaptation to life styles in the host country, combined with their genetic differences can account for their differences of morbidity and mortality attributable to complex chronic diseases. The conflicting observations regarding the Hispanic Paradox may also be explained by such heterogeneities. Hence, complex disease studies in these populations should consider the context of their cultural and demographic origin, particularly when the gene-environment factor is to be accounted for. (Research supported by NIH grant GM 41399).

Longevity Collection at Coriell Institute for Medical Research: a Resource for the Study of Aging. *D.L. Coppock, C.M. Beiswanger, B. Newman, B.A. Frederick, J. Leonard* Coriell Institute for Medical Research, Camden, NJ.

For studies on successful aging, new resources of lymphoblastoid cell lines/DNA from octogenarians, nonagenarians and centenarians are available from the Longevity Collection from the NIA Aging Cell Repository. The cell lines/DNA were prepared from blood samples that were donated by men and women who have reached great age and whose current health is good. Collection from additional donors over the age of 90 is continuing. Most donors have been interviewed to collect critical information about their age, medical history, family medical history, and demographics. The data are self-reported, although every effort has been made to verify the age. Data collected include history of major diseases and disorders including heart, lung, cancer, diabetes gastrointestinal, immune problems, smoking and drinking habits, demographic characteristics, and the number of family members who have lived over 90 years. Ages of those currently available are from 80 to 103 years old with 6 centenarians, 38 nonagenarians and 79 octogenarians including 66 women and 57 men. For each participant, a lymphoblastoid cell line has been prepared from a blood sample. Each line has been karyotyped. Constitutional abnormalities were detected in three cases [46,XX,inv(4)(p16q21), 45,XY,der(14;21)(q10;q10), 46,XX,t(11;22)(q23.3;q11.2)]. One third of the cultures presented with clonal abnormalities, which may have occurred in vitro. The majority of these were gains or losses of X or Y or trisomy 12. Of those donors reporting, 33% reported one or more cancers; 60% reported hypertension or heart disease; <9% reported lung disease and 6% reported diabetes. These samples provide a resource for the study of aging at the cellular and genomic level. Additional information about the Longevity Collection can be found at: http://ccr.coriell.org/nia/special/longevity.html.

Long-term benefits of early oral cysteamine therapy in cystinosis. *I. Bernardini, J. Balog, G. Golas, K. O'Brien, R. Kleta, W.A. Gahl* MGB, NHGRI, NIH, Bethesda, MD, USA.

The lysosomal storage disease, nephropathic cystinosis, presents with renal tubular Fanconi syndrome and growth delay in the first year of life. If untreated, glomerular failure occurs by 10 years of age; mortality and multisystemic morbidity occur after renal transplantation. The free thiol cysteamine can lower cellular cystine content by >90%. Early, diligent therapy with oral cysteamine (60-90 mg/kg/d dosed every 6h) has been shown to prevent or attenuate the growth retardation and glomerular deterioration of pre-transplant patients. It also helps prevent the disorders hypothyroidism and pulmonary dysfunction. We recently reported that the frequencies of swallowing difficulty, coronary artery calcification, and posterior segment ophthalmic complications varied directly with years off of cysteamine and inversely with years on cysteamine therapy. We now present data on 100 nephropathic cystinosis patients seen as adults at the NIH between 1986 and 2006 under our natural history protocol. Thirty-three are now deceased; their mean age at last visit was 26 +/- 1 (SEM) years and at death was 29 +/- 1 years. The most common causes of death were: 1) Unknown, sudden; 2) Sepsis; 3) Uremia. At least 30/33 required thyroid supplementation, 20/33 had significant myopathy; 9/33 exhibited diabetes mellitus, and 9/33 had retinal blindness; 12 of 20 males had primary hypogonadism. The mean serum total cholesterol was 201 +/- 12 mg/dL. In considering all 100 adult patients, the frequencies of death in relation to duration of cysteamine therapy were: On cysteamine for <10 y, 45% mortality; on for >20 y, 0% mortality; off cysteamine for <10 y, 4% mortality; off for >20y, 47% mortality. Similar results were found for the frequencies of myopathy and diabetes. We conclude that the late complications of cystinosis are devastating and that early oral cysteamine therapy exerts a dramatic salutary effect on the mortality and morbidity of cystinosis in adults.

Megacystis-Microcolon-Intestinal Hypoperistalsis Syndrome: Diagnosis and Intervention in Four Patients. *Q. Abu Ali[1], C. Kozma[2], S.S. Kaufman[3], T.M. Fishbein[3], C.S. Matsumoto[3], Y. Rekhtman[3]* 1) National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 2) Department of Pediatrics, Georgetown University Hospital, Washington, DC; 3) Georgetown University Transplant Institute, Georgetown University Hospital, Washington, DC.

Megacystis-Microcolon-Intestinal Hypoperistalsis syndrome (MMIHS), or Berdon syndrome, was first described in 1976. Inheritance is autosomal recessive. A genetic locus for MMIHS maps to 15q24. It is characterized by marked dilatation of the urinary bladder, external appearance of "prune belly", microcolon, dilated small intestine, and intestinal hypoperistalsis. Generally, it is lethal during the first year of life. We report four patients who were diagnosed with MMIHS. Patients A, B, and C were siblings to the same non-consanguineous Caucasian parents. Patient D was an offspring to non-consanguineous African American parents. Coincidentally, all four patients were born in the same geographical area. Patient A/male, diagnosed prenatally, was a still born at approximately 5 months gestation. Patient B/female was diagnosed prenatally and died at 5 months of age. Patients C/female and D/male were diagnosed prenatally, listed for multiple visceral organ transplants, and had normal karyotype, echocardiogram, and ganglion cells of the mesenteric plexus. Patient C had a classical "prune belly" appearance. MMIHS is a rare autosomal recessive disorder that manifests as gastrointestinal and genitourinary systems malformations. It should be differentiated from "prune belly" syndrome and chronic idiopathic intestinal pseudo-obstruction (CIPO) syndrome. Generally, there is a 3-4:1 female to male ratio, suggesting either underdiagnosis and/or a higher lethality prenatally in males. Prenatal diagnosis is possible, which enables parents to make informed decisions regarding the pregnancy. To date, no commercial diagnostic testing is available. Clinical differentiation between MMIHS, prune belly syndrome, and CIPO might not be simple, but carries a significant role in the extent of medical, surgical, and genetic counseling interventions offered. More clinical reports are needed to outline the natural history of this condition.

Angiotensin II Type 1 Receptor Blockade Improves TGF-induced Failure of Muscle Regeneration in the mdx Mouse Model of Duchenne Muscular Dystrophy. *R.D. Cohn[1], J.P. Habashi[2], B.L. Loeys[1], E.C. Klein[1], M. Gamradt[1], T.M. Holm[1], D.P. Judge[3], H.C. Dietz[1]* 1) Institute of Genetic Medicine and HHMI, Johns Hopkins Univ Sch of Med, Baltimore, MD, USA; 2) Division of Pediatric Cardiology, Dept of Pediatrics, Johns Hopkins University Sch of Med; 3) Division of Cardiology, Dept of Medicine, Johns Hopkins University Sch of Med, Baltimore, MD, USA.

We have previously shown that increased TGF activity causes abnormal muscle regeneration and myopathy in fibrillin-1 deficient mice, a model of Marfan syndrome. Systemic antagonism of TGF via administration of TGF-neutralizing antibody or the angiotensin II type 1 receptor (AT1) blocker losartan restores abnormal muscle regeneration and prevents subsequent development of myopathic features. Impaired satellite cell performance and muscle repair has also been shown to play a significant role in the disease progression of various forms of muscular dystrophy. Here we demonstrate that myopathic changes in the dystrophin-deficient mdx mouse model of Duchenne muscular dystrophy associates with excessive TGF signaling, as evidenced by increased phosphorylation of pSmad2/3. Systemic TGF antagonism via losartan improves the satellite cell response to toxin induced-injury in the mdx mouse. Losartan-treated mdx mice have improved steady-state muscle architecture and a near-normal amount of actively regenerating, neonatal myosin positive fibers four days after induced injury. After 18 days, losartan-treated mdx mice show only minimal amount of fibrosis, as demonstrated by vimentin expression, when compared to placebo-treated mice. Moreover, long-term administration of losartan leads to improved functional performance as demonstrated by increased muscle grip strength and decreased muscle fatigue in mdx mice. Together, our data demonstrate that treatment with the FDA approved medication losartan attenuates TGF-induced failure of muscle regeneration and represents a promising therapeutic strategy for the treatment of Duchenne muscular dystrophy. Given that losartan is widely used to treat hypertension and has an exceptional tolerance profile in all age groups, we propose that a clinical trial using losartan is warranted.

A Common Ancestral Haplotype Located at 4q13.2 Confers Susceptibility to ADHD. *M. Arcos-Burgos[1], M. Jain[1], S. Domene[1], S. Shively[1,2], D. Wallis[1], H. Stanescu[1], J.D. Karkera[1], M.T. Acosta[1], K. Berg[1], R. Kleta[1], E. Roessler[1], A. Vortmeyer[2], D. Pineda[3], J.D. Palacio[3], F. Lopera[3], J. Meyer[4], K.P. Lesch[5], J.E. Bailey-Wilson[1], F.X. Castellanos[6], M. Muenke[1]* 1) NHGRI, NIH, Bethesda, MD, USA; 2) NINDS, NIH; 3) Neurosciences Group, University of Antioquia, Medellín, Colombia; 4) Verhaltensgenetik, University Trier, Germany; 5) Department of Psychiatry and Psychotherapy, University of Wurzburg, Germany; 6) New York University Child Study Center, New York, NY, USA.

Attention-Deficit/Hyperactivity Disorder (ADHD) (OMIM 143465), the most common behavioral disorder of childhood, affects 8-12% of children worldwide. We described linkage of ADHD to a locus at 4q13.2 in families from a genetic isolate in Colombia, South America. Fine mapping and a second set of families (n=120) from this same isolate validated our linkage results and defined a minimal critical region (MCR). A common haplotype (frequency, 22.2%) within this MCR was found in LD to ADHD ($P < 2.7*10-5$). ADHD susceptibility haplotype variants reconstructed from 23 additional markers, iteratively validated the presence of the core haplotype in additional ADHD familial samples from the US (n=185, $P<3.1*10-3$) and from Germany (n=244, $P<3.8*10-5$) ($Ppooled< 3.01*10-8$) (RR 2.6). Variants complementary to the susceptibility haplotype conferred protection against ADHD. The haplotype defines a single gene. A phylogenetic reconstruction using the chimpanzee sequence for our gene as an out-group revealed the ADHD susceptibility haplotype ancestral to the protective variants. We also demonstrated that the gene we identified is expressed specifically in brain regions associated with ADHD. This is the first gene with variants conferring susceptibility to ADHD with an effect size (genotype relative risk) greater than 2.0. In addition, the evolutionary history and the high frequency of the susceptibility haplotype suggests that a selective advantage may be involved. The susceptibility gene will be fully characterized at the presentation of this work.

Pregnancy complications, risk perceptions, and reproductive planning in families affected with Neurofibromatosis Type 1. *J.L. Geurts[1], P.M. Veach[2], J. Kao[2], B.S. LeRoy[1]* 1) Genetics, Cell Biology and Dev, University Of Minnesota, Minneapolis, MN; 2) Educational Psychology, University of Minnesota, Minneapolis, MN.

Neurofibromatosis Type 1 (NF1) is a complex inherited condition, with variable expression. Published reports suggest that women with NF1 have an increased risk for pregnancy complications and neurofibroma growth during pregnancy. However, data regarding severity and frequency of problems are incomplete, contradictory and often outdated. In the present study 119 individuals with NF1 or their partners, recruited from two NF websites, responded to an on-line survey regarding pregnancy experience and reproductive planning. Major research questions were: 1) What is the incidence of pregnancy complications among women with NF1? 2) What percentage report NF1 symptom exacerbation during pregnancy? 3) How concerned are women about their health during pregnancy? 4) Are they knowledgeable about pregnancy risks? 5) How has NF1 affected reproductive planning decisions? and 6) To what extent has NF1 affected respondents relationships and daily life? Present findings indicate possible increased risk for pregnancy complications and symptom exacerbation for women with NF1, including: cesarean section (36.7%), low birth weight baby (31.9%), growth of existing neurofibromas (50%), and growth of new neurofibromas (68.8%). Across all women with NF1, mean ratings of concern about neurofibroma growth and effects of NF1 on their health during pregnancy were mild to moderate; t-tests indicated that those who had not been pregnant reported significantly greater concern than those who had been pregnant ($p < .001$). Sixty percent of women with NF1 reportedly had heard or read about NF1 possibly causing pregnancy complications, and 44.5% reported that this knowledge negatively affected their reproductive decision-making. Both females and males with more severe NF1 reported greater negative effects on relationships and on daily functioning. Samples of respondents written comments, and practice and research recommendations will be presented.

An algorithm for simulating genetics data based on linkage and linkage disequilibrium maps. *C. Chen[1,2], L.-Y. Wei[1], S.S. Shete[3]* 1) Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) Institute of Chinese Medical Science, China Medical University, Taichung, Taiwan; 3) Department of Epidemiology, University of Texas MD Anderson Cancer Center, Houston, TX, USA.

Recently, marker location databases that integrated linkage, linkage disequilibrium (LD) and physical maps provided more accurate estimations of locus-locations and inter-marker distances. In particular, the resolution of linkage maps, specifying distance in crossover counts, can be enhanced by interpolating dense locations from linkage disequilibrium (LD) maps, which specifies distance in LD unit (LDU). In this study, based on the linkage and LD maps, we proposed an algorithm to generate genetic data that retains the characters of target regions in every aspect for empirical calculation. The algorithm can be divided into five steps. (1) SNPs in the region of interests are assigned to blocks such that all SNPs within a block have the same location in the LD map. The SNP locations in LDU can be obtained from location databases or estimated from real data using the LDMAP program. (2) Haplotypes and haplotype frequency distribution in each block are estimated based on real data, which may be provided by user or obtained from publicly available databases, such as the HapMap project. (3) Each block is transformed as a pseudo multi-allelic marker and each haplotype acts as an allele. The inter-marker recombination is estimated by interpolating the locations of the last SNP of the first block and the first SNP of the second block from linkage and LD maps. (4) The information of the pseudo markers and recombination are then applied to generate genetic data using existing linkage simulation programs. (5) The simulated pseudo markers are finally translated back to the corresponding SNPs. The use of the existing linkage simulation programs that generated genotypes for specified pedigrees and genotypes conditional on phenotypes may sufficiently fulfill various sampling schemes used in family- and population-based genetic studies. In summary, data generated by this algorithm preserved not only the historic linkage disequilibrium structure among SNPs but also crossover events in the targeted data.

Phenylketonuria (PKU) disease knowledge and health locus of control. *M. Applegarth[1], V. Vandergon[1], M. Fox[2], N. Dorrani[2], S. Charnofsky[1], C. Palmer[2]* 1) California State University, Northridge, CA; 2) University of California, Los Angeles, CA.

Phenylketonuria (PKU) is a treatable inborn error of metabolism that occurs in approximately 1 in 10,000 to 20,000 live births. Untreated, PKU causes a build up of phenylalanine in the blood and brain resulting in neurologic damage and mental retardation. The current medical recommendation is to continue PKU treatment for life. Several studies have explored different psychosocial factors that may have an effect on diet compliance and metabolic control. The purpose of this study was to determine if a relationship existed between two such factors, PKU disease knowledge and health locus of control (HLOC). These two factors have been shown in earlier studies to independently affect metabolic control and medical adherence. The hypothesis stated that individuals with higher levels of internal HLOC would have higher levels of disease knowledge. Self-administered questionnaires were completed by 16 individuals with PKU and 38 parents of individuals with PKU from the National PKU News listserv and the California Coalition for PKU and Allied Disorders. The questionnaire was designed to measure PKU disease knowledge, Health Locus of Control, and individual health measures including overall health rating and blood phenylalanine level. Results of this study showed a positive relationship between disease knowledge and internal HLOC, and a negative relationship between disease knowledge and blood phenylalanine levels in individuals with PKU. These correlations were not statistically significant, possibly due to the small sample size. The results also showed that almost half of variance in health rating was explained by internal HLOC and disease knowledge in individuals with PKU. In conclusion, the results of this study suggest that disease knowledge and health locus of control are important variables in this population and warrant further investigation. Future research should expand on the current study with larger and more diverse samples and explore other independent variables that may account for the remaining variance in overall health ratings.

PRENATAL DIAGNOSIS OF TRISOMY 18. EXPERIENCE IN THE NATIONAL INSTITUTE OF PERINATOLOGY. *R. Baez-Reyes, G. Noceda-Rivera, G. Razo-Aguilera* Deparment of Genetics, National Institute of Perinatology, MEXICO.

OBJETIVE: To realize an analysis of the factors that guide to the diagnosis of trisomy 18 in prenatal form and to establish a work line for their detection. MATERIALS AND METHODS: We revised the records of 47 cases detected of trisomy 18 that were seen by the consultation of Prenatal Diagnosis, in one period that embraces since January 1st from 1989 to May 31th from 2006: 34 of the patients were prenatal death, 3 had early neonatal death and 10 were considerate by the commitee of perinatal damage. In all cases were confirmed the diagnosis for cytogenetic study: 43 for amniocentesis, 3 for chorionic villus sampling and 1 for fetal urine. RESULTS: Of those 47 cases studied, 46 were regular trisomy and 1 presented a mosaic form, being 30 male fetuses and 17 female fetuses; with a maternal age average of 36.1 years old and gestational age average for last menstruation of 23.9 weeks. We offered cytogenetic study in 31 cases (65.95%) for abnormal ultrasound, in 8 cases (17.02%) for maternal age bigger to 35 years old, mixed factor in 7 cases (14.89%) and previous child with somo defect in 1 case (2.12%). DISCUSSION: The trisomy 18 is associated with multiple severe structural abnormalities that involve to several organs. The ultrasound in this cases play an important paper, as well as the possibility to offer biochemical tests to the patients for a bigger sensibility. Howewer, the definitive diagnosis will always be for cytogenetic study, same that will give us the rule for an appropiate genetic advice.

Linkage disequilibrium in an isolated population from Norfolk Island. *C. Bellis[1], K.N. Begley[2], R.M. Hughes[1], S. Quinlan[1], R.A. Lea[1], S.C. Heath[3], J. Blangero[4], L.R. Griffiths[1]* 1) Genomics Research Centre, Griffith University, Gold Coast, Qld, Australia; 2) Division of Information Services, Griffith University, Australia; 3) Centre National de Génotypage, 2 Rue Gaston Cremieux, Evry, France; 4) Southwest Foundation for Biomedical Research, San Antonio, Texas, USA.

Genetic isolates provide a potentially powerful sample population for disease loci mapping of complex multifactorial traits, due to the combined effects of geographical isolation (and resulting inbreeding) and limited variation in environmental influences. Additionally, such populations generally arise from a small number of founding members, possibly from quite diverse cultural backgrounds, therefore introducing admixture affects. The Norfolk Island community is a population of approximately 1800 current permanent residents, of whom the majority are direct descendents of 18th century English sailors (Bounty mutineers) and Polynesian women. Such a population presents interesting and unique characteristics to a genomic investigation into complex disease analysis, due to the strong family groupings and well-documented family histories. Furthermore, the Norfolk population grew in isolation from other communities and as a result produced a relatively homogeneous genetic pool. We have collected samples from the Norfolk Island adult population, to investigate cardiovascular genes. Initial analysis identified a slightly higher prevalence of cardiovascular disease (CVD) and associated risk factors in the study sample of 602 Norfolk Island individuals when compared to the prevalence of CVD within Australia. Additionally, seven CVD risk associated phenotypes have been demonstrated as showing significant ($p<0.05$) heritability in the Norfolk population and the pedigree should have outstanding power (>90 percent) to localise QTL associated to these traits. The present study is primarily concerned with estimating the extent of linkage disequilibrium within the Norfolk population by analysing microsatellite markers from the Xq13.3 genomic region. The Xq13.3 genomic region has been extensively studied in other genetic isolates and presents a useful criterion for understanding levels of linkage disequilibrium within study populations.

Atypical 22q11.2 distal deletions detected by Chromosomal Microarray Analysis: Clinical significance and cytogenetic evaluation. *S. Ben-Shachar[1], M. Hummel,[2], J. Belmont[1], P. Eng[1], S. Bland[1], T. Appleberry[1], A.L. Beaudet[1], S. W. Cheung[1], A. Patel[1]* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Dep. of Pediatrics, W. Virginia University School of Medicine. Morgantown, WV.

Microdeletions within chromosome 22q11.2 cause a variable phenotype, including DiGeorge syndrome (DGS) and velocardiofacial syndrome (VCFS). About 90% of patients show a common recurrent ~3 Mb deletion and about 7% of patients have a smaller ~1.5 Mb recurrent deletion. Both deletions were found to occur as a result of non-allelic homologous recombination between low copy repeats (LCR) sequence substrates located in 22q11.2 region. Interestingly, although 9 different LCRs are located within this region only a few cases of atypical deletions have been described suggesting that such deletions are either less common than expected, do not have a clinical significance, cause a phenotype different than DGS/VCFS or may be lethal. Using Chromosomal microarray analysis (CMA)we have detected 3 unrelated cases of small deletions within 22q11.2, located distal to the 3 Mb common deletion. The BAC clones in the DGS/VCFS region have been selected to flank the majority of the LCR22s. All of the 3 patients had a small deletion of a single clone on the microarray. A deletion between LCR22-5 and LCR22-6 detected by clone RP11-36N5 was seen in 2 cases and a deletion between LCR22-4 and LCR22-5 detected by clone RP11-165F18 was seen in the 3rd case. The deletions were confirmed by FISH. Both patients with the deleted clone RP11-36N5 presented with FTT and facial dysmorphia. One of them had a common truncus arteriosus and developmental delay. They both had a *de novo* deletion suggesting potential pathogenecity. The 3rd patient was presented with FTT, atrioseptal heart defect and hypotonia. Parents were not available for study this patient. The fact that although *TBX1* was not included in these deletions, two of the patients did have a congenital heart defect may suggest a positional effect mechanism. We conclude that distal atypical deletions of chromosome 22q11.2 may be relatively common, clinically significant genetic aberration.

Comprehensive mutation analysis in Costello syndrome, CFC syndrome and Noonan syndrome : clinical and genetic overlap among three disorders. *Y. Aoki[1], T. Niihori[1], Y. Narumi[1], H. Kawame[2], K. Kurosawa[3], H. Ohashi[4], M. Filocamo[5], G. Neri[6], H. Cavé[7], A. Verloes[7], N. Okamoto[8], R.C.M Hennekam[9], G. Gillessen-Kaesbach[10], D. Wieczorek[10], M.I. Kavamura[6], L. Wilson[9], Y. Suzuki[1], S. Kure[1], Y. Matsubara[1]* 1) Dept Medical Genetics, Tohoku Univ Sch Medicine, Sendai; 2) Nagano Childrens Hosp, Nagano; 3) Kanagawa Childrens Med Ctr, Yokohama; 4) Saitama Childrens Med Ctr, Saitama, Japan; 5) IRCCS. G.Gaslini, Genova; 6) Istituto di Genetica Medica, Rome, Italy; 7) Hôpital Robert Debré (APHP), Paris, France; 8) Osaka Med Ctr & Res Inst for Maternal & Child Health, Osaka, Japan; 9) Inst of Child Health, London, UK; 10) Univ Essen, Essen, Germany.

Costello syndrome and cardio-facio-cutaneous (CFC) syndrome are Noonan-related syndromes characterized by heart defects, facial dysmorphism, ectodermal abnormalities and mental retardation. Recently we discovered proto-oncogene *HRAS* mutations in Costello syndrome and *KRAS* and *BRAF* mutations in CFC syndrome, establishing a new role of RAS/RAF/MEK/ERK pathway in human development. To elucidate the clinical and molecular characteristics of Noonan, Costello and CFC syndromes, we have analyzed *PTPN11*, *HRAS*, *KRAS*, *BRAF* and *MAP2K1/2* (MEK1/2) in 50 patients with Noonan syndrome, 35 patients with Costello syndrome and 59 patients with CFC syndrome. Mutations in *PTPN11* were identified in 42% of patients with Noonan syndrome, two patients with Costello phenotype and three patients firstly diagnosed as having CFC syndrome. *HRAS* mutations were detected in 57% of Costello patients. Mutations in *KRAS*, *BRAF* and *MAP2K1/2* were found in 61% of patients with CFC syndrome. Mutations in *BRAF* or *MAP2K1* were detected in two patients with Costello phenotype. No mutations were found in *MAPK3/1* (ERK1/2), downstream of MEK1/2, in mutation-negative CFC patients. The analysis of 81 clinical manifestations in 25 mutation-positive CFC patients showed that wrinkled palms and soles, hyperkeratosis and joint hyperextension, which are important diagnostic features in Costello syndrome, were present in 30-40% of CFC patients. These results suggest that there is significant clinical and molecular overlap among these three syndromes.

Severe Liver Disease and Urea Cycle Disorders. *K. Cusmano-Ozog[1], S.L. Rutledge[2], A. Boneh[3], G. Gottesman[4], M. Tuchman[5], L. Pickler[6], J. Van Hove[6], G.M. Enns[1]* 1) Stanford University School of Medicine, Stanford, CA; 2) University of Alabama, Birmingham, AB; 3) The Murdoch Childrens Research Institute, Melbourne, Australia; 4) St Louis University School of Medicine, St Louis, MO; 5) Childrens National Medical Center, Washington, DC; 6) The Children's Hospital, Denver, CO.

Urea cycle disorders (UCD) typically present in the neonatal period with lethargy and hyperammonemia. Although mild transaminase elevations are common, severe liver disease is relatively rarely reported. We present 8 probands with UCD and significant liver disease. Three were diagnosed with citrullinemia, one male and two females, ranging in age from 3 days to 15 months. Five females were diagnosed with ornithine transcarbamylase (OTC) deficiency and ranged in age from 1 to 7 years. All were noted to have elevated transaminases; AST levels ranged from 101 to 3642 u/L, and ALT from 778 to 2991 u/L. Six of the individuals had a coagulopathy with PT 7-37.1 sec, aPTT 33-74 sec, INR > 1.9-9.4. Hyperammonemia was common (levels 115-400 uM). Four individuals had liver biopsies. One patient was referred for transplantation secondary to acute liver failure. Biopsy did not demonstrate significant pathology. A second patient was initially diagnosed with cholecystitis and underwent cholecystectomy. Liver pathology showed focal bridging fibrosis. Two patients continued to have elevated transaminases despite standard treatment. Liver biopsy from the third patient demonstrated focal hepatocellular necrosis and she underwent transplantation. Biopsy from the fourth patient revealed glycogen distended hepatocytes and diffuse microvesicular steatosis; she is being evaluated for transplantation. The pathogenesis of severe liver disease in UCD is unknown. A diagnosis of UCD, especially OTC deficiency in a female manifesting heterozygote, may be missed if not included in the differential for unexplained liver failure. We recommend obtaining a complete metabolic screen, including quantitative urine orotic acid in any individual that presents with liver disease of unknown etiology. Also, any individual with a UCD should be monitored for possible hepatic disease.

Urinary 1-hydroxypyrene: a biomarker for the effects of genetic polymorphisms on the metabolism of PAHs following different degrees of exposure. *B. Chen[1], M. Shao[2], Y. Hu[1], L. Zheng[1], Q. Wang[1], H. Liu[2], Y. Liu[2], T. Jin[1], D. Lu[2]* 1) Department of Occupational Health, Fudan University, Shanghai, China; 2) State Key Laboratory of Genetic Engineering, Institute of Genetics, School of Life Sciences, Fudan University, Shanghai, China.

In this study 1-hydroxypyrene (1-OHP) was used as a biomarker to explore the metabolism of polycyclic aromatic hydrocarbons (PAHs) following different degrees of exposure. Genetic effects on urinary 1-OHP levels were determined in 19 polymorphisms of 11 metabolizing enzymes including AhR, CYP1A1, CYP1A2, CYP1B1, CYP2E1, EPHX1, GSTM1, GSTT1, GSTP1, UGT1A1 and UGT1A6. According to individual occupational pyrene exposure, 437 coke oven workers (COWs) were divided into two exposure groups as low exposed COWs and highly exposed COWs, both of whom presented higher urinary 1-OHP levels compared with 257 non-occupational exposed controls. Using multiple linear regression models to analyze effects of single polymorphisms or gene-gene interactions on urinary 1-OHP levels, a totally different pattern of modifications was found in three groups: CYP1A1 3801T>C and CYP2E1 RsaI- were marginally significant in the controls, CYP1A1 3801T>C and UGT1A1 12493C>T were marginally significant in the low exposed COWs, whereas eight polymorphisms had marginal or significant effects in the highly exposed COWs including AhR 554K, CYP1A2 5347C>T, CYP2E1 RsaI-, EPHX -362G>A, UGT1A1 -3263T>G, UGT1A6 R184S, GSTP1 I105V and GSTT1 deletion type. The tissue speciality of gene expression and the pathways of pyrene metabolism taken into account, our results suggested that certain polymorphisms appeared to affect the levels of urinary 1-OHP. Unlike CYP1A1 and CYP2E1 which seem to be two major PAHs-metabolizing enzymes in low exposure individuals, the metabolism of pyrene was rather complex in highly exposed workers, in whom urinary 1-hydroxypyrene level might be modified not only by the polymorphisms of phase I enzyme including CYP1A2, CYP2E1 and EPHX1, phase II enzyme including GSTT1, GSTP1, UGT1A1 and UGT1A6, but also by the AhR encoding gene.

Association and Haplotype analysis of GLI1 gene with simple congenital Heart Disease in 12q13. *G.R. Qiu, L. G. Gong, X.Y. Xu, N. Xin, K.L. Sun* Medical Genetics, Basic Medicine, China Medical University, Shenyang, Liaoning, China.

In the candidate region 12q13 of simple Congenital Heart Disease (CHD),we chose four single nucleotide polymorphisms (SNPs) in GLI1 gene and investigated individual SNP distribution and haplotypes analysis in simple CHD patients and normal controls, in order to identidy whether GLI1 gene was the candidate for CHD or not. All subjects were from Northeast Chinese Han. We analyzed genotypes of 4 SNPs, that were C9455A, A10691G, G11388A, and G11888C, in 180 simple CHD patients and 200 normal controls by RFLP and DHPLC. Statistic analysis included Hardy-Weinberg equilibrium Assay, Association study at individual SNP, Linkage Disequilibrium Test, and Haplotype analysis. Our results showed that there was no polymorphism at A10691G in the Northeast Chinese Han. The distribution of allele frequency and genotype frequency at G11888C which was located in the coding-region of GLI1 gene, had significant difference between two groups ($P<0.005$). Linkage disequlibrium Test showed that there existed linkage disequilibrium among C9455A, G11388A and G11888C. Haplotype analysis showed that the distribution of haplotype had significant difference between two groups ($P<0.0001$). C9455/G11388/G11888 and C9455/A11388/G11888 were the common haplotypes in the population, but C9455/G11388/G11888 were much higher in CHD groups than that in normal controls ($P<0.05$). Conclusion Together with our results in family-based association and Haplotype analysis, G11888C located in the coding-region of GLI1 gene was associated with simple CHD, person with G allele at G11888C has much more risk with simple CHD. C9455/G11388/G11888 might be linked with the susceptibility gene of simple CHD.

Congenital hyperinsulinism in Kabuki syndrome. *D. Genevieve[1], D. Sanlaville[1], C. Bellane[2], C. Bernardini[1], A. Munnich[1], M. Vekeman[1], B. Isidore[1], M. Rio[1], V. Cormier-Daire[1], S. Lyonnet[1], P. de Lonlay[3], J. Amiel[1]* 1) Dept de Genetique, Hosp Necker-Enfants Malades, Paris, France; 2) Service de génétique moléculaire, Hôpital Saint Antoine, Paris, France; 3) Service de Maladies Métaboliques, Hôpital Necker-Enfants Malades, Paris, France.

Congenital hyperinsulinism (CHI - MIM 256450), is characterized by profound hypoglycemia related to inappropriate insulin secretion due to focal adenomatous hyperplasia or diffuse pancreatic insulin hypersecretion. Recently, several gene involved in CHI have been identified namely the KIR6.2, SUR, Glucokinase, GLDh and S-CHAD genes. Syndromic forms of hyperinsulinism are rare and include Perlman, CDG-Ib, Simpson-Golabi-Behmel, and Wiedemann-Beckwith syndromes. Here we report on 4 patients with clinical and biological evidences of CHI, each of which presented with Kabuki syndrome (KS), a MCA/MR syndrome characterized by distinctive facial features, post-natal growth retardation, feeding difficulties, dermatoglyphic anomalies, skeletal dysplasia and mental retardation. Treatment with Proglicem allowed to normalize glycemia in all 4 patients. Hyperinsulinism was transitory in 2 patients and persistent in 2 others with an exhaust to Proglycem requiring other treatments such as somatostatin. CHI was associated with clinical features consistent with diagnosis of KS namely distinctive facial features, post-natal growth retardation, dermatoglyphic anomalies and mental retardation. The molecular bases of KS are hitherto unknown, and several unrelated chromosomal anomalies have been reported. KIR6.2 and SUR gene mutations were excluded by direct DNA sequencing in our patients. We believe that KS is a newly recognized cause of syndromic CHI and that this diagnostic should be envision in CHI patients presenting with feeding difficulties, mental retardation, malformations and/or facial dysmorphism.

A genome-wide association study in breast cancer. *D. Easton[1], P.D.P. Pharoah[2], A.M. Dunning[2], K. Pooley[2], D.R. Cox[3], D. Ballinger[3], D. Thompson[1], D.G.R. Evans[4], D. Eccles[5], N. Rahman[6], M.R. Stratton[6], J. Peto[7], O. Fletcher[7], B.A.J. Ponder[2]* 1) CR UK Genetic Epidemiology, University of Cambridge, Cambridge, United Kingdom; 2) Deparment of Oncology, University of Cambridge, Cambridge, United Kingdom; 3) Perlegen Sciences, Mountain View,CA; 4) St Mary's Hospital, Manchester, United Kingdom; 5) Wessex Clinical Genetics Service, Princess Anne Hospital, Southampton, United Kingdom; 6) Institute of Cancer Research, Sutton, Surrey, United Kingdom; 7) London School of Hygiene and Tropical Medicine, London, United Kingdom.

Breast cancer exhibits familial aggregation, consistent with variation in genetic susceptibility to the disease. Less than 20% of the familial risk of breast cancer is accounted for by known susceptibility genes such as BRCA1&2, and the residual genetic variance is likely to be due to variants conferring more moderate risks. To identify further breast cancer susceptibility alleles, we conducted a two stage genome-wide association study. In the first stage, 227,876 SNPs were genotyped in 408 breast cancer cases with a positive family history and 400 controls. These SNPs report on ~64% of known common SNPs in HapMap, with a minimum r2 of 0.8. 12,026 SNPs showing the strongest associations in stage I were genotyped in a further 4,037 cases and 4,012 controls. After adjusting for the potential confounding effects of population stratification using genomic control, 69 SNPs showed significant associations with breast cancer at Ptrend<.0001, and 24 at Ptrend<.00001, compared with ~36 and ~5 respectively that would be expected by chance alone. For the most significant SNP, the estimated ORs in stage II were 1.26 (95%CI 1.12-1.34) in heterozygotes and 1.56 (1.38-1.77) in homozygotes (combined Ptrend=1.6×10^{-18}). The 30 most significant SNPs are being further evaluated in >20,000 cases and matched controls in an international consortium (BCAC). None of these 30 SNPs were in previously suggested candidate genes, and 16 were not within any known gene. These results indicate that susceptibility to breast cancer may be largely mediated through a large number of variants each conferring a small risk of disease.

Elastogenesis in MPS IVA patients fibroblasts and identification of mutations in GALNS gene. *L. Carraresi*[1], *M.A. Donati*[1], *A. Caciotti*[1], *E. Procopio*[1], *C. Valleriani*[1], *R. Parini*[2], *G. Sersale*[2], *D. Antuzzi*[3], *R. Ricci*[3], *O. Gabrielli*[4], *G. Parenti*[5], *M. Sibilio*[5], *M. Filocamo*[6], *S. Tomatsu*[7], *A. D'Azzo*[8], *A. Morrone*[1], *E. Zammarchi*[1] 1) Pediatrics,Florence Italy; 2) S.Gerardo Hosp,Monza; 3) Catholic Univ,Rome; 4) Ped Clinic,Ancona; 5) Pediatrics,Naples; 6) Gaslini Inst,Genoa; 7) Pediatrics,St. Louis,USA; 8) Genetics,St.Jude,Memphis.

Mucopolysaccharidosis IVA (MPS IVA, Morquio A) is an autosomal recessive storage disorder caused by deficiency of lysosomal N-acetylgalactosamine-6-sulfate sulfatase (GALNS), that hydrolyses the sulphate ester groups of keratan sulphate (KS) and condroitin-6-sulfate (C6S). MPS IVA patients show a broad spectrum of clinical severity. Classical forms are characterised by severe bone dysplasia and normal intelligence. We describe 9 unrelated MPS IVA patients with different clinical phenotypes. Molecular analysis of the patients GALNS gene identified 9 known and the following 5 new mutations: H142N, C507L, K129X, c899-1GC, c1365-1GA. At RNA level, the mutation c899-1GC causes exon 9 skipping, resulting in a frameshift and early stop codon; instead, no transcript was detected in patients with the known c120+1GA mutation. The H142 is highly conserved among eukaryotic sulfatases, suggesting that H142N is responsible for a severe phenotype. It has been shown earlier that in patients with GM1-gangliosidosis and Morquio B disease a primary defect in the Elastin Binding Protein (EBP) important for elastic fiber assembly results in impaired elastogenesis. The latter phenotypic aberration was also demonstrated in patients with secondary EBP defect due to accumulation of C6S (Costello syndrome), dermatan sulphate (Hurler disease) and KS (GM1 gangliosidosis). Based on these results, we have now tested whether accumulation of KS and C6S in MPS IVA also leads to impaired elastogenesis. Quantitative Real-Time PCR analysis carried out on total RNA from patients fibroblasts showed no variations on EBP mRNA levels in comparison with controls. Otherwise, on Western blots probed with an anti-EBP antibody the expression levels of EBP appeared reduced in some patients fibroblasts, suggesting a secondary deficiency of this protein also in MPS IVA.

Long Distance Transcription Networks Characterize ENCODE Regions. *S.E. Antonarakis[1], F. Denoeud[2], A. Reymond[1,3], P. Kapranov[4], A. Frankish[5], J. Harrow[5], R. Guigó[2], T.R. Gingeras[4], ENCODE Project Consortium* 1) Genetic Medicine Dpt, University of Geneva Medical School, Switzerland; 2) Center for Genomic Regulation; Barcelona, Catalonia, Spain; 3) Center for Integrative Genomics; University of Lausanne, Switzerland; 4) Affymetrix, Inc.; Santa Clara, CA, USA; 5) European Bioinformatics Institute; Wellcome Trust Genome Campus, Hinxton, Cambridge, UK.

As part of the Encyclopedia of DNA Elements (ENCODE) project, the location of transcription sites across 1% of the human genome has been determined in multiple cell lines and tissues using tiling microarrays. There is a complex organization of lattice-like networks of transcription indicating that intergenic regions comprise only 10% of the genome. 15% of the ENCODE sequences are detected as processed transcripts, of which >30% correspond to previously unannotated transcription. These novel transcription sites are not under detectable selective evolutionary constraints. 5 RACE/microarray tiling array experiments of 359 annotated genes in 12 tissues, revealed the presence of many novel exons. Almost half (2,324 of 4,573) of all RACEfrags correspond to unannotated sites of transcription. Most of novel RACEfrags are cell lines/tissue specific. RACEfrags provide evidence of novel exons for 90% of the loci successfully assayed (316 out of 359). For 261 protein coding genes (66% of loci), RACEfrags detect distal novel exons, extending the loci beyond the annotated 5 boundaries. The average distance of these new distal RACEfrags is between 50-100 kb (in 20% >200 kb) from the "known" transription start site. In 224 (62% of loci), the extended RACEfrags reach to another upstream protein-coding gene, often transversing many intervening genes. In 180 of these (50% of loci) the elongated transcripts appear to incorporate exons of another upstream protein-coding transcript (chimeric transcripts). A total of 538 RACEfrags, were analyzed using RT-PCR, and tiling array resolution, cloning and sequencing. 314 (58%) of the 538 exons provided RT-PCR/microarray results confirming connectivity of the distal exon with a site within the downstream coding transcript.

FXR1P modulates FMRP affinity for G-quartets structure, increasing the dynamics of FMRP/FXR1P/RNA complex. *B. Bardoni[1], E. Bechara[1], L. Davidovic[1,2], M. Melko[1], M. Bensaid[1], E.W. Khandjian[2], E. Lalli[3]* 1) Dep.Human Genetics, CNRS UMR6543, Nice, France; 2) Dep. of Medical biology, Universite' Laval,Quebec, Canada; 3) IPMC, CNRS UMR 6097, Valbonne sophia Antipolis, Nice, France.

Fragile X syndrome is the most frequent form of inherited mental retardation and is due to silencing of the Fragile X Mental Retardation gene 1 (FMR1). FMR1 encodes FMRP, an RNA binding protein involved in several steps of mRNA metabolism: nucleocytoplasmic trafficking, translational control and also transport to specific locations for localized translation (e.g. synapses in neurons). Even if several hundreds of mRNAs have been identified as putative targets for FMRP, up to date only 3 sequences/structures have been shown to be bound with high specificity by FMRP: G-quartets forming structure, kissing complex and poly(U) stretches. FMR1 is a component of a family including two other genes: fragile X Related gene 1 (FXR1) and Fragile X Related gene 2 (FXR2), coding for the two proteins FXR1P and FXR2P, respectively, which have high homology with FMRP particularly at the level of functional domains: KH, RGG box, Nuclear Localization Signal (NLS) and Nuclear Export Signal (NES). In addition, the 3 proteins are able to interact forming heterodimers and larger complexes. FXR1P/2P are supposed to have a function very similar and probably overlapping with FMRP. As a consequence, they have been hypothesized to be able to partially compensate FMRP absence in Fragile X patients. In this study we test the ability of FXR1P to bind the G-quartet forming RNA structure and its ability to influence FMRP affinity for this structure. Indeed, FXR1P and FMRP mutually increase their affinity for G-quartet RNA, increasing the dynamics of the FXR1P/FMRP/RNA complex formation. These findings suggest that FXR1P has a non-redundant action with FMRP in modulating its RNA binding capacities. This result may be important to decipher the molecular basis of fragile X syndrome, through the understanding of the action of FMRP in the context of its containing multimolecular complex.

Mutational analysis of EFHC1 gene in Italian families with Juvenile Myoclonic Epilepsy. *F. Annesi*[1], *G. Annesi*[1], *A. Gambardella*[1,2], *Gruppo di studio Lega Italiana Contro lEpilessia*[3] 1) Inst of Neurol Sciences, National Research Council, Mangone, Cosenza, Italy; 2) Institute of Neurology, University Magna Graecia, Catanzaro, Italy; 3) LICE.

In this study, we screened for mutations in the EFHC1 gene 25 families from Italy in which at least two members had a typical form of Juvenile myoclonic epilepsy (JME). JME is a common form of generalized epilepsy starting in adolescence. Recently, a major JME locus was mapped to chromosomal region 6p12.p11 and it was associated with mutations in the EFHC1 gene. This gene contains 11 exons and encodes a protein of 640-amino acids that contains 3 DM10 domains and an EF hand calcium-binding motif. Twenty-five families were selected and families with fewer than two affected members were excluded from the study. In each patient the diagnosis of JME was done according to the ILAE criteria. After informed consent, DNA was isolated from peripheral blood lymphocytes by standard methods and each exon of EFHC1 gene was amplified and sequenced using intronic primers. We have identified three heterozygous mutations: the F229L mutation, previously described, and the novel mutations P429P and R353W among affected members of 5 unrelated families. EFHC1 gene has been associated with JME in six out 44 families from Belize, Los Angeles, and Mexico. The authors detected 3 heterozygous mutations (F229L, D210L, D253Y) and one double heterozygous mutation (P77T, R221H). The results of our study are important as they extend for the first time the distribution of EFHC1 mutations to Caucasian populations. Moreover, our data provide further evidence for the high level of genetic heterogeneity associated with JME, as most of our JME families did not carry any mutations. This study was partially supported by Italian League Against Epilepsy.

Sense and antisense RNAs are transcribed from a loosened heterochromatic structure at D4Z4 in Facio-Scapulo-Humeral Muscular Dystrophy. *M. Rossi[1], A. Cellini[1], E. Ricci[1], P. Tonali[1], L. Felicetti[2], P. Venditti[1]* 1) Neurology, Catholic University, Rome, Italy; 2) UILDM, Rome section.

The 3.3 kb KpnI tandemly repeated sequences are implicated in Facio-Scapulo-Humeral Muscular Dystrophy (FSHD). In healthy individuals the 4q subtelomeric repeat array (D4Z4) varies between 11 and more than 100 copies; a decrease in the number of units below the threshold number of 10 leads to disease. The underlying molecular mechanism still remains largely unsolved, even though several lines of evidence suggest a chromatin involvement in the pathogenesis of the disease. We probed healthy and FSHD affected individuals for specific hallmarks of condensed chromatin structure, namely DNA methylation and histone-H3 lysine-9 methylation. We could confirm DNA hypomethylation of D4Z4 in FSHD patients, even though bisulfite allele-specific genomic sequencing revealed a complex methylation pattern within the tandem repeat unit, with highly methylated regions alternating with undermethylated ones. Accordingly we found a relief in the histone-H3 lysine-9 methylation status in affected individuals versus unaffected at D4Z4. Finally we report that the loss of proper heterochromatinization in FSHD patients is associated with aberrant transcription of ncRNA species within the repeats that could deregulate the gene expression program of the cell.

The association of two SNPs in the brain-derived neurotrophic factor (BDNF) gene with depression: findings from the British Women's Heart and Health study. *L. Chen[1], D. Lawlor[1], I. Day[2], S. Lewis[1], S. Ebrahim[3], G. Davey-Smith[1], Y. Yao[1,4]* 1) Department of Social Medicine, University of Bristol, Bristol, UK; 2) Human Genetics Division, University of Southampton, School of Medicine, Southampton, UK; 3) Department of Epidemiology and Population Health,London School of Hygiene & Tropical Medicine, London, UK; 4) Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, USA.

Depression remains to be a significant health concern with a lifetime risk of up to 17%. Both clinical and pharmacologic studies have implicated the brain-derived neurotrophic factor (BDNF) gene as a susceptibility locus for the development of depression. However, previous genetic association studies have not consistently exhibited the relationship between the mutation allele of BDNF (Val66Met) and an increased risk of depression. This study aimed to test for an association between BDNF and depression status by examining the distribution of two functional single nucleotide polymorphisms within BDNF locus, -270 CT in the promoter region and Val66Met in the BDNF coding region in three case groups; 1) currently taking anti-depressant (n=406), 2) positive response to EuroQol mood question (n=811), and 3) ever diagnosed as depression (n=553) and 2,367 ethnically matched controls. All of the participants in the study were recruited into the British Women's Heart and Health Study (total participants =3548, aged between 60 and 79). The minor allele frequencies for -270 CT and Val66Met were 0.05 and 0.20, respectively. The D prime between the two SNPs is 1.0 with the R-square of 0.014. We did not detect evidence for positive associations between the above genetic polymorphisms and depression status when the two SNPs were analyzed separately. However, when we analyzed two polymorphisms together as a haplotype, the prevalence of case group reporting ever diagnosed as depression; was marginally higher in the women with the TG haplotype than among those carrying CG haplotype with an odds ratio of 1.25 (95% confidence interval = 0.94 -1.66) (after adjust for BMI). We would like to note that this potential finding needs further confirmation in samples of greater size.

Dynamics of GLB1 gene expression levels by RT PCR and molecular analysis of GM1 gangliosidosis and Morquio B patients. *A. Caciotti[1], M.A. Donati[1], E. Procopio[1], M. Filocamo[2], W. Kleijer[3], W. Wuyts[4], B. Blaumeiser[4], A. DAzzo[5], L. Simi[6], C. Orlando[6], F. McKenzie[7], A. Fiumara[8], E. Zammarchi[1], A. Morrone[1]* 1) Dept Pediatrics,Florence,Italy; 2) Gaslini Inst,Genoa,Italy; 3) Dept Clin Genet,Rotterdam,Holland; 4) Dept Med Gen,Wilrijk,Belgium; 5) Dept Genet,St Jude Childrens Hosp,Memphis,USA; 6) Dept Clin Physiopath,Florence,Italy; 7) Hunter Genet,Waratah,AU; 8) Dept of Ped,Catania,Italy.

The human GLB1 gene gives rise to two alternatively spliced transcripts that encode the -galactosidase (GLB1) and the elastin binding protein (EBP). Mutations at the GLB1 locus, responsible for the lysosomal disorder GM1 gangliosidosis and Morquio B, may affect both proteins or GLB1 only. EBP acts as a molecular chaperone, protecting tropoelastin from degradation and facilitating its assembly into a microfibrillar scaffold and, when affected, contributes to specific clinical features of GM1 gangliosidosis patients. We report the development of a quantitative assay based on Real-Time PCR for assessing levels of GLB1 and EBP transcripts in patients samples. We also characterized the GLB1 gene mutations in 1 Morquio B and 9 GM1 gangliosidosis patients in order to correlate the genetic lesions with mRNA expression levels and clinical phenotypes. Mutation analysis led to identification of 5 new mutations (c.18351836delCC; p.R148C; c.1068+1GT; c.245+1GA; p.P549L) 6 known (p.R59H; p.R201H; p.G123R, p.W273L, c.1480-2AG; c.75+2dupT) and 1 new polymorphism (c.1233+8TC). Quantitative RT-PCR on total RNA from all patients fibroblasts showed differential levels of GLB1 and EBP mRNA that correlated with the mutation type. The three GM1 gangliosidosis patients carrying splicing defects had reduced levels of both mRNA. One of these patients, homozygous for the c.245+1GA mutation, showed a complete absence of the EBP mRNA. Comparative analysis of the patients' phenotypes will enable a more thorough correlation between GLB1 genetic lesions and specific clinical manifestations. This fast method for detection of GLB1 gene alternatively spliced transcripts could be applied to other lysosomal disease-causing genes that give rise to multiple mRNAs.

Further evidence that D90A mutation is recessively inherited in ALS patients in Southern Italy. *F.L. Conforti, T. Sprovieri, R. Mazzei, C. Ungaro, A. Patitucci, A. Magariello, AL. Gabriele, M. Muglia, A. Quattrone* Institute of Neurological Sciences, Mangone, Cosenza, Italy.

Amyotrophic Lateral Sclerosis (ALS), an adult-onset motor neuron degeneration is caused by mutations in the Cu/Zn superoxide dismutase (SOD1) gene. All the SOD1 mutations are autosomal dominantly inherited with the exception of D90A, very frequent in Scandinavian population, and D96N which can act as recessive. Only few cases of D90A heterozygous ALS in Belgian and Russian patients have been reported, all of them inherited as dominant trait. Up to now, in Italy, only two sporadic ALS cases carrying the D90A mutation have been reported in homozygous state. The aim of this study is to investigate the presence of D90A mutation in ALS patients in Southern Italy. One hundred and fifty-four ALS patients (8 familial ALS and 146 sporadic cases, 76 men and 78 woman; mean age 60.32 years, SD 10.22, range 36 to 88 years) from Southern Italy were screened for SOD1 mutations in exon 4. DNA was extracted by standard procedure and PCR products were subjected to enzymatic digestion with Fnu4H I and analyzed by direct sequencing. In our study, of 154 cases investigated three ALS patients (2%)with mild phenotype showed the homozygous D90A mutation. Two out of three patients were familial cases(FALS)and the remaining patient was an apparently sporadic case (SALS). We also screened the available members of one of the FALS cases. In this family we identified an affected and an unaffected individual carrying the D90A mutation in homozygous and heterozygous state respectively. Our study provides further evidence that D90A is an autosomal recessively inherited mutation in ALS patients in Southern Italy. Previous data reported that all ALS patients homozygous for the D90A show a phenotype characterized by slow progression of the disease, with little variation between patients. According to the literature, our patients show a mild phenotype with a prolonged survival. An explanation for the recessive and the dominant inheritance of the D90A, and the different expressed phenotype was suggested by Parton and coll. who indicated the presence of a cis-acting disease modifier in the recessive haplotype.

Neurofibromatosis type 2 (NF2) in children under 1 year of age: a clinical and molecular study. *A.L. Gabriele[1], M. Ruggieri[2,3], C. Nucifora[3], A. Patitucci[1], T. Sprovieri[1], A. Magariello[1], R. Mazzei[1], F.L. Conforti[1], C. Ungaro[1], M. Muglia[1], A. Quattrone[1,4]* 1) Institute of Neurological Sciences (ISN)- CNR, Mangone, Cosenza, Italy; 2) ISN, CNR, Section of Catania, Italy; 3) Department of Paediatrics, University of Catania, Italy; 4) Institute of Neurology, University of Magna Graecia, Catanzaro, Italy.

To identify the earliest (i.e., disease onset < 1 year of age) clinical presentations of neurofibromatosis type 2 (NF2) 1,2, to characterise its natural history and to investigate the NF2 mutations at this young age 3.Two NF2 children (1 boy, 1 girl), out of the 28 (aged 4-18 years) diagnosed as having the disease according to current NF2 criteria 1, had their first disease manifestations under 1 year of age: both were prospectively followed up and investigated according to our protocol for paediatric NF2 2. Molecular analysis of the NF2 gene was carried out by means of Denaturing High Performance Liquid Chromatography (DHPLC) and sequence analysis.The boy (aged 7 years) presented at age 4 months with right lens opacities and imaging evidence of bilateral colpocephaly and high signal (calcified) lesions in the posterior periventricular regions. MRI scans at age 8 months confirmed these findings and revealed bilateral vestibular schwannoma. He developed multiple (> 40) skin NF2-plaques in the limbs with no neurological or hearing dysfunction. At his last head and spinal MRI the schwannoma and the periventricular lesions did not progress with any additional lesions. The girl (aged 10 years) presented at age 4 months with pigment retinal hyperplasia misdiagnosed as retinal folds. She was first referred to us at age 7 years for a pelvic bilateral plexiform schwannoma (operated). At that age the child had multiple (> 20) NF2-plaques in the four limbs; head and spinal MRI scan revealed bilateral vestibular schwannoma, high signal lesions in the temporal periventricular regions and a cervical meningioma (operated). At his last MRI control the vestibular schwannoma and the high signal lesions were stable. No neurological or hearing deficits ensued. Molecular genetic analysis carried out in both children revealed so far a novel mutation in the exon 3 of the NF2 gene in the boy: this mutation was a small insertion of 4 bases pair (c.281_282 ins CCTT). This mutation was not detected in 100 control chromosomes from matched healthy individuals. In 5 previous NF2 cases disease onset was < 1 year of age : to the best of our knowledge however this is the first time 2 that: (1) bilateral eight nerve tumours in NF2 children do not show progression after a long follow-up period; and (2) NF2 children develop large numbers of skin NF2-plaques in atypical localisations.

Natural history of 1p36 deletion syndrome: experience with 60 cases. *A. Battaglia[1,2], J.C. Carey[2], H.E. Hoyme[3], ICSG* 1) Stella Maris Inst. Pisa, Italy; 2) Medical Genetics, Dept. Pediatrics, University Utah, SLC, USA; 3) Medical Genetics, Dept. Pediatrics, Stanford University, USA.

From the recent literature it appears that 1p36 deletions account for 0.15-1.2% of idiopathic DD/MR. 1p36 deletion syndrome is a newly recognized segmental aneusomy condition with an estimated incidence of 1/5,000 newborns. Although about 90 cases have been reported so far, there is still little data on its natural history. Information given to parents at the time of diagnosis tends to be skewed to the extreme negative. To help delineate more thoroughly the natural history of monosomy 1p36, and to obtain better information to answer parents questions in a clinical setting, we evaluated 60 patients (female/male ratio: 2/1), in different centers, with the syndrome. One third of them were detected by standard cytogenetics, whereas the other two thirds by subtelomeric FISH analysis. OFC was at/below the 2 centile, and height/weight ranged between <3rd-50th centile. All patients had a distinct craniofacial appearance with tower skull, prominent forehead, deep-set eyes, straight eyebrows, epicanthus, midface hypoplasia, broad nasal root/bridge, long philtrum, pointed chin; associated with brachy/camptodactyly, and short feet. 70% of the patients had CNS anomalies (ventricular dilatation; cortical atrophy; multifocal white matter T2 hyperintensity); 70% had heart defects (ventricular myocardium non-compaction [n=15]; ASD; VSD; PDA; tetralogy of Fallot); 50% had seizures (infantile spasms; partial and generalized seizures); 40% had hearing impairment; 40% had skeletal anomalies; 100% had mild/profound DD/MR, and 95% had hypotonia. 80% had no speech, whereas the remaining 20% pronounced either isolated words or simple words associations. Just over 25% was able to walk alone, and 70% had a behavior disorder. A slow, but constant progress in development was observed in all cases overtime. The non-compaction of the ventricular myocardium, and all seizure types were well controlled by the usual pharmacotherapy. In conclusion, the combined cases of our sample represent considerable experience, providing new information on several aspects of this important and frequent deletion syndrome.

UNUSUAL CO-EXISTENCE OF MILD PKU AND FABRY DISEASE: CASE REPORT. *D. Concolino[1], M. Rapsomaniki[1], M.T. Moricca[1], E. Disabella[2], E. Arbustini[2], P. Strisciuglio[1]* 1) Department of Pediatrics, University of Catanzaro, Catanzaro, Italy; 2) Department of Cardiology, I.R.C.C.S. Policlinico San Matteo, Pavia, Italy.

Phenylketonuria is inborn error of the metabolism resulting from a deficiency of phenylalanine hydroxylase. Fabry disease (FD) is an X-linked lysosomal storage disorder due to a deficiency of the enzyme alpha-galactosidase A (GALA) with subsequent accumulation of globotriaosylceramide in the lysosomes of various tissues leading to clinical symptoms. The GALA gene is localized on Xq22.1 and over 300 mutations have been reported.The disease affects kidney, myocardium, central nervous system, skin and, in many patients, the gastrointestinal tract. FD usually becomes clinically manifest in childhood with generalized pain, acroparaestesia, non-specific GI symptoms and hearing problems. We report here the discover of Fabry disease in a 3 year- old boy with mild PKU detected at birth by neonatal screening.The patient was first seen in our department at the age of 20 days because affected by mild PKU and was subjected to a dietetic treatment with a good compliance. Growth, neurological and psychological development, were normal. At the age of 21 months he showed a reduction of the weight, lack of appetite, abdominal pain and periodically appearance of not specific attacks of gastroenteritis resolved spontaneously and not correlated with the dietetic treatment. These symptoms associated to a familiar history of renal failure and cerebral stroke on young familiars, suggested a possible Fabry disease. The reduced plasma a-galactosidase A, decreased GALA activity in leucocytes and the molecular analysis (Arg 112 His in exon 2) supported our hypothesis.This co-existence of mild PKU and Fabry disease is very rare and made very difficult to attribute the clinical symptoms of lack of appetite, abdominal pain and non-specific gastroenteritis attacks to an other disease different from PKU.This case helps to define the clinical phenotype of Fabry disease in children for early diagnosis. The ERT treatment should be initiated at an early stage, prior to the onset of irreversible complications.

Autosomal dominant seborrhea-like dermatitis caused by a mutation in *ZNF750*, a novel putative C2H2 zinc finger protein. *R. Birnbaum, A. Zvulunov, D. Hallel-Halevy, R. Ofir, E. Cagnano, O.S. Birk* The Morris Kahn Laboratory of Human Genetics, National Institute for Biotechnology and Soroka Medical Center, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel.

Seborrheic dermatitis (SD) and Psoriasis are common chronic papulosquamous dermatoses. Occasionally, distinction between these entities can be obscured by overlapping clinical features, particularly when the manifestations are limited to the scalp and face. An Israeli Jewish Moroccan family (44 affected individuals in 5 generations) presented with autosomal dominant seborrhea-like dermatosis with psoriasiform elements: enhanced keratinocyte proliferation, parakeratosis, follicular plugging, *Pityrosporum ovale* overgrowth, and CD4 lymphocyte infiltrate. Through genome wide linkage analysis we mapped the disease gene to a 0.5cM region on 17q25 (maximum lod score 8.8 at theta=0), within a locus previously associated with psoriasis (*PSORS2*). Sequencing of candidate genes within that locus identified a mutation in *ZNF750* ,a novel putative transcription factor. The mutation fully abrogates the putative active domain of the encoded protein. The gene is normally expressed in keratinocytes but not in fibroblasts or CD4 lymphocytes (activated or not), suggesting that the primary event leading to the disease is in keratinocytes. *ZNF750* is the first gene shown to be associated with SD, and the first gene associated with Mendelian heredity of a non-arthritic variant of psoriasis.

Germline and somatic mosaicism at the PHOX2B locus in Late-Onset Central Hypoventilation syndrome. *J. Amiel, D. Trochet, L. de Pontual, A. Munnich, S. Lyonnet* Genetics, Hôpital Necker, University Paris5, Paris, France.

Idiopathic late-onset central hypoventilation syndrome (LOCHS) is a rare disorder occurring from early childhood to adulthood. The physiopathological relationships between LOCHS and congenital central hypoventilation syndrome (CCHS) have been debated and the question of whether both disorders can result from heterozygous PHOX2B gene mutations. Here we report a series of 25 patients with LOCHS referred from 6 months of age to adulthood. We identified a heterozygous PHOX2B gene mutation in 13/25 patients, the most frequent mutant allele resulting in a +5 alanines expansion of the series of 20 alanines C terminal to the homeodomain of the protein (10 cases). In vitro studies showed a significantly reduced PHOX2B transactivation for all mutations except the +5 alanines expansion. However, spontaneous formation of oligomers occurred starting at +5 alanines expansion suggestive of misfolding mutant proteins. Most mutations were germline while some were somatic mosaics. This has major consequences in terms of genetic counselling. In this series, one adult with LOCHS had a child with CCHS. This raises the question of the follow-up of apparently healthy parents of a CCHS child and found to harbour a somatic mosaic fro a PHOX2B gene mutation (5% of the parents in our series). Among patients with no PHOX2B mutation identified, 3 presented also hypothalamic-related endocrinopathies and behavioral problems, suggesting genetic heterogeneity of idiopathic LOCHS. Altogether, these data demonstrate a genetic link between CCHS and, at least, a subgroup of LOCHS. Genetic heterogeneity of LOCHS is however likely. Furthermore, combined genetic and environmental factors may explain the variability of disease onset ranging for neonatal period to adulthood for an identical PHOX2B gene mutation (+5 alanines expansion).

Methylation Specific PCR (MSP) analysis of APC tumor suppressor gene promoter in adenocacinoma of stomach. *M.R. Alivand[1,2], F. rastgar[1], M. zare[1,2]* 1) biochemistry, National Institute of Genetic Engineering and Biot, Tehran, Tehran, Iran; 2) khatam Institute of Higher Education;depattment of biology,Tehran.

Adenocarcinoma of stomach is among cancers with high mortality rate in the world, for which a high incidence rate has also been reported from Iran. The cancer could only be diagnosed when it is well developed and diffused out to the adjacent tissues which make treatment almost impossible. In order to diagnose and study the molecular basis of the disease, recently attentions were focused on the methylation status of promoters of tumor suppressor genes including Adenomatous Polyposis Coli (APC) both in tissues and preferentially serum. By applying Methylation Specific PCR (MSP), here we studied methylation status of the APC promoter. Our results indicate methylation of APC promoter in %66.6 of tissue samples. We further extended our study to the serum of such patients and the results indicate almost the same percentage of promoter methylation. This report is among the first reports that indicate MSP is also applicable for analyzing APC promoter methylation status in serum samples which clinically is important for diagnosis and evaluation of treatment.

Single nucleotide polymorphisms of DNA repair gene *XPC* and risk of lung cancer in a Chinese population. *Y. Bai[1], J. Yuan[1], Z. Hu[2], X. Yang[1], F. Wang[1], L. Xu[3], M. Shao[4], Y. Wang[4], W. Yuan[3], J. Qian[4], H. Ma[2], Y. Wang[3], H. Liu[4], F. Chen[2], Y. Liu[4], L. Jin[4], Q. Wei[5], H. Shen[2], D. Lu[4], T. Wu[1]* 1) Institute of Occupational Medicine and Ministry of Education Key Lab for Environment and Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China; 2) Department of Epidemiology and Biostatistics, Cancer Research Center of Nanjing Medical University, Nanjing, China; 3) Department of Genetics, Chinese National Human Genome Center at Shanghai, Shanghai, China; 4) State Key Laboratory of Genetic Engineering, School of Life Sciences, Fudan University, Shanghai, China; 5) Department of Epidemiology, The University of Texas M. D. Anderson Cancer Center, Houston, TX, USA.

DNA repair is a major defense against environmental damage to the genome and protect the cell from carcinogenesis. In the nucleotide excision repair (NER) pathway, xeroderma pigmentosum C (XPC) repair protein participates in recognition of DNA damage and initiation of DNA repair. Polymorphisms of the *XPC* gene are thought to have an effect on DNA repair capacity and susceptibility to cancer. In this study, we investigated five *XPC*-related tagSNPs in a case-control study of 1010 patients with newly diagnosed lung cancer and 1011 controls matched on age and sex. In individual tagSNP analysis, we found that the rs3731055AG+AA genotype was associated with a significantly decreased risk of lung adenocarcinoma, but an increased risk of small cell carcinomas. Accordingly, we also found that the haplotype ACCCA was associated with decreased risk of lung adenocarcinoma, but increased the risk of small cell carcinomas, which reflected the presence of rs3731055A allele in this haplotype. Further combined analysis of all five polymorphisms, we found that compared with subjects with 0-3 risk alleles, those having 4-6 risk alleles had a significantly 1.26-fold increased risk of lung cancer, and this association was more evident in the subgroups of young peoples (age < 60), males, light smoker and patients with lung adenocarcinoma. These results suggest that the inherited variation in *XPC* may modulate the risk of lung cancer, especially lung adenocarcinoma.

Estimating the age of four common CFTR mutations in Brittany (western France). *Y. Fichou*[1], *E. Génin*[2], *C. Le Maréchal*[1,3], *V. Scotet*[1], *C. Férec*[1,3] 1) INSERM U613, Brest, F-29220 France; UBO, Faculté de Médecine de Brest et des Sciences de la Santé, F-29238 France; 2) INSERM U535, Villejuif, F-94817 France; 3) EFS-Bretagne, Brest and Centre Hospitalier Régional Universitaire (CHRU) Brest, Hôpital Morvan, Brest, F-29220 France.

Cystic fibrosis (CF) is the most common autosomal recessive disorder in Caucasian populations, characterized by inflammation and chronic infections progressively leading to alteration of pulmonary functions, abnormal exocrine gland secretions, digestive troubles and pancreatic insufficiency. To date, more than 1,400 mutations and sequence variations have been reported (www.genet.sickkids.on.ca/cftr) within the CF transmembrane conductance regulator (CFTR) gene, the CF-causing gene which encodes a cAMP-dependent chloride channel. The F508 mutation, which consists of the deletion of a phenylalanine aminoacid at position 508 of the protein, accounts for 70% of the total mutated alleles and only four additional mutations have a frequency 1% in the general population. However, disparities exist between specific populations depending on their ethnical and/or geographical origins. We have used a simple likelihood-based method to date the most recent common ancestors carrying four CFTR mutations that are preferentially observed in Brittany (western France): $W846X_2$, 1078delT, G551D and F508 mutations. This approach, which assumes that all haplotypes have diverged from a common ancestor, has proven useful for estimating rare mutations (Génin *et al.*, 2004). Ten microsatellite markers flanking the CFTR gene were systematically genotyped in CF patients and relatives (200 individuals) carrying at least one of the mutations of interest. Following phase reconstruction and calculations of haplotype frequencies, it was estimated that the most recent common ancestors lived 625 [375-1,025], 1,000 [750-1325], 1,200 [900-1,550] and 2,875 [2275-3700] years ago, respectively, with a 95% confidence interval. These estimations suggest, as expected, that the F508 mutation is an old event. Furthermore, they also give evidence for important migrations of populations in western Europe in the second part of the first millenium.

An improved genomic control method for association studies: LGC. *W. Fu*[1], *Y. Wang*[1], *L. Jin*[1, 2] 1) State Key Laboratory of Genetic Engineering, School of Life Sciences, Fudan University, Shanghai, China; 2) CAS-MPG Partner Institute for Computational Biology, Shanghai, China.

The presence of undetected population structure with differential disease prevalence among subpopulations can lead to either false positive results or failures to detect genuine associations. One method for correcting such problem is genomic control (GC). The GC method is based on the assumption that the variance inflation factor () is a constant across the genome for all null loci. This assumption leads to under- or over-estimation of which varies greatly across the genome. We therefore propose a locus-specific genomic control method (LGC) to circumvent the problem. We first estimated the , a constant that reflects the difference of the prevalence among subpopulations. The along with the locus-specific Fst can be used to estimate the locus-specific variance inflation factor $_L$ for correcting for the population structure for each locus in association studies. Using simulation, we will show that the improved genomic control method (LGC) performs better than its original version in association studies.

Molecular characterization of the reciprocal translocation t(4,7)(q26;p15) in a patient with mental retardation and congenital defects. *C. Fusco[1], L. Micale[1], S. Calvano[1], B. Augello[1], A. Reymond[2], M. Rocchi[3], L. Zelante[1], G. Merla[1]* 1) Medical Genetics Unit , IRCCS Casa Sollievo della Sofferenza Hospital, S.G. Rotondo, Foggia, Italy; 2) Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland; 3) Department of Genetics and Microbiology, University of Bari, Bari, ItalySwitzerland.

De novo translocations are a powerful tool for the identification of genes associated with complex phenotype and specific disease. We report the case of a 2 years-old subject affected by mental retardation, speech delay and congenital defects. The patient was hypotonic and showed facial phenotype characterized by unilateral strabismus, horizontal palpebral fissures, open mouth with cupids bow, folded down helix. Other congenital defects were bilateral cryptorchidism, pulmonic stenosis and syndactyly of 2nd and 3rd toes. High resolution chromosome analysis showed an apparently balanced reciprocal translocation t(4,7)(q26;p15). Karyotype analysis revealed that parental chromosomes were normal, indicating that the patient's translocation was de novo. FISH analysis demonstrated that the clones RP11-954B12 and RP11-281H13 mapped to both the derivative chromosomes indicating that these BACs spanned the chromosomes 7 and 4 breakpoints, respectively. The HSA7 region covered by BAC RP11-954B12 is rich in Alu repeats and devoid of annotated genes and mapped ESTs, while clone RP11-281H13 encompasses two genes. Using Long PCR, we created overlapping fragment to perform additional FISH analysis to narrow the breakpoint. This approach identified a 10 kb fragment hybridizing to both derivatives. It spans exons 9 to 12 of a novel gene, LOC91431 that encodes a putative helicase. Preliminary characterization showed that this gene is expressed in several tissues such as spleen, colon, muscle, lung, fetal liver, kidney and heart and that its product has a diffuse cytoplasmic and nuclear distribution in transfected HeLa cells. We propose that the complex phenotype observed could be caused by lack of LOC 91431 gene integrity with consequent deregulated gene expression and protein level.

Combining case-control association and allele frequency differentiation to detect malaria resistance genes. *G. Ayodo[1,2,3], A. Ajwang[3], A. Keinan[1,2], A. Price[1,2], M.F. Otieno[3], N. Patterson[2], A.S.S. Orago[3], D. Reich[1,2]* 1) Dept. of Genetics, Harvard Medical School, USA; 2) Broad Institute of MIT & Harvard, USA; 3) Dept. of Pre-Clinical Sciences, Kenyatta University, Kenya.

Nineteen genetic variants have been identified as conferring resistance to severe malaria, but only a few have been replicated, and most studies have been limited to West Africans. We report a replication study in 460 cases from the Luo ethnic group and 470 Luo controls. We confirm that heterozygosity for the sickle-cell trait HbAS ($P<0.02$), heterozygosity for the GT genotype at CD36-1264 ($P<0.03$), and homozygosity for the T allele at NOSA-1659 ($P<0.01$), are all associated to malaria resistance. We do not replicate 16 previously reported associations, suggesting that some may have been spurious, and emphasizing the importance of systematic replication. Failure to replicate associations at these 16 genotypes may also be due to limited sample size, differences between the Luo and the populations in whom the associations were originally found, or differences between the phenotypes we and other groups studied. We also compared the frequency of malaria resistance variants in the Luo to the ethnically closely related Masai. We hypothesized that there would be a higher frequency of malaria resistance alleles in the Luo, due to selection for malaria resistance since the two populations split (the Masai have lived in a high-altitude, low malaria environment since they split from the Luo). Indeed, the sickle cell variant, the CD36 variant, and the Duffy null variant were all strikingly differentiated in frequency. We assessed the statistical significance of the allele frequency differentiation by genotyping 1,536 random SNPs in the Luo and Masai, demonstrating empirically that the level of differentiation at the malaria resistance alleles stands out. We propose that in future, variants conferring resistance to malaria and other infectious diseases may be found not only by case-control analysis, but by combining this information with evidence that the alleles are highly differentiated in frequency comparing exposed and unexposed populations.

X-APL: A family-based association test for the X chromosome. *R.-H. Chung[1,2], R.W. Morris[3], L. Zhang[1,2], Y.-J. Li[2], E.R. Martin[2]* 1) Bioinformatics Research Center, North Carolina State University; 2) Center for Human Genetics, Duke University Medical Center; 3) Department of Anesthesiology, Duke University Medical Center.

Linkage analyses have identified regions on the X chromosome for several diseases, such as Parkinson disease and autism. Association methods can further localize disease susceptibility genes in the linkage regions, however, few family-based association methods are available for testing markers on the X chromosome. Two such tests, XS-TDT and XRC-TDT [Horvath et al. 2000, AJHG], are valid tests of association in family triads or discordant sib pairs, but are not theoretically valid in multiplex families when linkage is present. We have developed a family-based method for testing association in nuclear families, X-APL, which extends the APL method [Martin et al. 2003, AJHG] to X-linked markers. X-APL is based on the difference between the observed number of a specific allele in affected siblings and the expected number conditional on parental genotypes and sibling sex under the null hypothesis of no association or no linkage. Sharing properties of APL, X-APL can use singleton and multiplex families and properly infers missing parental marker genotypes in linkage regions. X-APL was extended to haplotype association analyses. To allow for different penetrances in males and females, separate tests for males and females are provided in X-APL. We used Monte Carlo simulations to verify that X-APL has correct type I error rate. Power analyses showed that X-APL can have substantially more power than XS-TDT. The X-APL test using all data from males and females can have more power than separate tests for males and females when the disease penetrance is similar in both sexes. Tests in males and females alone can have more power than a test using both sexes if the X-linked locus only has effects on one sex. X-APL identified a SNP marker and 2-SNP haplotypes in the MAOB gene significantly associated with Parkinson disease in females but not in males. In summary, X-APL provides a powerful tool for association analysis on the X chromosome using single loci or haplotypes in nuclear family data.

Synergistic effects of nucleotide content on SNP formation. *E. Arehart, N. Barney, W. Holden, J. Hwa, J.H. Moore*
Computational Genetics Laboratory, Dartmouth Medical School, Lebanon, NH.

The fidelity of DNA replication serves as the nidus for both genetic evolution and genomic instability fostering human disease. Single nucleotide polymorphisms (SNPs) constitute greater than 80% of the genetic variation between individuals. A new theory regarding the mechanism for DNA replication fidelity has emerged were selectivity is governed by base-pair geometry and interactions between the selected nucleotide, complementary strand and the polymerase active site. The goal of this study was to identify nucleotide patterns that may predispose specific sites to mutation. We investigated the role of flanking regions within known SNP sequences using the novel multifactor dimensional reduction (MDR) method that was designed specifically to detect synergistic interactions among multiple discrete attributes. The MDR method was used to analyze 2194 human DNA sequences sampled from the Human SNP Database. Each mutation position was evaluated for its flanking nucleotide content (A, G, T, and C) as well as its purine/pyrimidine content. MDR was utilized to identify the best combination of two, three, or four nucleotide content measures that are predictive of each of the twelve possible transition/transversion SNPs. Cross-validation and permutation testing were utilized to minimize overfitting and false-positives due to multiple testing. Information theory was used to decompose each multifactor model into redundant, independent, and/or synergistic effects for statistical interpretation and visualization. We found statistically significant evidence for the presence of independent and synergistic nucleotide position effects on SNP formation. The results of this study highlight the importance of local nucleotide content in biasing certain sites towards mutation. Further, this study demonstrates that multiple nucleotides may work in concert to increase this position bias. Embracing the complexity of nucleotide effects on mutation may prove to be a useful research strategy for understanding the role of flanking regions in DNA polymerase fidelity.

Genetic modifiers of cystic fibrosis-related diabetes. *S.M. Blackman[1], S. Hsu[1], K. Naughton[2], B. Coleman[2], T. Lai[2], A. Bowers[2], D.J. Cutler[2], G.R. Cutting[2]* 1) Division of Pediatric Endocrinology, Johns Hopkins Hospital, Baltimore, MD; 2) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins Hospital, Baltimore, MD.

Diabetes is the most common extrapulmonary complication of cystic fibrosis (CF), a multi-system genetic disease caused by defects in an epithelial chloride channel, the cystic fibrosis transmembrane conductance regulator (CFTR). While some loss of exocrine and endocrine function is typical in CF, a subset (20-25%) of CF adults develop defects in insulin secretion, insulin resistance, and an accumulation of islet amyloid polypeptide, features typical of type 2 diabetes in the general population. Using a twin study design, we tested whether modifier genes might contribute to CF-related diabetes (CFRD). CF patients were classified as diabetic based on physician diagnosis and appropriate daily insulin dosage. CFRD was present in 14 of 128 monozygous (MZ) twins, 1 of 45 dizygous (DZ) twins, and 44 of 697 siblings. The relative absence of CFRD in DZ twins is likely due to a lower mean age (8.3 yr) than that of the MZ twins (15.5) and siblings (12.5). Concordance for CFRD was 56% in MZ twins (5 of 9 pairs), and was 27% in siblings (8 of 30 pairs; p=0.1). To account for variation in defects in CFTR, concordance was calculated using patients homozygous for the F508 mutation. Then, 4 of 6 MZ twin pairs (67%) and 3 of 16 sibling pairs (19%) were concordant (p=0.05; Fisher exact). If siblings are used as a proxy for DZ twins in a heritability estimate defined as $h^2=2(MZ\ concordance\ rate - DZ\ concordance\ rate)$, these twins and siblings estimate heritability of CFRD to be 0.6-0.9, which is comparable to heritability estimates for type 1 and type 2 diabetes. An initial genome-wide linkage study has been performed using 402 short tandem repeat markers in 24 individuals from 7 families concordant for CFRD. In addition to CFTR on chromosome 7 (peak LOD score 2.5), regions with similarly high degrees of allele sharing (LOD 2.4-3.0) were identified on chromosomes 3p25 and 10p13-14. Thus, it appears that genes other than CFTR affect the risk of diabetes in CF, and two possible genetic loci have been identified.

G72/G30 transgenic mice and gene expression profiling. *L. Cheng, E. Hattori, A. Nakajima, Y. Tang, E. Gershon, C. Liu* Dept Psychiatry, Univ Chicago, Chicago, IL.

The G72/G30 gene region has multiple reports of association with both bipolar disorder and schizophrenia. We have introduced a human BAC clone containing G72/G30 gene into B6/CBA mice. In the F4 generation of the transgenic mice, real-time PCR revealed stable transmission of BAC DNA between generations. We performed G72/G30 expression study in cDNAs from 16 different human tissues, fetal brain, five brain subregions (amygdala, substantia nigra, thalamus, celebral cortex, hippocampus), and RNA from human fetal and adult whole brains. Eight new alternative splicing forms of G72 were identified, including two isoforms from testis; two from brain (substantia nigra and amygdala); four from transgenic mice brain cortex tissues. G72 expresses at very low level in human testis and brain. In fourteen other human tissues no G72 expression was detected using multiple methods. G72 expresses at a high level in transgenic mouse brain cortex. The transgenic mice may form a living model to study G72 function and its role in disease etiology.

Regulation of RUNX2 at the nuclear lamina. *P. Fonseca[1], G. Zhou[1], P. Hermanns[1], E. Munivez[1, 2], Q.P. Zheng[1], B. Lee[1, 2]* 1) Dept Human Molecular Genetics, Baylor Col Medicine, Houston, TX; 2) Howard Hughes Medical Institute.

RUNX2 is a member of the RUNT family of transcription factors. It is essential for osteoblast formation and chondrocyte maturation. Loss of function mutations in RUNX2 result in the human skeletal disorder cleidocranial dysplasia (CCD). RIP (CBFA1/RUNX2-Interacting Protein) was isolated in a screening of a human osteosarcoma cDNA library using a yeast two-hybrid approach. Two families with rearrangements involving chromosome 8q - where RIP maps - have been reported to have a CCD-like phenotype. In transient transfection experiments, RIP down-regulated the transactivation by RUNX2 in a dose-dependent manner in both 10T1/2 cells and ROS17/2 cells. RIP is mainly localized in the nucleus and it was identified as a component of the nuclear envelope in proteomic screens. Interestingly, mutations in LaminA/C result in several laminopathies, including mandibuloacral dysplasia (MAD). MAD clinical features include hypoplastic clavicles and in some cases delayed closure of cranial sutures, partially phenocopying CCD. Hence, the skeletal defects observed in these patients may be due to disruption of interactions of the nuclear lamina with transcriptional regulators relevant in bone formation such as RIP and RUNX2. GST pull-down experiments showed an interaction between LaminA/C and RIP, as well as the RUNT domain of RUNX2. The tail domain, which is mutated in MAD patients, is responsible for both the interaction between RIP and RUNT. Moreover, LaminA/C could repress RUNX2 transactivation of reporter genes in COS7 cells in a dosage-sensitive way. Mutant versions of lamin carrying MAD mutations seem to lose their repressive action. A GST-pulldown assay revealed that these mutants had altered RUNT domain and RIP binding properties. These data suggest a novel mechanism for transcription regulation of skeletogenesis involving sequestration of a RUNX2 complex at the nuclear lamina.

Alpha-synuclein promoter haplotypes and dementia in Parkinsons disease. *E.V. De Marco[1], P. Tarantino[1], G. Provenzano[1], D. Civitelli[1], F.E. Rocca[1], F. Annesi[1], I.C. Cirò Candiano[1], S. Carrideo[1], V. De Luca[2,3], F. Condino[1], N. Romeo[1], G. Nicoletti[1], R. Marconi[4], M. Zappia[5], G. Annesi[1]* 1) Inst of Neurological Sciences, National Research Council, Cosenza, Italy; 2) Dept of Neurosciences, University of Naples "Federico II", Italy; 3) Dept of Psychiatry, University of Toronto, Canada; 4) Dept of Neurology,Misericordia Hospital, Grosseto,Italy; 5) Dept of Neurosciences, University of Catania, Italy.

Mutations of the alpha-synuclein gene (SNCA) were linked to rare familial forms of Parkinsons disease (PD), while association studies on the promoter polymorphisms gave conflicting results in sporadic patients. No works were produced to test the association of SNCA and dementia in sporadic cases. We performed a case control study to investigate whether genetic variability in the promoter of SNCA could predispose to dementia in PD. A total of 114 demented patients with sporadic PD and 114 non demented patients, matched for age, duration of the disease and origin, were included in the study. The haplotype analysis was based on six polymorphic loci (five single nucleotide polymorphisms and the Rep1 microsatellite) in the promoter of the SNCA gene. Genotyping was performed using different methods: enzymatic digestion, allelic discrimination (ABI PRISM 7900HT), fragment sizing (ABI 3130xl). Statistical analysis were performed using Haploview 3.2 and UNPHASED, treating the multiallelic marker Rep1 as a two-allele system after pooling the rare alleles. Each marker, taken individually, did not show association to dementia. No significant differences were observed in the inferred haplotype frequencies of demented and non demented patients ($p=0.73$). In a previous study we did not find association between the Rep 1 polymorphism of the SNCA promoter and PD in our population, confirming the results obtained by some researchers. Since haplotype analysis has proven to be a more reliable method in association studies, in this work we analyzed a more extended region of the promoter of the SNCA gene . Our data suggest the lack of involvement of the SNCA promoter in the pathogenesis of dementia in PD. Further studies are needed to confirm these results in other populations.

Novel candidate genes for late-onset Alzheimers disease from a focused genome-wide association study of 20K functional variants: A follow up study. *R. Abraham[1], A. Grupe[2], Y. Li[2], P. Hollingworth[1], A. Morgan[1], L. Jehu[1], N. Cope[1], K. Dowzell[1], P. Nowotny[3], D. Rubensztein[4], C. Brayne[4], M. Gill[5], B. Lawlor[5], L. Thal[7], S. Lovestone[6], M. O'Donovan[1], A. Goate[3], M. Owen[1], J. Williams[1]* 1) Dept Psychological Medicine, Cardiff Univ, Cardiff, United Kingdom; 2) Celera Diagnostics, Alameda, CA; 3) Departments of Psychiatry, Neurology & Genetics Washington University School of Medicine, St. Louis, MO; 4) University of Cambridge, Cambridge, UK; 5) Department of Psychiatry, Trinity College, Dublin, Ireland; 6) 6Department of Neuroscience, Institute of Psychiatry, Kings College London, London, UK; 7) 7Department of Neurosciences, University of California, San Diego, La Jolla, CA.

Background We performed a focussed genome-wide association study using independent AD case-control samples collected from the UK and USA (1738 cases,2000 controls). 20,421 putatively functional SNPs, in 12,500 known and predicted genes from the Celera human genome database were tested for allelic association with LOAD. This identified 4 SNPs that showed significant association ($p<0.0003$) in a combined meta analysis across all 4 independent case-control samples. The SNPs were located on chromosomes 14,17,19 and 20. Aim To test the hypothesis that the 4 significant SNPs may be in linkage disequilibrium with causal variants for LOAD, we genotyped additional SNPs around these loci. Methods 63 TagSNPs across 7 genes ($R2 > 0.8$)were genotyped in 1124 cases and 1329 controls from the UK. Results We found increased evidence for association with LOAD with one intronic SNP in a gene encoding a neuropeptide on chromosome 19 (tag SNP p-value 0.0006, original SNP p-value 0.021. In a second gene coding for a key enzyme involved in insulin signalling, 5 out of 13 tag SNPs showed some evidence of association with LOAD (p-value range 0.01 - 0.04). The strength of these associations was comparable to the original significant SNP (p-value 0.009). Conclusions We observed further evidence for a novel susceptibility gene for LOAD on chromosome 19 and suggestive evidence supporting at least one additional loci.

A common founder for the Lrrk2 Gly2019Ser mutation in Italian PD patients. *D. Civitelli, P. Tarantino, G. Nicoletti, I.C. Cirò Candiano, F. Annesi, E.V. De Marco, S. Carrideo, G. Provenzano, P. Spadafora, F.E. Rocca, G. Annesi* Inst Neurological Sciences, National Research Council, Mangone Cosenza, Cosenza, Italy.

The LRRK2 Gly2019Ser mutation was founded in both autosomal dominant parkinsonism linked to PARK8 locus and in sporadic forms of Parkinsons disease (PD). A peculiar chromosome 12q12 haplotype is shared by the European and North African carriers, indicative of a common ancestor. We tested the Gly2019Ser mutation in unrelated Italian PD patients: 450 sporadic and 38 patients with autosomal dominant parkinsonism. Age-at-onset was 30-78 years. Subsequently, we genotyped 14 intragenic and flanking markers (the microsatellite D12S2514, D12S2515, D12S2516, D12S2518, D12S2519, D12S2520, D12S2521, D12S2522, D12S2523, D12S2517 and the SNPs rs1896252, rs1427263, rs11176013, rs11564148) We found the heterozygous Gly2019Ser mutation in 12 sporadic PD patients (2.7%). Moreover, two familial patients (5.2%) carried the Gly2019Ser substitution. One of them was heterozygous and the second one was homozygous. Both mutation carriers and non-carriers exhibited similar clinical features. The age-at-onset ranged from 40 to 77 years. The age-at-onset of homozygous subject was 47. Since the DNA of the family members was not available, the haplotype was unambiguously determined for the single PD patient homozygous for all the examined markers, including the G6055A mutation. In the remaining PD cases, parental phases could not be unequivocally reconstructed, but their genotypes were compatible with a common founder. The minimum shared haplotype (256-G-T-G-A-A-153), including a ~127 Kb region, spans from marker D12S2516 (intron 30) to D12S2519 (3 flanking). Concerning the D12S2515 microsatellite, we found two subclasses of PD patients: the first group carrying al least one 226 bp allele, end the second one with at least the 230 bp allele. This observation could support the recent hypothesis, that a mutation occurring within this polymorphic marker could produce a subhaplotype in the contest of the ancestral haplotype extending beyond the D12S2515 marker, but is also compatible with a recombination event.

Deficiency of Prolyl 3-Hydroxylase (Leprecan) Causes a Novel Recessive Metabolic Disorder of Bone Resembling Lethal/Severe Osteogenesis Imperfecta. *W.A. Cabral[1,2], W. Chang[1], A.M. Barnes[1], D.R. Eyre[3], M.A. Weis[3], S. Leikin[4], E. Makareeva[4], N.V. Kuznetsova[4], K.N. Rosenbaum[5], C. Kozma[6], C.J. Tifft[5], P. Smith[7], J.C. Marini[1,2]* 1) Bone & Extracellular Matrix Branch / NICHD / NIH, Bethesda, MD; 2) MOCB Program, Univ of Maryland, College Park, MD; 3) Orthopedic Research Laboratories, Univ Washington, Seattle, WA; 4) SPB / NICHD / NIH, Bethesda, MD; 5) CNMC, Dept Genetics, Washington, DC; 6) Georgetown Univ Hosp, Dept Pediatrics, Washington, DC; 7) Shriners Hospital, Dept Orthopedics, Chicago, IL.

The majority of cases of Osteogenesis imperfecta (OI) are caused by dominant mutations in COL1A1 and COL1A2, the two genes that code for type I collagen. A recessive form of lethal OI, not caused by collagen mutations, has long been suspected. Recently, a KO mouse for CRTAP (CASP, cartilage associated protein) with severe bone disease and several cases of severe OI with recessive CRTAP mutations have been reported. CASP co-purifies with leprecan, a matrix glycoprotein which is also known as prolyl 3-hydroxylase and believed to modify the Pro986 residue in the 1(I) chain of type I collagen and probably sites in other collagen types. This suggests that leprecan and CASP form a complex which modifies collagen in the ER. Since the enzymatic activity of the complex resides in leprecan, we postulated that leprecan deficiency might result in a severe bone dysplasia. We have identified the first five cases of lethal and severe OI-like dysplasia caused by recessive defects in leprecan (LEPRE1). All probands have null mutations in both alleles with heterozygous parents. Proband leprecan mRNA is absent on real-time RT-PCR, as is leprecan protein on Western blots and 3-hydroxylation of type I collagen on mass spectrometry. Proband leprecan deficiency results in excessive lysyl hydroxylation and subsequent excess glycosylation of type I collagen, showing 3-hydroxylation is essential for helix formation. A mutant allele from West Africa, also found in African-Americans, is present in four of five cases in this report. These insights define a novel metabolic bone disorder and open new avenues of investigation into the role of leprecan in development of bone, lung and other organs.

Differential gene expression of cardiac muscle to ischemia in Atkins Diet dogs using Microarray data. *C.A. Gregory, T.N. Masters, A.A. Fokin, F. Robicsek* Heineman Medical Research Labs, Carolinas Medical Center, Charlotte, NC.

Background: According to the American Obesity Association, obesity is the second leading cause of preventable death in the U.S. Low carbohydrate, high protein and fat diet such as the Atkins diet has been the diet of choice for many dieters. Physicians concerned with overweight cardiac patients have prescribed the Atkins diet. However, little is known how basic heart metabolic reactions may be altered. The purpose of the study was to delineate myocardial response to ischemia in cardiac muscle of dogs on the Atkins vs. normal diet. Methods: Eight dogs were maintained on Atkins Diet for 7 weeks while 9 on a normal diet served as a control. Temporary occlusion of the circumflex coronary artery was used for ischemic challenge of the left ventricular myocardium. Samples of endocardium/epicardium were processed for gene expression profiling using the Affymetrix Canine GeneChip. Results: Using ArrayAssist, 162 genes were significantly (p=0.01) up or down regulated in experimental animals. To delineate genes of interest involved in cardiac energetics, a subset of metabolism genes were created. Significant analysis at p0.05 of a subset of 240 metabolic genes, revealed 12 upregulated genes and 1 down regulated gene. The 12 upregulated genes were analyzed by PathwayAssist and functions were assigned to 8 of the 12 upregulated genes namely FABP1, APOC-1, APOC-2, PRPS1, CROT, IDI1, PTPN6, and PGS1. Conclusions: Energy requirements of the cardiac muscle response appear to be handled by upregulation of FABP1 which roles include fatty acid uptake, transport, and metabolism. Additional energy may have been provided by activation of LPL by both APOC-1 and 2. LPL is known to hydrolyze triglycerides thus providing free fatty acid to the cell. APOC-1 and 2 also play a role as a ligand/bridging factor for receptor-mediated lipoprotein uptake. CROT catalyzes the transfer of fatty acyl groups between CoA and carnitine, critical for mitochondrial transport. The predominance of upregulated genes in lipid metabolic pathways accompanying the Atkins Diet would seemingly reduce the ability of the heart to adequately metabolize carbohydrates during ischemic episodes.

Metabolically Biotinylated Helper Dependent Adenovirus: a new and rapid approach for targeting of High-Capacity Adenoviral Vector. *V.C. Cerullo[1], M.C. Seiler[1], C.C. Clarke[1], A.E. Erez[1], M.B. Barry[2], B.L. Lee[1]* 1) HUMAN MOLECULAR GENETICS, BAYLOR, HOUSTON, TX; 2) Department of Immunology,Baylor, Houston, Texas.

Developing cell-targeting vectors is an important goal in gene therapy. There are several advantages in using helper-dependent adenoviral vectors (HD-Ad) instead of first-generation vectors; HD-Ad contain only the noncoding termini of the viral genome, can deliver large DNA fragments of up to 36 Kb into target cells, elicit reduced toxicity and generate prolonged transgene expression in vivo. To enhance the potential of HD-Ad to transduce different cell types, we constructed a novel metablically biotinylated Helper Virus (Fib2102) to package HD-Ad vectors. Thus, co-infection of a helper-dependent packaging cell line with the fiber-modified helper virus and various HD-Ad constructs would allow the production of fiber-modified HD-Ad expressing different transgenes, obviating the requirement to fiber-modify each individual transgene-expressing HD-Ad. To this end, we modified the c-terminus of the Ad5 fiber by addition of a 70 amino acid biotin acceptor peptide (BAP) in our helper virus backbone by homologous recombination. Adenovirus particles bearing the BAP were metabolically biotinylated during vector production by the endogenous biotin ligase to produce covalently biotinylated virions. The resulting biotinylated vectors can subsequently be used to transduce different cell type receptors by conjugation to specific biotinylated antibodies. In particular, we tested whether a biotinylated HD-Ad generated with this system expressing the LacZ transgene could transduce chondrocytes in vitro. We found that a fiber-modified HD-Ad coupled to the chondrocyte specific -10 integrin antibody was more efficient at transducing a chondrocyte cell line than vectors bearing wild type fiber. In summary, we show the novel construction of a fiber-modified helper adenovirus which can be used to propagate high titers of fiber-modified HD-Ad, which, when coupled to cell-specific antibodies, results in improved transgene expression in vitro. This study demonstrates progress in retargeting strategies for helper-dependent vectors.

Two uncommon dystrophin deletions associated with Becker muscular dystrophy (BMD). *G. Galluzzi[1, 2], J. Mela[1, 2], L. Colantoni[1, 2], R. Verardo[3], S. Servidei[3]* 1) Neuromuscular Disorders, UILDM - Lazio Section, Rome, Italy; 2) IRCCS S.Lucia Foundation, Rome, Italy; 3) Department of Neurosciences, Catholic University, Rome, Italy.

Deletions in the dystrophin gene are the most common disease causing mutations in Xp21-Linked muscular dystrophies: out of frame deletions cause Duchenne phenotype while deletions maintaining the reading frame, are associated with BMD. Out of more than 600 cases of dystrophinopathy we found BMD patients with unusual dystrophin deletions reported only once in literature. An in frame deletion of the region encompassing exon 35 to 44 was found in two oligosymptomatic brothers with high CK (now 18 and 14 year-old) that both had an isolated episode of myoglobinuria at age 12. Western blot demonstrated a smaller and slightly reduced muscle protein. RT-PCR performed on muscle RNA with primers F/R located in exons 33 and 46 showed a cDNA fragment of 634 bp instead of 2,227 bp , consistent with the deletion. The second unusual deletion was detected in a severe BMD patient (current age 17) that showed reduction of dystrophin immunostaining in muscle specimen with N-terminus and rod domain antibodies and a total absence with C-terminus, suggesting a deletion in the distal region of the gene. By DNA analysis we found the deletion of the penultimate exon (78): RT-PCR on muscle RNA with primers F/R located in exon 77 and in the 3UTR showed a cDNA fragment lacking 32 bp corresponding to exon 78 sequence. The mutation results in a frame shift which creates a novel open reading frame in exon 79 and substitutes the normal C-terminus 14 amino acids with 32 new ones. This deletion, although out of frame, due to the particular position at the 3 end of the gene allows the production of an abnormal protein still able to assembly on the membrane and interact with the other dystrophin-associated-proteins. Our findings suggest that in cases of dystrophinopathies the whole gene must be investigated: many genomic regions are not included in most screening protocols and rare deletions such as that reported here, might remain undetected.

Variable size and occurrence timing of CREBBP microdeletions in Rubinstein-Taybi syndrome. *C. Gervasini[1], P. Castronovo[1], A. Bentivegna[1], A. Selicorni[2], F. Mottadelli[1], ML. Uzielli[3], F. Faravelli[4], A. Pessagno[5], E. Lucci-Cordisco[6], AM. Pinto[6], G. Neri[6], L. Larizza[1]* 1) Medical Genetics University of Milan; 2) I Clinica Pediatrica Fondazione Policlinico Milan; 3) Genetics Unit University of Florence; 4) Human Genetics Galliera Hospital Genoa; 5) Molecular Genetics Gaslini Institute Genoa; 6) Medical Genetics Catholic University Rome.

Rubinstein-Taybi syndrome (RSTS) is a rare congenital disorder with a wide severity phenotype spectrum characterized by mental and growth retardation, broad and duplicated distal phalanges of thumbs and halluces, typical facial dysmorphisms and increased risk of tumors. RSTS is associated with microdeletions and point mutations in the gene encoding the CREB-binding protein (CBP) located in 16p13.3. CBP is an histone acetyl transferase which functions as transcriptional coactivator. Here we report on 5 deletions affecting the CBP genomic region identified in a cohort of 31Italian patients (pts), 14 of them carrying a CBP point mutation. By genotyping 3 STR and 1 SNP we identified 2 deletions likely encompassing the whole gene. By FISH analysis using region-specific BAC clones and small-sized CBP-probes we estimated the extent and approximate boundaries of both deletions:one appears to extend fom exon 2 to exon 31 with its proximal breakpoint in the highly unstable region where 16p bkps underlying rearrangements in RSTS and leukaemia are clustered. The second deletion appears to extend both 5 and 3 beyond the gene with a deletion interval more than 500Kb. By FISH analysis we detected 3 additional deletions, all in a mosaic condition: 2 affect the 5end of the gene and one a significant portion of the gene and its 3 flanking region. In all three cases the percentage of deleted cells in the lymphocytes is 50percent, a finding correlated with a moderate phenotype. The clinical presentation of the 2 patients carrying whole gene deletion is severe, with extra signs manifested in the patient with the larger one. In conclusion complementary analyses allowed us to detect 5 deletions, 3 in a mosaic condition, accounting for a 16percent deletion rate, a finding emphasizing underscore of both CBP microdeletions and mosaicism in RSTS pts.

In Vivo Evaluation of Coagulation Factor VIII Variants for the treatment of Hemophilia A by Helper-dependent Adenoviral Vectors. *A.E. Erez[1], V. C. Cerullo[1], M. M. McCormack[1], R.G. Garcia[1], C.C. Clarke[1], S.P. Pipe[2], B.L. Lee[1,3]* 1) Human molecular genetics, Baylor, Houston, TX; 2) Pediatrics and Communicable Diseases, University; 3) Howard Hughes Medical Institute.

Hemophilia A is an X-linked bleeding disorder resulting from a deficiency of coagulation factor VIII (FVIII). FVIII expression after gene transfer is limited by unstable mRNA, interaction with endoplasmic reticulum (ER) chaperones, a requirement for facilitated ER to Golgi transport through interaction with the mannose-binding lectin LMAN1, and the instability of the activated form of FVIII (FVIIIa). Bioengineering of different variants of recombinant FVIII molecules by rational design can overcome these limitations. In this study we evaluated the in vivo efficacy of six different variants of recombinant FVIII. To this end, we generated six different helper dependent adenoviral vectors carrying human cDNAs regulated by the liver tissue restricted PEPCK promoter. To compare the short term efficacy and duration of hFVIII expression and infectivity of the different HDV hFVIII variants, FVIII deficient mice were treated with each vector at a dose of 5X1012 vp/kg via tail vein injection. Plasma was collected at baseline, two and four weeks post-injection and the FVIII biological activity was quantified by Coatest chromogenic bioassay. In this experiment, all vectors expressed functional FVIII at two weeks post-injection. FVIII activity varied from 2-8% in WT FVIII to up to 70% in other constructs. Functional FVIII activity dropped significantly in all vectors by 4 weeks except in the mice treated with an inactivation resistant rFVIII (HDV IR8 hFVIII) suggesting that this variant may exhibit an improved immunological profile. Interestingly, another construct containing the F309S substitution, which decreases binding to the ER protein BiP to increase the rate of secretion of the FVIII variant, appears to significantly improve activity over WT hFVIII in vivo. By using these engineered FVIII variants, longer-lived protein with improved immunological profiles can be generated for gene transfer and protein therapy.

Very early somatic genetic origins of cancer: Can somatic mosaicism of androgen receptor CAG repeat length in early stages of prostate growth and development be a predictor of future cancer? *B. Gottlieb[1,4,6], K. Sircar[2], C. Alvarado[1], A. Aprikian[5], L.K. Beitel[1,3], M. Alam-Fahmy[2], L. Begin[5], M. Trifiro[1,3,4]* 1) Dept Cell Genetics, Lady Davis Institute for Medical Research,; 2) Department of Pathology, McGill University Health Center; 3) Department of Medicine; 4) Human Genetics; 5) Urology, McGill University; 6) Department of Biology, John Abbott College, Montreal, Quebec, Canada.

In recent years the genetic origins of many cancers have been found to be somatic rather than germline. In particular this is true of prostate cancer (PCa). This has been partly due to the use of techniques such as laser capture microdissection (LCM), which has allowed for genetic analysis of specific cancer tissues. Shorter CAG repeat lengths in the androgen receptor gene (*AR*) have been correlated with increased *AR* transactivation activity and are associated with increased risk for PCa. We analyzed and sequenced *AR* CAG repeat lengths in human prostatic tissues from 13 PCa patients, as well as from disease-free individuals as young as I year old. Microdissected PCa lesions, high grade prostatic intraepithelial neoplasia (HGPIN), benign peripheral, transitional and central prostate zones, as well as leukocytes from peripheral blood were examined. Many prostate sections showed AR CAG repeat length heterogeneity in the form of multiple repeat lengths, i.e. somatic mosaicism. These consisted of normal and shortened CAG repeat lengths and even complete CAG repeat deletions. Blood leukocytes, representing germline DNA, predominately showed a single *AR* CAG repeat length per patient. CAG repeat length heterogeneity in both histologically benign tissues from diseased prostates and non-diseased prostates suggests that genetic heterogeneity of the *AR* CAG repeat precedes microscopically recognizable malignant changes and may occur very early in the growth and development of prostate tissue. Whether such genetic heterogeneity is a feature of all prostates must await a much more detailed survey of normal prostate tissues, but it raises some intriguing questions about the very early origins of prostate cancer susceptibility which will be further discussed.

Mutation of a putative potassium channel tetramerization domain gene in familial progressive myoclonic epilepsy. *M. Abramowicz[1], R. Azizieh[1], A. Aeby[2], L. De Meirleir[3], F. Christiaens[4], J. Desir[1], P. Van Bogaert[2]* 1) Dept Genetics, Hosp Erasme - ULB, Brussels, Belgium; 2) Pedi Neurology, Hosp Erasme - ULB, Brussels, Belgium; 3) Pedi Neurology, AZ-VUB, Brussels, Belgium; 4) Pedi Neurology, UCL-St Luc, Brussels, Belgium.

Progressive myoclonic epilepsy (PME) consists of myoclonus, seizures, and a progressive clinical course. PMEs are usually mendelian and transmitted as autosomal recessive traits, e.g., Unverricht-Lundborg and Lafora disease. We investigated three female patients from two consanguinity loops in a large inbred Morroccan family. Epilepsy started at 18-24 months after normal psychomotor development. Seizure types were multifocal myoclonus affecting limbs and face and aggravated by movements, and febrile generalized tonic-clonic in one patient. EEG showed slow dysrythmia, multifocal epileptiform discharges without reproducible temporal relationship with myoclonus and occasional generalized epileptiform discharges. Photosensibility was present in one patient. Giant somatosensory evoked potentials were not found. Cerebral MR imaging and fundoscopy were normal. Known PME-associated diseases were thoroughly excluded. GeneChip analysis of 11K SNPs showed a 15 Mb homozygous haplotype common to the three patients. Further analysis using microsatellite markers confirmed linkage of the disease to a 5 cM pericentromeric region of chromosome 7, with a maximum multipoint LOD of 4.2 at D7S663. In silico inspection showed the gene for a Potassium Channel Tetramerization Domain homolog, *KCTD7*, close to D7S663 within the segment. *KCTD7* contains a BTB/POZ-type domain and has strong homology with the T1 domain of the voltage-gated potassium channels. Direct sequencing of *KCTD7* revealed a C to T transition creating a stop codon in exon 2, homozygous in patients and heterozygous in parents. We conclude that loss of *KCTD7* function in PME is consistent with a defect of membrane repolarization.

Improving the safety of Helper-Dependent Adenoviral Vector gene transfer for Crigler-Najjar Syndrome. *D. Dimmock, N. Brunetti-Pierri, V. Mane, D. Palmer, A. Beaudet, P. Ng* Dept Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.

Crigler-Najjar syndrome is an inborn error of metabolism characterized by severe unconjugated hyperbilirubinemia caused by a deficiency of uridine diphospho-glucuronosyl transferase 1A1 (UGT1A1). Current treatment relies on phototherapy to prevent kernicterus, and liver transplantation to prevent long term complications.

Life-long correction of hyperbilirubinemia by liver directed gene therapy using a helper-dependent adenoviral vector (HDAd) has been recently reported in the Gunn rat model. However, in that study complete phenotypic correction was only achieved using a dose of $3x10^{12}$ vp/kg and only partial correction was observed at $6x10^{11}$ vp/kg. These doses are above the range used safely in human trials.

To increase the therapeutic index, we injected Gunn rats with an HDAd bearing an improved expression cassette containing the PEPCK promoter, the hUGT1A1 cDNA, the WPRE and a liver specific enhancer (LCR). We observed complete phenotypic correction with $5x10^{11}$ vp/kg and partial correction with $1x10^{11}$ vp/kg. These are the lowest therapeutic doses reported to date.

We have previously shown that hydrodynamic injection of HDAd results in increased expression of secreted proteins compared to conventional injection. Combining this method of delivery with our improved construct, complete correction was achieved with $5x10^{10}$ vp/kg for the duration of the observation period (at least 12 months).

Although hydrodynamic injection is not feasible in large animals, we have developed methods to mimic hydrodynamic injection of HDAd into larger animals which have yielded encouraging results (see Brunetti-Pierri abstract).

Taken together these strategies may offer clinical gene therapy for Crigler-Najjar syndrome.

CGH-array study of 40 holoprosencephaly patients: the observed large scale quantitative alterations continuum is not correlated with phenotypic severity. *C. Bendavid[1,2], C. Dubourg[1,2], S. Le Gallou[1], I. Gicquel[1], L. Pasquier[3], M.R. Durou[2], C. Henry[4], S. Odent[3], V. David[1,2]* 1) UMR 6061 CNRS; 2) Laboratoire de Génétique Moléculaire; 3) Service de Génétique Médicale; 4) Laboratoire de Cytogénétique, CHU de 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. We previously reported our results for point mutations screening and gene dosage in known HPE genes (*SHH*, *SIX3*, *ZIC2* and *TGIF*), describing genomic defects in up to 27% fetuses and 30% newborns. Recently, we screened subtelomeres by MLPA and found alterations in known HPE candidate loci but also in new regions, including many unbalanced translocations. Therefore, we decided to screen HPE patients using CGH-array to assess the frequency, location and size of large scale quantitative alterations through the entire genome.

We used Agilent CGH-array 44K technology. Out of 40 patients, we screened 10 DNA samples with known quantitative anomalies and 30 samples with no alterations identified to date. First we localized the breakpoints of the 10 DNA with known alterations (loss and/or gain in specific loci) and showed no correlation between the size and severity of the defect. Such variable penetrance and genomic plasticity imply a transcriptomic or post-transcriptomic regulation that still needs to be investigated. Out of the 30 DNA with no genetic etiology, we found 5 new rearrangements involving new potential HPE loci located on chromosomes 21q, 20p, 15q, 6q, 5q, 17q and 18q. We observed both isolated or associated genomic loss and gain, the latter suggesting an unbalanced translocation from parental origin. Detected alterations ranged from less than 1Mb to 16 Mb and were not further considered if they involved less than 3 consecutive spots on the array.

Our data strongly reinforce the multigenic and multihit origin in HPE and participate in the explanation for the wide phenotypic spectrum described in this developmental disorder.

Reference DNA in CGH-array studies of inherited anomalies: pool of normal DNA can generate background due to copy number polymorphisms. *V. David[1,2], S. Le Gallou[1], I. Gicquel[1], C. Dubourg[1,2], L. Pasquier[3], M.R. Durou[2], C. Henry[4], S. Odent[3], C. Bendavid[1,2]* 1) UMR 6061 CNRS; 2) Laboratoire de Génétique Moléculaire; 3) Service de Génétique Médicale; 4) Laboratoire de Cytogénétique, CHU de Rennes, France.

CGH-array technique checks on chromosomal imbalances using comparative hybridization on arrays where tailed sequences of the chromosomes have been spotted. Consequently, a quantitative anomaly is detected when patient DNA presents a cluster of chromosomal sequences with a gain or a loss. The aim of this work was to study if gains or losses for non clustered sequences (detected by CGH-array on Agilent 44K technology) could be confirmed, so that to know if these isolated anomalies could be considered as background and /or real copy number variations.

We studied three non- related families (father, mother and one child) to detect gains and losses in the child and their possible parental origin. We tested each DNA against a sex matched DNA pool (made of 10 unrelated "healthy" controls) and compared the status of the children alleles with copy number variations to the copy number of these alleles in their respective parents. We selected "familial" sequences that were quantitatively abnormal for at least the child and one of his parents and tried to confirm these results with duplex quantitative PCR (qPCR). Parents and child qPCR profiles were compared to 5 different control DNA, each extracted from a single "healthy" donor. Out of the "familial" sequences tested, many revealed a normal status with the qPCR method. Therefore, we studied the status of the "healthy" controls used in the pools. We found that some of the controls sequences presented enough copy number polymorphisms to impair the CGH-array familial analysis results.

Consequently, we strongly recommend to test patients DNA against one single "healthy" control at a time and to do the dye swap against another control. Interpretation will be easier if the two controls have been previously compared together and discrepancies validated by qPCR.

The aetiology of ectopic maxillary canine teeth. *S. Camilleri*[1], *C. Lewis*[2], *F. Mcdonald*[1] 1) Department of Orthodontics, Dental Institute of King's College London, London, United Kingdom; 2) Reader in Genetic Epidemiology and Statistics, Division of Genetics and Molecular Medicine, King's College London School of Medicine at Guy's, King's College and St Thomas' Hospitals.

Objectives: To elucidate the mode of inheritance of ectopic canines.

Introduction: The aetiology of ectopic maxillary canines has been proposed to be genetic and is associated with incisor-premolar hypodontia as well as with various other dental anomalies. The Maltese population has a high prevalence of ectopic teeth, especially ectopic canines, as compared to other populations. This has been ascribed to the Founder Effect, the population having grown from under 20,000 to over 400,000 in the past 500 years.

Methods: Thirty consecutive probands with a family history of ectopic canines were identified. 152 first, 51 second and 113 third degree relatives were contacted and their dental status ascertained by direct examination, anamnestic records, or written or telephone questionnaire. Pedigrees were constructed; the risk to the relatives was determined and plotted against the degree of relation.

Results: Analysis of the pedigrees suggests autosomal dominant transmission. 12 percent of first degree relatives had ectopic canines, 3.9 percent had transposed canines and a further 9 percent exhibited hypodontia, in particular upper lateral and lower central incisors. There is an appreciable relative risk in second and third degree relatives. The female to male ratio of the sample is 2.06, with no difference in the incidence of relatives of male or female probands. Penetrance is highly variable as is expressivity, with wide variation in the number and severity of ectopicity of teeth.

Conclusion: The genetic aetiology of ectopic canines is supported by this study. The data points to the action of a single major gene with incomplete penetrance and variable expressivity.

Selecting SNPs for Association Studies with SNPbrowser Software. *F.M. De La Vega, C.R. Scafe, H. Isaac* Applied Biosystems, Foster City, CA.

The design of genetic association studies using single-nucleotide polymorphisms (SNPs) requires the selection of subsets of the variants providing high statistical power at a reasonable cost. SNPs must be selected to maximize the probability that a causative mutation is in linkage disequilibrium (LD) with at least one marker genotyped in the study. The HapMap project performed a genome-wide survey of genetic variation with about a million SNPs typed in four populations, providing a rich resource to inform the design of association studies. A number of strategies have been proposed for the selection of SNPs based on observed LD, including construction of metric LD maps and the selection of haplotype tagging SNPs. Here, we demonstrate the typical workflows for the selection of markers for association studies utilizing the SNPbrowser Software, a freely available, stand-alone application. In our current release we integrated the complete the HapMap project dataset, together with a number of selection algorithms into a single, stand-alone application that provides a fast query capability and swift visualization of SNP and gene annotations, statistical power, haplotype blocks, and LD map coordinates. The wizards provided in the tool enable workflows for the selection of SNPs by spacing on the physical or LD map coordinates, or by selecting tag SNPs that capture the haplotype diversity of a region by a number of methods. These SNP sets have already been filtered by their assay conversion potential to either TaqMan SNP Genotyping Assays or the SNPlex Genotyping System, thus expediting the set-up of genetic studies with an increased probability of success. In addition of the HapMap validated SNPs, SNPbrowser provides access to up to 5 million candidate SNPs, including over 60,000 non-synonymous coding SNPs. The latter represent putative functional variants that can be used on its own for direct association studies, or to supplement mapping studies, allowing researchers to rapidly generate and test functional hypothesis when significant association are found.

Burden of genetic diseases in Colombia: 1996 - 2025. *J. Bernal, F. Suárez* Inst Genetica Humana, Univ Javeriana, Bogota, Colombia.

The public and private health care system in Colombia suffered a reform in 1992, since this reorganization, a better register system of mortality and morbidity was established. Knowing the expected population growing, life expectancy, and the reported incidence and prevalence of the principal genetics diseases (chromosomal and Mendelian) according to previous studies in the country, we calculated the genetic burden of genetic disease after the reform and projected to 2025. We calculated, incidence, prevalence, and disability adjusted life years (DALYs) for fourteen dominant Mendelian diseases, 5 recessives diseases, five X recessive linked diseases and seven main chromosomal disorders. Despite uncertainties about mortality and burden of disease estimates, we found that, with the decrease of childhood infectious mortality and the increase quality of medical attention in the elderly, with a life expectancy of 71.8 years, the burden of genetic disease is increasing in the country, expecting a frequency of 37.3 to 52.8 per every 1.000 habitants. The data also show that in the year 2000 the number of accumulated patients affected with genetic disease was about 2.000.000 and the number is going to increase to 2.866.000 people, with a heavy burden of DALYs of 1.1000.000 every 5 years and of 6.345.000 years during 1994 to 2025. The projected burden of the genetic disease can not be faced with the actual health care system, which lacks of genetics services, follow up of special patients, genetic counseling specialist and modern diagnostic and laboratory methods.

Pairwise relatedness estimation in structured populations. *A.D. Anderson, B.S. Weir* Department of Biostatistics, University of Washington, Seattle, WA.

A maximum likelihood estimator for pairwise relatedness is presented for the situation in which the individuals under consideration come from a large outbred subpopulation of the population for which allele frequencies are known. We demonstrate via simulations that a variety of commonly-used estimators that do not take this kind of misspecification of allele frequencies into account will systematically overestimate the degree of relatedness between two individuals from a subpopulation. A maximum-likelihood estimator that includes Fst as a parameter is shown to work fairly well in this type of situation, even when the value of Fst is misspecified.

Novel, complex interruptions of the GAA repeat in the small expanded alleles of two affected siblings with a mild, late onset form of Friedreich's ataxia. *E.C. Frackelton[1], D.R. Lynch[2], J.M. Farmer[2], J. McCallum[1], C.A. Stolle[1]* 1) Pathology & Laboratory Medicine, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Dept of Neurology, UPenn School of Medicine, Philadelphia, PA.

Friedreich's ataxia (FA) is an autosomal recessive disorder characterized by progressive ataxia and onset usually before the age of 25 years. Most patients have expanded GAA repeats in intron 1 of the FRDA gene. Normal alleles contain 5-33 repeats; premutation alleles contain 34-65 uninterrupted GAA repeats. Disease causing alleles contain between 66-1700 repeats, with the majority of alleles having 600-1200 repeats. Long stretches of GAA repeats assume a novel DNA structure that interferes with transcription, resulting in decreased expression of the gene. The severity of the phenotype in most patients correlates with the size of the smaller expansion. Late onset (26-39 years) and very late onset (>40 years) cases represent atypical presentations of FA. Almost all patients, even with the shortest (<100) GAA repeats, have some symptoms by age 40. Two siblings with an atypical presentation of FA had onset at >60 years of age and a relatively mild form of the disease. Both had a large expansion (~600-1000 repeats) and a small expansion (~120-130 repeats) as determined by long-range PCR. DNA sequence analysis of the small allele revealed mulitple, complex interruptions in the GAA repeats of the composition (GAA)>72+[GAGAAGAAAA]+(GAA)x20+[GAAAAGAA]+[GAGGAA]x4+(GAA)x10. One of these interruptions (GAGGAA) has been reported previously, but two others (GAGAAGAAAA and GAAAAGAA) are novel. Interruptions of trinucleotide repeats are thought to stabilize the repeat sequence and decrease the probability of expansion. In these patients, the longest stretch of pure GAA repeats is ~72, an allele size more consistent with their age of onset and mild disease course. The presence of interruptions in the GAA repeats may not only stabilize the repeat sequence, but also prevent formation of the DNA structure that results in silencing of the expanded allele. These data suggest that DNA sequence analysis of small expanded alleles may provide better phenotype correlation than sizing of the alleles by long-range PCR alone.

Oromandibular limb hypoplasia: report of two patients in Colombia. *R. Garcia, P. Paez, F. Suarez* Inst de Genetica Humana, Pontificia Univ Javeria, Bogota, DC, Colombia.

Oromandibular limb hypoplasia (Hanhart syndrome), is a rare disorder described for the first time in 1950 by Hanhart. The classical features of this disorder affect oral cavity (oromandibular hypoplasia) and limbs (adactylia, hypodactyly ectrodactyly). We present two unrelated female patients with classical phenotype of this syndrome. Case 1: 24 days-old girl born in Bogotá (Colombia) of non consanguineous parents, with micrognathia, hypoglossia, left hand adactylia, right hand ectrodactyly, bilateral clubfoot with oligodactylia. The patient was the product of mother's first 31-week pregnancy. No know teratogenic exposure. Case 2: A 39 years old woman born in Simijaca (Boyaca Colombia) of non consanguineous parents with moderate short stature, severe micrognathia, inexpressive fascies, hypoglossia, anodontia, limbs abnormalities including left hand agenesia, right hand olygodactylia, right food syndactylia and left food syndactylia with olygodactylia. Perinatal history is unknown. No apparent teratogenic exposure. Mental developmental was normal. Both of the patients had a complete radiological assessment which confirmed bone development defects. Two cases were diagnosed with Oromandibular Limb Hypoplasia Syndrome. As far as we know these are the first reported patients in whom clinical and radiological comparisons can be achieved, between a new born and an adult affected with Hanhart syndrome in Colombia.

First 285 Human Subjects of Genetics of Left Ventricular Outflow Tract Malformation Study. *S.M. Fitzgerald, G.A. Zender, K.L. McBride* Center for Molecular & Human Genetics, Children's Research Institute, Columbus, OH.

Congenital heart defects are among the most common of all medically significant birth defects and are a leading cause of infant mortality. Left ventricular outflow tract (LVOT) obstruction malformations, include aortic valve stenosis (AS), coarctation of the aorta (CoA), mitral valve stenosis (MS), hypoplastic left heart syndrome (HLHS) and bicuspid aortic valve (BAV). They are thought to arise embryologically from reduced flow through the LVOT and therefore likely have related genetic and environmental etiologies. To be able to determine the specific causes of LVOT malformations, consent, demographic information and blood samples are collected on affected probands and both parents (trios). Additional data such as family history, pregnancy exposures, maternal health and LVOT diagnoses must be obtained on each proband and other affecteds. We have designed a Microsoft Access database to facilitate gathering, storing and analyzing this data on 500 probands (1500 subjects), using the National Birth Defects Prevention Study questionnaire as a template. We have enrolled 285 subjects in 104 families over the first 13 months; 36% of subjects are probands, 33% mothers, 25% fathers and 6% other relatives. Of probands, 23% have HLHS, 7% Shone complex, 42% CoABAV, 19% ASBAV, 9% BAV, 1% IAA-A. We have blood samples on 67% of the all subjects and 64% of probands. Currently, 66 trios have consented and 35 trios also have blood samples on all members. The pregnancy exposure questionnaire has been completed by 43 families or 41%. Reported prenatal exposures include: medication (56%), cigarette smoking (36%), chemical exposure (19%), hot bath/Jacuzzi (14%), radiation exposure (12%), alcohol (10%), fever (5%), and heavy metal exposure (5%). As these mothers have reported higher than expected rates of exposure during pregnancy, when we reach our recruitment goal of 500 probands we should be able to link genetic with exposure data to establish one or more specific gene-environment interactions contributing to LVOT malformation.

More than you bargained for: Unsolicited Information in Direct to Consumer (DTC) Genetic Tests. *K.A.B. Goddard[1], R.P. Igo, Jr.[1], J. Fishman[2]* 1) Department of Epidemiology & Biostatistics; 2) Department of Bioethics, Case Western Reserve Univ., Cleveland, OH.

DTC genetic tests have recently exploded onto the market, with immediate access available to consumers over the internet. However, regulation is lagging behind, and DTC genetic testing raises novel concerns beyond those of traditional clinical testing. One consideration is the issue of unsolicited information, defined as information revealed by a genetic test that is not in response to its intended purpose. Unsolicited information is a concern because of the potential for others to misuse the information, and the potential for personal distress or interpersonal strife. We illustrate our approach to evaluate unsolicited information using CATGee, produced by DNA Products, Inc. The intended purpose of CATGee is to show an individuals uniqueness, and the product materials explicitly state that no scary health or paternity information is revealed. Consumers are encouraged to compare their CATGee profile with other individuals and with aggregated data for populations. Through a literature review, we found no evidence of pleiotropy for the six microsatellite markers in the CATGee profile. Using published allele frequencies for US Hispanics, US Caucasians, and African-Americans (AA), we computed the posterior probability of each ethnic group for simulated genotypes. Although the median posterior probability was higher than the prior probability for each ethnicity, the ability to predict ethnicity with certainty (e.g., posterior probability > 99%) was limited except for the AA group. We devised two tests to detect common relationship errors for putative full-siblings using the CATGee profile. Using simple allele counting methods comparing the profiles of two putative full siblings, the power was surprisingly high to detect adoption (~60%) and half-sibling (~30%) relationship errors. Using more sophisticated likelihood ratio tests, the power increased to ~80% for adopted siblings and ~40% for half-siblings. The potential for unsolicited information should be evaluated for any DTC genetic test, and policies detailing when and how to reveal such information need to be developed.

Balloon Occlusion Catheter-Based Delivery of HDAd into the Baboon Liver: Stable, High Level Transgene Expression with Minimal Toxicity. *N. Brunetti-Pierri[1], G. Stapleton[2], C. Mullins[2], D. Palmer[1], Y. Zuo[1], M. Finegold[3], A. Beaudet[1], P. Ng[1]* 1) Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Dept Pediatric Cardiology, Baylor Col Medicine, Houston, TX; 3) Dept Pathology, Baylor Col Medicine, Houston, TX.

Helper-dependent adenoviral vectors (HDAds) hold tremendous potential for liver-directed gene therapy as they can mediate long-term transgene expression without chronic toxicity. However, due to a nonlinear dose-response, high doses are required to achieve hepatic transduction resulting in dose-dependent acute toxicity. To overcome this important obstacle, we developed a minimally invasive method to preferentially deliver low dose HDAd into the liver of baboons to achieve efficient hepatic transduction. Briefly, a single balloon occlusion catheter was percutaneously positioned in the inferior vena cava of baboon 1 to occlude hepatic venous outflow. $1x10^{11}$ vp/kg of a HDAd expressing the baboon -fetoprotein (bAFP) marker was injected directly into the occluded liver via a percutaneously placed hepatic artery (HA) catheter and left to dwell in the liver for 15 min before balloon deflation. As controls, $1x10^{11}$ vp/kg was administered to baboon 2 by peripheral intravenous injection and baboon 3 by HA injection without balloon occlusion. All procedures were well tolerated, and all three animals returned to their normal pre-injection states with no clinical manifestations of toxicity. Only mild transaminitis was seen in all animals, peaking at 24 to 48 h post-vector but returning towards baseline the next day. Importantly, serum bAFP levels increased 425-fold over baseline for baboon 1, while only a modest increase of 12-fold and 5.6-fold were observed for baboon 2 and 3, respectively. To date, bAFP levels have been sustained (84 days). These results suggest the therapeutic index of HDAd can be significantly improved by delivering the vector preferentially into the liver using a minimally invasive balloon occlusion catheter technique and may be a first step towards clinical application of HDAd for liver-directed gene therapy.

Waardenburg syndrome type 3 or Klein-Waardenburg syndrome. A Mexican family. *G. Garcia-Sanchez[1], C.F. Martínez-Cruz[2,3], D.M. Mendoza-Ugalde[1], M. Díaz-García[1], M.R. Baez-Reyes[4], A. Garcia-Huerta[1]* 1) Instituto Nacional de Rehabilitación. Genética. Mexico; 2) Instituto Nacional de Perinatología.Departamento de Seguimiento Pediatrico.Comunicacion Humana; 3) Instituto Mexicano del Seguro Social. Hospital General de Zona 53; 4) Instituto Nacional de Perinatología.Genética. Email guillegs@yahoo.com.mx.

Waardenburg syndrome type 3 (WS-III; MIM 148820) or Klein-Waardenburg syndrome is characterized by the presence of musculoskeletal abnormalities in association with clinical features of Waardenburg syndrome type 1 (WS-I). Since the description of the first patient in 1947 (D. Klein, Arch Klaus Stift Vererb Forsch 1947: 22: 336-342), a few cases have been reported. Only occasional families have demonstrated autosomal-dominant inheritance of WS-III. In a previous report, a missense mutation in the paired domain of the PAX3 gene has been described in WS-III. In this report, we present a Mexican family (mother and son) with clinical findings of WS-III. Son: 12 years old with, poliosis and premature graying, dystopia canthorum, blue eyes, hyperpigmented spots on thorax and left arm and camptodactily (moderate-severe) and short fingers. Hearing loss, was suspected at 2- 8/12, ABR at 3 years old demonstrated bilateral non-response at 100dB, audiogram al 12 years old shows: bilateral sensorineural profound hearing loss, Father. 42 years old, poliosis and premature graying, dystopia canthorum, blue eyes and mild bilateral flexion contractures of the fingers. Right sensorineural profound hearing loss, onset at 11 years old, aparently non progressive.

X chromosome DNA methylation: a new quantitative assay and preliminary results in women with premature ovarian failure. *K.L. Bretherick[1], M.R. Fluker[2,3], C.J. Brown[1], W.P. Robinson[1]* 1) Dept of Medical Genetics, UBC; 2) Genesis Fertility Center; 3) Dept of Obstetrics & Gynecology, BC Childrens and Womens Hospital; Vancouver, BC, CANADA.

One of the hallmarks of X chromosome inactivation is DNA methylation of the promoter regions of inactivated genes. Since women have one X chromosome inactive in each cell, the proportion of DNA methylation expected at any given locus is 50%. However, the degree of skewed X chromosome inactivation assayed by methylation-sensitive restriction digestion does not always correlate when measured at different loci, suggesting that there may be deviation from the expected 50% on some regions of the X chromosome. We have developed a new assay to assess the percent methylation at a single locus using methylation-sensitive restriction enzyme digestion and quantitative PCR (q-PCR). Digested DNA is amplified by q-PCR with primers that flank a restriction site at the locus of interest, and with primers for an internal control locus that does not have a restriction site. Quantity determined at the locus of interest is normalized to the quantity at the internal control locus, to control for the amount of input DNA. The normalized quantity of digested DNA is then expressed as a percentage of the normalized quantity of an undigested sample, to determine the % DNA that is methylated at the locus of interest. Preliminary results assaying the degree of DNA methylation at the Androgen Receptor gene (AR) promoter show that deviation from the expected 50% methylation is common; with reproductively healthy (RH) controls having an average of 37.010.4% methylation (N=20, range 20-57%). In female controls of ages 5-81 years, there is a trend toward increased methylation at the AR locus with age, with an increase of 0.1% per year (N=31, p=0.26). In addition, women with premature ovarian failure (POF) show increased methylation at AR compared to RH controls; 44.8% in POF patients (N=18) vs. 36.9% in controls (N=20) (p=0.05, 2-tailed t test). These results indicate that there may be a change in AR promoter methylation with age and suggest that there are methylation abnormalities on the X chromosome of women experiencing POF.

Discovery of low abundance sequence variants in mitochondrial heteroplasmy with a Quantitative Sequencing method. *S. Chen, A. Lakdawalla, K. Hunkapiller, C. Brown, E. Gerber, K. Livak* Applied Biosystems, Foster City, CA.

Mitochondrial heteroplasmy has been associated with variability in mtDNA associated disease phenotypes.

The discovery and quantification of mitochondrial heteroplasmy is performed by cloning PCR amplicons of the mtDNA sample and sequencing multiple clones to discover low frequency mutations in a normal background. By improving the detection limit of standard DNA sequencing to low amounts of mixed bases in the sequence the need for cloning can be circumvented.

We will present the quantitative analysis of mixed human mtDNA hypervariable regions and identify low abundance SNPs from the higher abundance background DNA pool.

The quantitative sequencing method would also simplify the discovery of variants in mixed DNA samples (somatic mutations), for the quantitative discovery of DNA methylation sites in heterogeneous samples, and for increasing sequencing efficiency by pooling samples.

The Optimal Minor Allele Frequency (OMAF) for a SNP-Disease Association Studies. *I.P. Gorlov[1], O.Y. Gorlova[1], S. Sunyaev[2], M.R. Spitz[1], C.I. Amos[1]* 1) Epidemiology, UT M. D. Anderson Cancer Center, Houston, TX; 2) Division of Genetics, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

The ultimate goal of any case control association study is identifying causal polymorphisms modulating risk of common human diseases. Missense mutations are most plausible candidates for causal polymorphisms. In this study we sought to find out whether there is a Minor Allele Frequency (MAF) that maximizes the chances to identify the causal missense mutation in a case-control association study. The MAF can affect the chances to identify a causal SNP because (i) statistical power depends on MAF, an also because (ii) there is an inverse relationship between MAF and the probability for a SNP to be functional. In this study we used PolyPhen and SIFT bioinformatics tools for predicting SNP functionality to identify functional (protein-damaging) SNPs. We found a significant negative correlation between MAF and the probability that a SNP is functional. Joint probability that a SNP is a functional and that it will be detected as significant in a case-control association study is a useful measure to characterize chances of detecting a causal SNP. We named this joint probability the Probability to Detect a True Association (PDTA). We sought to identify MAF for which the PDTA is maximal. This MAF was called Optimal MAF (OMAF). We found an inverse relationship between OMAF and sample size. Deviation of MAF from OMAF decreases chances that a causal mutation will be detected. The effect of the deviation of MAF from the OMAF is much stronger for dominant model compared to the recessive one. Our study suggests that for a given sample size and Odds Ratio (OR) there is a single OMAF. Our study predicts OMAFs for different sample sizes.

Oxidative stress in treated Maple Syrup Urine Disease patients. *A.G. Barschak[1,3], A. Sitta[1,3], M. Deon[1,3], M.C. Pigatto[1], T. Terroso[1], A. Barden[1], C.S. Dutra-Filho[3], M. Wajner[1,3], R. Giugliani[1,3], C.R. Vargas[1,2,3]* 1) Serviço de Genética Médica, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil; 2) Departamento de Análises, Faculdade de Farmácia-PPGCF, UFRGS, Porto Alegre, RS, Brazil; 3) Departamento de Bioquímica, ICBS, UFRGS, Porto Alegre, RS, Brazil.

Maple syrup urine disease (MSUD) or branched-chain -keto aciduria (BCKA) is an inherited disorder caused by deficiency in the branched-chain -keto acid dehydrogenase complex (BCKAD) activity. The blockage of this pathway leads to tissue accumulation of the branched-chain amino acids (BCAA) leucine, isoleucine and valine and their respective keto-acids. The symptomatology includes ketoacidosis, hypoglycemia, poor feeding, convulsions, coma, psychomotor delay and mental retardation. The mechanisms of the neurological dysfunction are still poorly understood. In the present work we evaluated some parameters of oxidative stress named thiobarbituric acid-reactive substances (TBARS), total antioxidant reactivity (TAR) and total antioxidant status (TAS) in plasma from MSUD patients under dietary therapy presenting different leucine plasma levels (groups I, II and II). We verified a significant increase of TBARS as well as a decrease of TAR in plasma of the three groups of treated MSUD when compared to control group. However, no difference of TBARS was found between the patients during treatment and at diagnosis. These results suggest that the dietetic therapy do not change the oxidative stress presented by MSUD patients, suggesting that leucine blood levels could not be directly related to oxidative stress in this disease. Financial support: CNPq/PROPESQ-UFRGS/FAPERGS/PROREXT.

A novel gene derived from a segmental duplication shows perturbed expression in Alzheimers disease. *D. Avramopoulos[1], R. Wang[1], M.D. Fallin[2], S.S. Bassett[2]* 1) Psychiatry, Johns Hopkins Univ, Baltimore, MD; 2) Public Health, Johns Hopkins Univ, Baltimore, MD.

Alzheimers disease (AD) is a disabling neurodegenerative disorder with an onset most commonly in late life. Three genes have been identified to date causing earlier onset AD and one other has been shown to be a risk factor for late onset AD (LOAD). The existence of many more is suspected for LOAD yet they remain undetected. A number of studies have reported linkage to a LOAD locus on human chromosome 10, and we have identified a parent of origin effect for that region. We present here a gene on chromosome 10 showing an expression profile that suggests its involvement in LOAD. Its expression is significantly reduced in females and with increasing age, both of which are risk factors for LOAD, and it is further decreased in patients. Interestingly the latter effect is more pronounced in patients with an affected mother, in concordance with our linkage results. Although we originally studied this transcript attempting to measure the product of the alkaline ceramidase gene (ASAH2), a good candidate gene for AD, we found that the transcript is the product of a new gene that we call ASAH2L. This gene is the result of a partial duplication of ASAH2 ~500 Kb away and next to promoter sequences. It carries a polymorphic start codon derived from a single nucleotide change of the original ASAH2 sequence but also other putative translation start sites that would produce novel proteins. ASAH2L is an interesting example of the generation of a new gene, and data from genome databases suggest that the duplication that generated the gene is human specific. Although its function remains unknown our expression data suggest that the ASAH2L transcript might play a role in preventing neurodegeneration.

Imatinib-induced CML remission as evidenced by automated FISH analysis predicts good outcome but is not achieved in patients with der(9) deletion within 30-month follow up. *G. Calabrese[1,2,3], D. Fantasia[2], P. Guanciali Franchi[1,2], R. DiGianfilippo[2], E. Morizio[2], M. Alfonsi[1,2], A. Di Tecco[2], A. Marzoli[2], L. Stuppia[1,3], R. DiLorenzo[4], G. Palka[1,2,3]* 1) Scienze Biomed/Genetica Medica, Univ G D'Annunzio, Chieti, Italy; 2) U.O. Genetica Umana, Pescara Hospital, Pescara, Italy; 3) CESI, Center for Aging, D'Annunzio Foundation, Chieti, Italy; 4) Dept. Hematology, Pescara Hospital, Pescara, Italy.

Seventy patients with CML, 63 in chronic phase (CP), 1 in accelerate phase (AP), and 6 in blastic crisis (BC), were treated with imatinib mesylate (IM). The patients were studied for a 18-61 months follow up period using clinical and haematological features, cytogenetics and FISH analysis with a dual-fusion BCR-ABL probe, and an automated FISH imaging system for rare cell events (BioView-Duet). Before IM treatment 51 patients showed Ph chromosome in all examined cells while in the remaining 19 patients a normal clone was also found. FISH analysis showed BCR-ABL fusion gene in >95% of cells in 49 patients including 9 with a partial deletion on der(9) at band q34. In 18 patients BCR-ABL rearrangement was observed in 5-95% of cells while in 3 it was present in <5% of nuclei. Complete or partial cytogenetic remission rate [CCR or PCR: t(9;22) absent or <35% of cells, respectively] overlapped complete or partial FISH remission rate (CFR or PFR: BCR-ABL absent or <35% of cells, respectively) along all the follow up period being observed in 72% of patients. However, CFR achievement within 12 months of treatment resulted in a disease-free second year of treatment in 94% of patients, while 33% of samples with a CCR actually showed >1% leukaemic cells at FISH analysis. Moreover, FISH unravelled leukaemic cells in 21% of samples unsuitable for cytogenetic investigation from 9 patients who subsequently developed haematological relapse. In patients with partial deletion on der(9), no CCR/CFR was achieved. Clinical and haematological relapse was observed in 4 cases, while in the remaining 5 a PCR/PFR was observed only after 30 months of treatment supporting a negative role of der(9) deletion on IM effect in CML patients as previously reported with other therapy protocols.

Association study of the GABA(A) receptor cluster at 15Q12 with autism in the French Canadian population.

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Chromosomal region 15q11-13 has been consistently implicated in autism through numerous avenues such as maternal duplications(3), multiple types of chromosomal abnormalites in the region(2), and presence of autism in patients with Prader-Willi and Angelman syndrome(5). Furthermore, linkage and assocation to this region has been previously described and replicated in different populations of autistic individuals(1,4). 140 trios, of which 70-75% are of French Canadian origin were used in a TDT based association study. 26 SNPs, with an average spacing of 30 Kb, spanning 820Kb of the GABA(A) cluster at 15q12 were selected from the dbSNP and Celera databases based on minor allele frequency > 0.05, spacing and haplotype structure. Three markers were shown to be in transmission disequilibrium including the cytosine of SNP rs2873027 contained within intron 3 of GABRB3 which replicates a previous study4.

Repeated Analysis of Limited Amount of DNA/RNA Samples Using an Irreversible Binding Matrix. *C. Brown, V. Boyd, E. Gerber, K. Hunkapiller, S. Chen, A. Lakdawalla* Applied Biosystems, Foster City, CA.

Substantial libraries (> 100K samples) of characterized formalin-fixed paraffin-embedded tissue sections and the associated information provide an exceptional resource for clinical research. Molecular analyses of these samples are limited to the small amounts of extractable RNA and DNA. Methods like pre-amplification, whole genome amplification, and cloning have been used to augment the quantity of nucleic acids. An alternative approach is to recycle the extracted RNA and DNA for an increased number of analyses.

A binding matrix, based on aluminum and zirconium oxides, can bind to RNA/DNA in an irreversible manner under defined conditions. The bound nucleic acid can be used for multiple enzymatic reactions (reverse transcription and/or PCR) without depleting the bound nucleic acid. The amplified product does not bind to the matrix under the reaction conditions and can be removed for downstream analysis (real-time PCR, sequencing, fragment analysis).

We will present performance data on the sequential PCR amplification and sequencing of limited amounts of genomic DNA in the hypervariable region of mitochondrial DNA.

Linkage studies in an Ecuadorian population with Keratoconus. *B.A. Bejjani[1,4], D. Winters[1], A. Molinari[2], J.A. Pitarque[2], M.H. Chahrour[3], S.M. Leal[3], M. Gajecka[1], R.A. Lewis[3]* 1) Health Research & Education Center, Washington State University, Spokane, WA; 2) Hospital Metropolitano, Quito, Ecuador; 3) Baylor College of Medicine, Houston, TX; 4) Sacred Heart Medical Center, Spokane, WA.

Keratoconus (KC) 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. Although both genetic and non-genetic factors have been associated with KC, its molecular basis is still elusive. We identified an Ecuadorian cohort in which KC without other ocular or systemic features is transmitted as an autosomal dominant trait with incomplete penetrance. Here we present the results of sequencing and linkage analyses which were performed to verify the association between KC in these Ecuadorian families and the previously reported KC loci. To date, we have examined, collected blood, and purified DNA from 181 individuals from 25 multiplex families with KC. Subjects were diagnosed clinically with KC by slit lamp examination and corneal topography. We excluded previously assigned KC loci on chromosomes 3, 15, 16, and 20 by linkage analysis. Additionally, the coding exons of *VSX1* in 18 individuals from the Ecuadorian families (1 individual from each family with multiple affected individuals) and 2 ethnically matched control individuals (Ecuadorian individuals with no ocular abnormalities) were sequenced. Three single nucleotide polymorphisms (SNPs) in the *VSX1* coding region (18GT, 174GT and 542AG) were identified, but no mutation was found in the gene. Next, we performed a genome-wide screen with fluorescent markers with an average spacing of 10 cM spanning all chromosomes. No evidence for linkage between KC and the analyzed markers was observed. We initiated a second genome-wide screen for a KC locus with fluorescent markers with an average spacing of 5 cM spanning all chromosomes. Genotyping is in progress. Keratoconus in our families is not linked to any of the previously defined KC loci. We have excluded *VSX1* as a candidate for KC in this population.

A high-throughput method for direct sequencing from bacterial cultures. *T. Ganguly, P. Chen, R. Teetsel, L. Zhang*
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Large scale screening of bacterial colonies from shotgun plasmid libraries, cDNA or other libraries by DNA sequencing requires a rapid and cost effective method. DNA sequencing technologies have significantly improved over the years giving rise to enhanced sequence quality, higher throughput, and a substantial reduction in cost. However, in large-scale sequencing projects, the preparation of templates involving cellular growth from bacterial colonies and the subsequent plasmid purification continues to be one of the most costly steps in terms of reagents and labor. Previous attempts to sequence directly from bacterial colony or culture have yielded poor quality data and short sequence reads. The latest advancements in sequencing instrumentation, chemistry, and data analysis tools, available from Applied Biosystems (ABI), have significantly increased the quality and efficiency of direct sequencing as demonstrated by a recent report from ABI that used a proprietary buffer for cell lysis. This prompted us to initiate an effort to reduce or eliminate the reagent cost involved in plasmid DNA preparation for direct sequencing. We would describe a simple, high throughput, and inexpensive method for direct sequencing of high copy number plasmids by heat lysis of bacterial cells in water. The sequence quality and efficiency is comparable to that obtained by using commercial reagents. Furthermore we would present data on the suitability of the cellular lysates for other applications including PCR, restriction digestion and transformation.

A novel dicentric chromosome 13 - a familial variant? *K. Chun, S. Trevisiol, E. Capua, L. Hunniset, D. Allingham-Hawkins, L. Velsher* Genetics Program, North York General Hospital, Toronto, ON, Canada.

A 37.5-year-old woman of Turkish descent and her husband were seen for genetic counseling for advanced maternal age. The couple has four healthy children and is 15 weeks into their fifth pregnancy. Due to neonatal deaths of siblings of both the patient and her husband, chromosome studies were ordered for the couple. The husbands chromosomes were normal. However, both homologues of chromosome 13 for the patient appeared identical and dicentric. C-banding showed two centromeres and NOR staining showed two stalk regions on both chromosomes. FISH analysis using the alpha-satellite probe set for chromosomes 13/21 showed two signals on each chromosome 13. Meanwhile, an MSS result came back positive for Down syndrome and an amniocentesis was performed. The fetus karyotype was normal except for having inherited one copy of the dicentric chromosome 13. Family history revealed that the patients parents are consanguineous. Although UPD studies for chromosome 13 were considered, due to the family history of consanguinity and no abnormal clinical features in the patient and her four children, this novel dicentric chromosome 13 is most likely a very interesting familial variant.

SOX9cre1: a distal *SOX9* regulator that interacts with Hh signaling factors. *G.A. Bien-Willner[1], P. Stankiewicz[1,2], J.R. Lupski[1, 3, 4]* 1) Department of Molecular and Human Genetics, BCM, Houston, Texas; 2) Department Medical Genetics, Institute of Mother and Child, Warsaw, Poland; 3) Texas Childrens Hospital, Houston, Texas; 4) Department of Pediatrics, BCM, Houston, Texas.

Chromosomal rearrangements, such as translocations, can sometimes alter a nearby genes expression resulting in disease, despite the rearrangement breakpoints mapping at great distances from the disease-causing gene. Such phenomena can be defined as position effects, and have led to speculation of the importance of chromatin interactions occurring upwards of a megabase upstream or downstream to certain genes. Position effects have been reported with *SOX9*, which encodes for a transcription factor essential in male sexual development and bone formation. Although haploinsufficiency of *SOX9* results in campomelic dysplasia (CD), several clinical cases of this phenotype have been described with translocation breakpoints mapping as far as 932 kb upstream from the gene. Studying one such case (900 kb upstream to *SOX9*) in the context of an apparent clustering of breakpoints has led to the prediction of a possible cis-acting regulatory element 1.1 Mb upstream of *SOX9*, named SOX9cre1, which may be responsible for a subtle CD phenotype when displaced. We investigated the role of this putative regulator in *SOX9* expression using reporter assays in a *SOX9*-expressing chondrosarcoma cell line, and evaluated the tissue-specific activation of the gene. SOX9cre1 increases the activity of a minimal *SOX9* promoter in reporter constructs nearly three-fold, in a tissue-specific manner, consistent with an enhancer role. In silico studies identified a Gli1 binding site in the 2.1 kb SOX9cre1, suggesting that *SOX9* may be activated directly through the Sonic hedgehog (Shh) or related Hh cell signaling pathways known to be involved in *SOX9* expression. EMSA and functional assays in which reporter constructs are co-transfected with Gli1 and Smad1 of the bone morphogenic protein (Bmp) signaling pathway suggest these transcription factors may directly interact with SOX9cre1. These data support a link between the Hh signaling pathways and *SOX9* regulation, suggesting a mechanism of regulation through distal chromatin interactions.

Fine mapping and confirmation of linkage to chromosome 9q22 in colorectal neoplasia kindreds. *C. Gray-McGuire[1,4], R.C. Elston[1,4], S.D. Markowitz[4,5,6], J. Potter[7], N. Lindor[8], G.L. Wiesner[2,3,4,5]* 1) Dept Epi and Biostat, Case Western Reserve Univ, Cleveland, OH; 2) Dept Genetics, Case Western Reserve Univ, Cleveland, OH; 3) Center for Human Genetics, Case Western Reserve University and Univ Hospitals of Cleveland, Cleveland, OH; 4) Ireland Comprehensive Cancer Center, Western Reserve University and Univ Hospitals of Cleveland, Cleveland, OH; 5) Dept of Medicine, Case Western Reserve University and University Hospitals of Cleveland, Cleveland, OH; 6) Howard Hughes Medical Institute, Cleveland, OH; 7) Fred Hutchinson Cancer Institute and Colon Cancer Family Registry, Seattle, WA; 8) Mayo Clinic and Colon Cancer Family Registry, Rochester MN.

Colorectal Cancer is the second leading cause of cancer in American adults and presents a substantial public health concern. While ~5% of cases are accounted for by familial adenomatus polyposis and hereditary nonpolyposis colon cancer, 20% of cases have a family history of colon cancer. This study is a follow-up of a genome scan for colorectal neoplasia predisposing genes in a European American sample comprising 185 sibling pairs ascertained through two or more sibs with a history of colorectal cancer (Wiesner et al. PNAS, 2003). The primary area of interest from the genome scan was chromosome 9q22.2-31.2. For this study, genotypes for 10 additional markers were generated for 1) a revised version of the sample mentioned above (182 sibling pairs), referred to as ROC and 2) a confirmation collection (CC) of 90 sibling pairs. These fine mapping regions were analyzed using the weighted Haseman-Elston regression (dependent variable is a weighted average of the squared sib-pair trait difference and the squared mean-corrected sum) and the sibling pair mean test. The marker with the strongest signal in the ROC and ROC+CC was D9s1786 (-log p-values =3.25, and 3.38, respectively) the same marker indicated in the genome scan. The mean test yielded -log p-values of 3.30, and 3.79 for the ROC and ROC+CC collections, respectively. These results both confirm the presence of a susceptibility gene on chromosome 9 as well as narrow the region most likely to contain this susceptibility gene to 9q22.31-9q22.33.

Four Genes in the Hypertension Pathway are confirmed to be Genetic Markers for Insulin Resistance. *X. Guo[1], K.D. Taylor[1], S. Cheng[2], B. Fang[1], J. Cui[1], A.H. Xiang[3], M.J. Quiñones[4], J.C. Ramirez[4], W.A. Hsueh[4], 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, Inc; 3) USC; 4) UCLA.

Insulin resistance (IR), though most often considered in terms of the pathophysiology of type 2 diabetes, also influences blood pressure and is a risk factor for hypertension. Previously, in Mexican-American families ascertained via a coronary artery disease proband (MACAD), we found evidence for association between IR and variations in 6 previously reported hypertension genes: angiotensinogen (AGT), adducin 1 (ADD1), natriuretic peptide precursor A (NPPA), beta-2-adrenergic receptor (ADRB2), sodium channel, nonvoltage-gated 1 alpha (SCNN1A), and nitric oxide synthase 3 (NOS3). We have now genotyped the same SNPs in these 6 genes in an independent cohort of 566 non-diabetic offspring from 150 Mexican-American families ascertained through a hypertensive proband in our Hypertension-Insulin Resistamce project (HTN-IR). As in MACAD, IR was assessed by the euglycemic clamp, in which the glucose infusion rate (GINF) and insulin sensitivity index (SI) were determined. The program QTDT was utilized to evaluate the associations between genetic variations and IR, adjusting for age, sex and BMI. ADRB2, NPPA, NOS3, and SCNN1A were each associated with indices of IR (p= 0.017, 0.008, 0.034, 0.016 resp.). Haplotype analysis with 2 SNPs in NOS3 demonstrated that one haplotype was associated with insulin sensitivity (IS) in both MACAD and HTN-IR samples, providing a strong confirmation for the association. Similarly, specific haplotypes in NPPA and SCNN1A were associated with IR in both samples, while another haplotype in NPPA was associated with IS. In conclusion, we were able to replicate association with insulin resistance in 4 out of the 6 genes, ADRB2, NOS3, NPPA, and SCNN1A. This supports a genetic basis for the known associations among diabetes, hypertension, and insulin resistance, and emphasizes the importance of consistency in phenotype assessment and ethnicity when attempting to confirm genotype-phenotype associations.

Histone deacetylation enhances qkI transcription, an essential factor for promoting oligodendroglia differentiation. *Y. Chen[1], C. Kitchen[1], M. Xia[1], D. Osterhout[2], Y. Feng[1]* 1) Pharmacology, Emory University, atlanta, GA; 2) Cell and Developmental Biology,SUNY Upstate Medical University.

The dynamic regulation of histone acetylation modulates transcription through remodeling the chromatin structure. Although increased histone acetylation is often associated with enhanced transcription, emerging evidence suggests opposite scenarios in which histone deacetylation can also up-regulates transcription. In particular, a global reduction of histone acetylation is accompanied with up-regulated differentiation markers of oligodendroglia, a special type of glia for myelination of the CNS. Moreover, inhibition of histone deacetylase (HDAC) abrogates oligodendroglia differentiation, suggesting that histone deacetylation may enhance transcription of genes that promote oligodendroglia differentiation. However, which genes are directly regulated by such mechanism still remains elusive. Here we report that histone deacetylation up-regulates transcription of the quakingI (qkI) gene, which encodes a selective RNA-binding protein essential for oligodendroglia differentiation. We show that qkI transcription is vigorously elevated upon induced differentiation. In addition, we identified several genetic fragments that mediate differentiation-induced qkI transcription. Interestingly, histone acetylation at the qkI promoter/regulatory elements is reduced during oligodendroglia differentiation. Deletion of these regulatory fragments in the quakingviable (qkv) mutant results in abnormally increased histone acetylation of the qkI promoter and diminished qkI transcription. We further show that HDAC inhibition increases histone acetylation at the qkI promoter, markedly reduces qkI transcription, and abrogates oligodendroglia differentiation, all can be reversed after the inhibitor is removed. In contrast, histone acetylation of the promoter of an adjacent gene, pacrg, is not affected under these conditions. Together, our data suggest that the qkI promoter is sensitive to the dynamic changes of histone acetylation, and qkI transcription is up-regulated upon histone deacetylation, which is a conceivable factor that promotes oligodendroglia differentiation.

Assessing Genetic Literacy in Undergraduate Students. *E.E. Acra[1], C.A. Huether[1], B.V. Bowling[1], H. Bender[2]* 1) Univ Cincinnati, OH; 2) Univ Notre Dame, IN.

Genetics issues play a large role in health and public policy, and new knowledge in the field will continue to have significant implications for individuals and society. It is therefore important to continue improving genetics education at all levels to promote greater genetic literacy. This study assessed both the level of genetic literacy in undergraduate students, and their attitudes toward genetics issues. It also evaluated the effectiveness of different courses in increasing genetics knowledge and the impact of the courses on students attitudes. 416 undergraduate students in seven courses at the University of Cincinnati participated in this study representing both biology and non-biology majors. Data were collected utilizing a modified 35-item inventory of multiple choice and true/false questions from the University of Notre Dame. The inventory was completed by students in general biology and genetics courses: students in a general psychology course were used as a control. Pre-course and post-course scores were compared among courses as well as within each course. Overall students in courses for biology majors scored higher on the pre-course instrument (69.83%) than students in the general psychology course (65.21%) and those in courses for non-biology majors (60.53%). The effect each course had on genetic literacy was also assessed by comparing post-course scores to pre-course scores. Significant increases in knowledge scores were seen in only two of the four courses emphasizing genetics, one course for non-majors and one honors course for majors. The psychology course showed a significant decrease in knowledge scores. Very little change in attitude scores was observed in any of the courses. The results indicate that some genetics courses are not effective in increasing levels of genetic literacy. Course content and pedagogy need to be considered in these courses. However, some of the results, particularly the significant change in the control group, raise concerns that the instrument may not be as reliable as desired. A valid and reliable instrument designed specifically for testing genetic literacy in undergraduates would allow a more accurate measure of students knowledge and course impact.

Phenotypic Spectrum of Adducted Thumbs: Case Report. *M. Descartes, D. McIlvried* Dept Genetics, UAB, Univ Alabama at Birmingham, Birmingham, AL.

Adducted thumbs is a mandatory feature of several syndromes, including whistling face syndrome, congenital contractural arachnodactyly, multiple pterygium syndrome, distal arthrogryposis type I, X-linked recessive hydrocephalus, MASA syndrome, Christian-Adducted thumb syndrome, and adducted thumb-club foot syndrome. All of these conditions can be differentiated on a clinical basis and all have a neuropathic and/or myopathic origin. We report a male patient with isolated adducted thumbs and learning disabilities. The patient is the second child born to a 38 year old G2P1 mother. He was noted at birth to have bilateral adducted thumbs without additional associated complications. At age 8 years, he had normal growth parameters. On physical examination he was noted to have frontal bossing, wide set eyes, downslanted palpebral fissures, overfolded helices and mild joint laxity. Other features included bilaterally adducted thumbs, decreased hypothenar eminence, fifth finger clinodactyly, and plantar gap. He has been diagnosed with ADHD and dyslexia, and his IQ score was reported to be in the 90s. His evaluation has included chromosome analysis in peripheral blood, spine MRI, head CT scan and skeletal survey. The skeletal survey showed adducted thumbs and bilateral hind foot valgus. The other tests were reported as normal. The family history was noncontributory. LICAM mutation analysis and chromosome microarray analysis are pending. X-linked hydrocephalus and MASA syndrome are associated with mutations of the L1CAM gene on Xq28. L1CAM mutations affect mainly the central nervous system and are characterized by a variable phenotype that includes mental retardation, abnormal brain development, spastic paraplegia and adducted thumbs. Recently, Frints et al. (2003) reported a 76 year old male with non-specific mental retardation, bilateral adducted thumbs and a Xp22.1;3p26.3 translocation. The 3p breakpoint disrupted the neural Cell Adhesion L1-Like (CALL) gene, which has been previously linked to non-specific MR and is closely related to LICAM both structurally and functionally. Adducted thumbs may represent a mild manifestation of a phenotypic spectrum associated with mutations in the neural cell-adhesion gene. .

MLS syndrome evaluated by prenatal karyotyping, FISH and array CGH. *C.C. Cain[1], D.O. Saul[1], L. Attanasio[1], A. Hamosh[2], E. Oehler[1], K. Blakemore[1], G. Stetten[1,2]* 1) Department of Gynecology and Obstetrics, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, MD.

Microphthalmia with linear skin defects (MLS) syndrome is a rare developmental disorder inherited as an X-linked dominant and associated with monosomy of the Xp22.3 region. We describe a severe case of MLS syndrome that presented prenatally with multiple anomalies including cystic hygroma, micropthalmia, intrauterine growth restriction and a complex congenital heart defect. Chromosome analysis of amniocytes revealed a de novo translocation between chromosomes X and Y [karyotype 46,X,der(X)t(X;Y)(p22.3;q11.2).ish der(X)(DXZ1+,DMD+,KAL-,STS-,SRY-),22q11.2(Tuple1x2)]. MLS diagnosis was made at birth and the prenatal karyotype was confirmed. Replication studies showed the translocated X chromosome was inactive in all 20 fibroblasts examined. Comparative genomic hybridization to BAC arrays (array CGH) was used to explore the genomic imbalance and refine the breakpoints of the rearrangement. Array CGH confirmed the X and Y imbalances seen in the karyotype and also showed eleven probes in the MLS region were deleted as a result of the translocation. FISH with BAC clones verified the array findings and placed the X breakpoint in Xp22.2, just proximal to the MLS critical region. In conclusion, the sensitivity of array CGH was valuable in detecting monosomy of the MLS critical region. Array CGH should now be considered for the prenatal diagnosis of MLS syndrome.

Genome-wide strategies to identify DNaseI hypersensitive sites. *G.E. Crawford[1], S. Davis[2], P.C. Scacheri[3], G. Ranaud[2], P.S. Meltzer[2], T.G. Wolfsberg[2], F.S. Collins[2]* 1) Institute for Genome Sciences & Policy, Duke University, Durham, NC; 2) National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 3) Department of Genetics, Case Western Reserve University, Cleveland, OH.

Mapping DNaseI hypersensitive (HS) sites is a highly accurate method of identifying the location of active gene regulatory elements, including promoters, enhancers, silencers, and locus control regions. While Southern blots have been the traditional method of identifying DNase HS sites for the last 25 years, this standard manual method is not readily scalable to studying large chromosomal regions, much less the entire genome. We have developed two high-throughput strategies to identify DNase HS sites across the genome. One method uses next-generation sequencing strategies developed by Solexa and 454 Life Sciences to sequence DNase-treated nuclear DNA, recovering a very large number of short sequence tags at the sites of cleavage. A second method, called DNase-chip, identifies DNase HS sites using NimbleGen tiled microarrays. Both methods have been shown to be highly accurate at identifying valid DNase HS within the representative 1% of the genome identified by the ENCODE consortium. We have recently scaled up the number of tag sequences (1.1 million per cell type) as well as increased the number of tiled microarrays to analyze the entire non-repetitive portion of the human genome. While many DNase HS sites appear immediately upstream or in the first intron of known genes, others appear in intergenic regions or even in gene deserts. Genes that have a DNase HS site nearby are more likely to display elevated gene expression than genes that do not. While many DNase HS sites are found to be present in more than one cell type studied, a number are cell type specific, indicating we have identified tissue-specific gene regulatory elements. As genome-wide sequencing and tiling microarrays become increasingly affordable, it will be possible to apply these methods globally to any tissue from any species with a sequenced genome.

104,268 SNP whole genome association results for alcohol dependence converge with genome scanning data from other addictions. *T. Drgon, C. Johnson, Q.R. Liu, D. Walther, G.R. Uhl* Dept Molecular Neurobiology, NIDA-IRP/NIH, Baltimore, MD.

Association genome scanning can identify markers for the allelic variants that contribute to vulnerability to complex disorders, including alcohol dependence. To improve the power and feasibility of this approach, we have validated 100k microarray-based allelic frequency assessments in pooled DNA samples. We then used this approach to analyze 100k genome scanning data from unrelated alcohol dependent vs control individuals sampled from pedigrees collected by the Collaborative Study on the Genetics of Alcoholism (COGA). Allele frequency differences between alcohol-dependent and control individuals were assessed in quadruplicate at 104,268 autosomal SNPs in pooled samples. Positive SNP clusters nominate interesting genes whose products are implicated in cellular signaling, gene regulation, development, cell adhesion and Mendelian disorders. These data converge with association results from association genome scans for amphetamine dependence, polysubstance abuse and nicotine dependence to extents that are not seen by chance in Monte Carlo simulation studies. These convergent data support polygenic contributions to vulnerability to dependence on a number of different studies, as suggested by classical genetic twin data. Studies using higher marker densities and studies in other samples of alcohol dependent individuals will help assess whether the findings that do not replicate in samples from other addictions represent loci in which allelic variants selectively impact vulnerability to alcohol dependence. The reproducibly-positive SNPs identified here provide new tools to aid the understanding, prevention and treatment of substance abuse and dependence. (Support: NIH IRP, NIDA).

Characterizing karyotypic evolution in adenocarcinoma of the pancreas. *C. Griffin[1,2], J. Kowalski[2], L. Morsberger[1], A. Hawkins[1], A. Blackford[2], C. Yeo[3], R. Hruban[1,2]* 1) Pathology; 2) Oncology; 3) Surgery, Johns Hopkins University, Baltimore, MD.

The high level of karyotypic complexity found in epithelial tumors makes it difficult to characterize cytogenetic evolution. Derivation of such pathways in adenocarcinoma of the pancreas also has been limited because virtually no tumors are resected at an early stage of disease. In addition, the number of primary tumors for which analysis of abnormal karyotypes has been reported is small. We report clonal karyotypic abnormalites from G-band analysis of 36 primary pancreas carcinomas. 91% of the tumors were diploid or triploid. Numerical alterations were common. All chromosomes were involved in gain and/or loss in at least 8 and up to 28 tumors. Chr 18, 17, 6, 21, 22, Y & 4 were most commonly lost in 28, 20, 16, 15, 15, 13 & 12 tumors respectively. Gain of chr 20 was found in 10 carcinomas. Structural abnormalities were common, with a median number of 7 imbalances (excluding whole chromosome loss) per tumor (range 1-15). 16 tumors had dms and/or hsrs, indicating gene amplification. Adding these tumors to those published in the Mitleman database (http://cgap.nci.nih.gov/Chromosomes/CytList) we used statistical methods to begin to determine pathways of karyotypic evolution. Based on analyses of 105 tumor samples (44 with 2N ploidy; 61 with not 2N ploidy) from 9 studies, the number of imbalances (sum of the number of losses and/or gains) per chromosome & tumor was calculated & categorized as either none or at least one imbalance. A test of the null hypothesis of no difference in the percent imbalances between 2N ploidy vs. not 2N ploidy tumors, among all chromosomes was rejected ($p=0.005$), indicating a higher percent of imbalances among not 2N ploidy tumors (60%) vs. 2N ploidy (25%). Chromosomes 10, 16, 22, 8 & 15 were identified as contributing to such a difference. In this analysis, information from ploidy was used as a marker of evolution to address the question of whether there is any particular evolutionary advantage to being diploid. Results from analyses of the temporal evolution of these samples, irrespective of ploidy will be compared.

Mandibulo-facial dysostosis, acral anomalies, and frontonal dysplasia: a new form of Acrofacial dysostosis.
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Acrofacial dysostosis (AFD) is a condition that involves mandibulo-facial dysostosis (MFD) associated with limb defects in varying degrees [Rodríguez et al., 1990].The more severe forms, considered lethal AFD can be subdivided, more or less confidently, into Nager, POADS, and Rodríguez [Opitz et al., 1993].Nager AFD is characterized by malar, mandibular, and maxillary hipoplasia, macrostomia, abnormal ears, and radial defects. The lower limbs are usually normal. Is an autossomal dominant syndrome with variable expression [Merrer et al., 1989; Goldstein and Mirkin.,1988]. Opitz [1987] has suggested that Nager AFD should be considered an anomaly rather than a syndrome due to the apparent causal heterogeneity of this AFD. POADS differs from the preceding by preferential involvement of ulna and fibula with inconstant involvement of radius. Is an autossomal recessive trait. Lethal cases in the fetal or neonatal period are unusual and show more severe anomalies [Rodríguez et al., 1990; Merrer et al., 1989]In 1990, Rodriguez et al., described three affected sibs with severe, apparently lethal AFD. This syndrome type Rodríguez envolves MFD, predominantly preaxial limb deficiencies, rare postaxial limb anomalies, absent fibulae and ribs, shoulder and pelvic hipoplasia, cardiac malformations, CNS malformations (arrhinencephaly, triventricular hydrocephaly due to aqueductal stenosis, corpus callosum agenesis), absence of lung lobulation and urogenital anomalies [Petit et al., 1992]. Its an autossomal recessive trait.We described a female stillborn with acrofacial dysostosis and frontonasal dysplasia. The patient had protrusion of the forehead with accentuated hypertelorism and nose absence with rhinencephalo. The autopsy showed wide cranial sutures, severe hydrocephaly with distinction of the hemispheres right and left of the brain and preservation of the olfactory bulb, first and second cranial nerves, small kidneys bilaterally, and anal atresia with recto-vaginal fistula. Therefore, for our patient, we suggested to treat in a new form of AFD.

Expanding spectrum of CDG-Ig: Siblings with prenatal-onset skeletal dysplasia, B-cell lymphopenic hypogammaglobulinemia, cardiac and genital malformations, and fatal outcome. *A.A. Basinger[1], C. Kranz[2], M. Gucsavas-Calikoglu[1], C.M. Powell[1], F.W. Henderson[1], H.H. Freeze[2], A.S. Ayslworth[1]* 1) Pediatrics, UNC Chapel Hill, Chapel Hill, NC; 2) Burnham Institute for Metabolic Research, La Jolla, CA.

The congenital disorders of glycosylation (CDGs) are a diverse group of disorders caused by disruption in production of lipid-linked oligosaccharides (LLOs). We present siblings with prenatal-onset skeletal dysplasia, polyhydramnios, and prematurity. Dysmorphic features included broad nose, thick ears, thin lips, micrognathia, inverted nipples, ulnar deviation, long fingers with spatulate tips, fifth finger camptodactyly, nail hypoplasia, and talipes equinovarus. Other features included psychomotor retardation, hypogammaglobulinemia, normal fat distribution, deafness, intestinal malrotation and poor gastrointestinal motility, edema, persistent hyponatremia, and intermittent hypoglycemia and thrombocytopenia. Cardiac malformations include PDA, VSD, biventricular hypertrophy, and arrhythmias. Small penis with hypospadias, hypoplastic scrotum, and non-palpable testes were noted. Radiographic studies showed short limbs, absent ossification of cervical vertebral bodies, short, thin ribs with flared metaphyses, and short humeri, radii, femurs, and tibiae. Both died in infancy, one of overwhelming sepsis and the other of cardiorespiratory failure. Metabolic and molecular studies reveal a type I pattern of abnormal transferrin glycosylation. Activities of phosphomannomutase and phosphomannose isomerase in fibroblasts were normal. HPLC analysis showed that fibroblasts synthesized truncated LLOs, primarily $Man_7GlcNAc_2$, suggestive of CDG-Ig. Mutation analysis of *ALG12* showed that both sibs were compound heterozygotes for a novel mutation 301 A>C (G101Arg) and previously described 437 G>A (R146Q). Congenital disorders of glycosylation should be considered in the etiology of prenatal-onset skeletal dysplasias with cardiac and genital malformations. Precise diagnosis will require collaboration with a laboratory experienced in testing for these novel and complex conditions. (Supported by R01 DK55615 to HHF).

Genome-wide analysis of gene expression in transformed lymphocytes from sib pairs discordant for type 2 diabetes (T2DM). *S.C. Elbein[1,2], W.S. Chu[1,2], S.K. Das[1,2]* 1) Internal Medicine, University of Arkansas for Medical Sciences, Little Rock, AR; 2) Central Arkansas Veterans Healthcare System, Little Rock, AR 72205.

T2DM shows replicated linkage to chromosome 1q21-23, and work from our laboratory has mapped that linkage to the D1S484-D1S2705 region. To further map genes for this region or under control of this region, we examined the differential expression in total RNA isolated from E-B virus transformed lymphocytes (TLs) from 4 sib pairs discordant for both T2DM and marker D1S2705, and selected from families linked to chromosome 1q. All TLs were grown in 10% FBS supplemented RPMI-1640 medium at 5.6 mmol/l glucose (physiologic). GE Healthcare CodeLink Bioarrays (Human ~55K) were hybridized with the biotinylated cRNA target, stained with Cy5-streptavidin and scanned on an Axon GenePix 4000B scanner (performed by GenUS BioSystems, Inc., Northbrook, IL). Data was analyzed with CodeLink and GeneSpring software packages. Intensity was normalized to the median intensity of each array. Comparison of T2DM subjects with controls showed the expression of 28,212 genes in at least 3 members of each group and with <1 standard deviation, whereas pairwise analysis of the discordant siblings showed the expression of 28,864 genes in at least 3 discordant pairs. Groupwise analysis showed 132 differentially expressed genes (>1.5-fold difference and ANOVA p-value <0.05), whereas only 38 genes differed (>1.5-fold difference) between all 4 sib pairs. In total, 20 genes were common to pairwise and groupwise comparisons (11 up regulated and 9 down regulated). Differentially expressed genes mapped to previously reported regions of linkage on chromosomes 1q(2 genes), 11q (2 genes), and 12q (4 genes). Several genes, including DNAJC12 (HSP40; 10q22.1), which showed 4.2-6.8 fold over-expression, are potential candidates for T2DM. A putative gene (Hs.127310) with serine/threonine kinase activity in the linked region at 1q23.3 also showed significant up-regulation (2.4 fold) in the T2DM group. Studies are in progress to confirm these findings with real time PCR and to further examine the role of these genes in T2DM pathophysiology.

Effects of urban migratory patterns on a contemporary regional gene pool of Quebec. *C. Bherer[1, 2], B. Brais[2], H. Vézina[1]* 1) Interdisciplinary Research Group on Demography and Genetic Epidemiology (GRIG), University of Quebec at Chicoutimi, Canada; 2) CHUM Research Centre, University of Montreal, Canada.

Interregional mobility towards urban areas and their periphery has become increasingly important throughout the 20th century. Because of its geographic location (north-east of the Montreal area) and a recent demographic expansion, the Lanaudiere region offers a favorable context to explain and illustrate the effects of these migratory patterns on the structure of its gene pool. This study aims at characterizing intraregional variability and temporal transformation of the Lanaudieres gene pool. A second objective is to gain a better understanding of the role of settlement history in the spread of two founder mutations causing recessive hereditary sensory and autonomic neuropathy type 2 (HSAN2, OMIM 201300). We sampled 400 individuals from 2 subregional populations (north and south) and married in 2 periods (1945-55 and 1985-95). Ascending genealogies of selected individuals were reconstructed using the BALSAC database (average depth: 10 generations). For the 1945-55 period, analyses of kinship and consanguinity in the northern part of Lanaudiere point toward a stronger endogamy than in the southern part, while the distribution and genetic contribution of ancestors are quite similar in the two subregions. For the 1985-95 period, the genealogical indices display much more heterogeneity in the southern part of the region which is consistent with the recent demographic boom in this territory. Overall, a marked reduction of kinship and consanguinity levels in Lanaudiere is observed in this fifty-year period and it can clearly be linked to interregional migratory trajectories toward urban areas. Lastly, we integrated a particular genetic story to this diversity pattern by analyzing the introduction, diffusion and current distribution of the 2 French Canadian mutations causing HSAN2. Analyses of ascending genealogies of carriers and controls allowed us to identify ancestral clusters with the highest probability of having introduced the mutations in the Quebec population.

Molecular analyses of duplications on human chromosome 17p. *W. Bi[1], X. Shi[1], C.M.B.C. Fonseca[1], C. Shaw[1], G. Weissenberger[1], L. Potocki[1,3], J.R. Lupski[1,2,3]* 1) Dept Molecular & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Dept Pediatrics, Baylor Col Medicine, Houston, TX; 3) Texas Children's Hospital, Houston, TX.

Dup(17)(p11.2p11.2) syndrome is a genomic disorder caused by an interstitial duplication in chromosome 17 sub-band p11.2. We previously reported 7 patients with a common duplication of ~4 Mb that is reciprocal to the common deletion present in >70% of Smith-Magenis syndrome (SMS) patients. Both common deletions and common duplications are mediated by meiotic homologous recombination between non-allelic low-copy repeats, the distal and proximal SMS-REPs. We analyzed the breakpoints in 26 new duplication patients by pulsed-field gel electrophoresis, fluorescence in situ hybridization, and array comparative genomic hybridization. We found that 60% have a common duplication, the reciprocal to the common SMS deletions, and the remaining have uncommon duplications ranging from 1.1 Mb to 15.1 Mb. The patient with 1.1 Mb duplication has the typical features of dup(17)(p11.2p11.2) syndrome, narrowing the critical region of this syndrome from ~4 Mb to 1.1 Mb. The dosage sensitive retinoic acid inducible 1 gene (*RAI1*) that is responsible for the majority of SMS features is located within this region, implicating *RAI1* as the gene responsible for clinical features in the dup(17)(p11.2p11.2) syndrome. Most of the proximal breakpoints in the uncommon duplications mapped within two adjacent BACs very close to the centromere. This contrasts with half of the proximal breakpoints in the uncommon deletions mapping within or adjacent to the proximal SMS-REPs and none close to the centromere. The distal breakpoints in duplications did not cluster and distributed from p11.2 to p13. Therefore, the uncommon duplications in chromosome 17p may be stimulated by centromeric structure and mediated by non-homologous end joining. Note worthily, although uncommon recurrent deletions have been identified in 6 SMS patients, no recurrent reciprocal duplications were identified. Our results suggest that the mechanism applied in the uncommon duplications of 17p may differ from that in the uncommon deletions of 17p.

Molecular and cytogenetic elucidation of a familial case of Prader-Willi syndrome: evidence of a rare imprinting centre mutation. *D. Allingham-Hawkins, C. Gibbons, D. Kennedy, J. Tokunaga, M. Bedford, A. Hunter, K. Chun*
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A 14-year-old boy was referred for genetic assessment due to developmental delay and behavioural problems. Clinical history revealed failure to thrive in the newborn period followed by global developmental delay. A female paternal half-sibling of the patient was reported to have a similar clinical phenotype and had been diagnosed with Prader-Willi syndrome (PWS). In addition, there were other male relatives on the paternal side of the family who reported to have a PWS-like phenotype although these diagnoses have not been confirmed. Methylation studies of the SNRPN gene confirmed a diagnosis of PWS in the patient. Fluorescence *in situ* hydridization studies showed no deletion on either chromosome 15. Because the father was unavailable, uniparental disomy (UPD)studies were performed using a specimen from the patient, his mother and a full male sibling who is also developmentally delayed but does not clinically have symptoms of PWS. UPD studies revealed that the patient does *not* have UPD of chromosome 15 thereby suggesting that an imprinting centre (IC) mutation is present. To further support this, the patient and his unaffected brother appear to share a common paternal chromosome 15 until within the Prader-Willi critical region (PWCR) at which point a crossover event occurs. Mutation studies for an IC mutation are being pursued as are specimens from the affected paternal half-sibling and her mother to further elucidate the haplotype on which the mutation resides. Imprinting mutations occur in <1% of PWS patients and approximately 15% result from small deletions in the PWCR while the majority is epimutations or alterations of the *imprint* rather than the DNA. However, the presence of multiple affected individuals in a family, as in the present case, favours the presence of a deletion (Buiting *et al.*, 2003. *Am J Hum Genet* 72:571). Therefore, although more work is needed for confirmation, this case most likely represents a rare familial case of PWS caused by an imprinting centre mutation.

Genetic associations with response variability on the continuous performance task in AD/HD families. *A.E. Ashley-Koch[1], J.N. Epstein[2], S. Tonev[3], D. FitzGerald[3], E.M. Kane[4], A.D. Anastopoulos[4], A.M. Lachiewicz[5], M.E. Kail[1], L. Exelbierd[1], M.L. Cuccaro[1], J.R. Gilbert[1], S.H. Kollins[3]* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Division of Behavioral Medicine and Clinical Psychology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 3) Department of Psychiatry, Duke University Medical Center, Durham, NC; 4) Department of Psychology, University of North Carolina, Greensboro, NC; 5) Department of Pediatrics, Duke University Medical Center, Durham, NC.

Reaction time and reaction time variability on neuropsychological tests have been proposed as endophenotypes for unraveling the genetics of AD/HD. AD/HD children are usually slower and more variable in their reaction times than control children. We have examined genetic associations with four measures of response variability from the continuous performance task (CPT) including commission errors, mean response time (mu), standard deviation of response time (sigma), and tau, an alternative measure proposed by Hervey et al. (in press) which extracts the ex-Gaussian part of the response distribution. CPT data were collected from 53 nuclear families with at least one child aged 5 to 11 years meeting AD/HD DSM-IV criteria. Using a haplotype tagging approach, families were genotyped for 123 SNPs in neurotransmitter candidate genes. CPT measures of tau and sigma were log-transformed for analysis. Preliminary results with QTDT suggest that mu may be associated with genetic variation in the BDNF gene ($p<0.05$ for rs1048221, rs7124442 and rs7934165) while tau may be associated with genetic variation in the DBH gene($p<0.05$ rs2073833, hcv3274654, rs1108580, hcv2253949 and rs1541333). The association of tau with DBH is particularly exciting as hcv2253949 is a coding SNP. Both genes belong to the dopaminergic pathway. These data suggest that both traditional and alternative measures of response variability in CPT performance are associated with genetic variation and that such endophenotypes may be useful for better understanding the biological mechanisms that contribute to the clinical diagnosis of AD/HD.

Identification of a novel major BBS gene (BBS10) coding for a vertebrate specific chaperonine-like protein and mapping of BBS12. *H. Dollfus[1], C. Stoetzel[1], J. Muller[2], V. Laurier[2], E. Davies[3], N. Salem[4], E. Chouery[4], S. Corbani[4], N. Jalkh[4], S. Rix[5], JL. Badano[3], C. Leitch[5], A. Verloes[6], O. Poch[2], D. Bonneau[7], A. Mégarbané[4], P. Beales[5], N. Katsanis[3], JL. Mandel[2]* 1) Laboratory EA 3949, Faculté de Médecine, Université Louis Pasteur, Strasbourg, France; 2) IGBMC, Strasbourg, France; 3) Institute of Genetic Medecine, John Hopkins University, MD, USA; 4) Genetics laboratory, Université Saint Joseph, Beyrouth, Lebanon; 5) Molecular Medecine Unit, Institute of Child Health, University College, London, UK; 6) Service de Génétique, CHU Robert Debré, Paris, France; 7) Départment de génétique, CHU Angers, France.

The phenotype of Bardet-Biedl syndrome (BBS) is defined by the association of retinitis pigmentosa, obesity, polydactyly, hypogenitalism, renal and cognitive impairment. The extensive genetic heterogeneity of this condition was supported by the identification, initially by classical linkage analysis and more recently by comparative genomics, of 9 genes up to the beginning of 2006 (BBS1 to 9) that appear implicated in cilia assembly or function. SNP homozygosity mapping performed on an extended consanguineous Lebanese family permitted us to identify the new BBS10 gene on chromosome 12: unexpectedly patients in different sibships were either homozygous or compound heterozygotes for BBS10 mutations while one sibship carried homozygous missense mutations in BBS2. 311 additional BBS families where tested (from French, US and British cohorts). 16 truncating mutations and 18 missense mutations were found. The C91fsX95 mutation accounts for 46% of the mutational load. Bioinformatic studies revealed that BBS10 codes for a chaperonine-like protein. Unlike other BBS genes, the new gene is vertebrate specific and would have been missed by comparative genomics approaches that were successful for other BBS genes. This gene is mutated in 20 % of families, similar to the BBS1 gene and much higher than for the other BBS genes. Using the same strategy we also mapped a new BBS locus in two consanguineous unrelated families, that share a extended common haplotype over 6 Mb. Testing of candidate genes in this region will be reported.

Clinical and cytogenetic findings in a child with mosaic 45,X/47,X, pseu idic(Y)(q11.2)x2 karyotype. *S. Gupta[1], P. Koduru[1], D. Carey[2], L. Palmer[3], L. Mehta[4]* 1) Laboratory Medicine, North Shore Univ Hosp, Manhasset, NY; 2) Div. of Endocrinology; 3) Pediatric Urology; 4) Medical Genetics, Schneider Children's Hospital, Albert Einstein College of Medicine and NYU School of Medicine, NY.

A newborn infant was evaluated because of genital abnormalities. There were minor dysmorphisms with low set ears, single transverse palmar creases and a sacral dimple. Genitalia were ambiguous, with a short phallus, chordee, penoscrotal hypospadias, a left scrotal sac with a palpable gonad and a flat right hemiscrotum with no palpable right testis. Pelvic ultrasound did not show a uterus or intrabdominal gonads. Serum testosterone was 159 ng/dl, DHT 12 ng/dl, and 17- OHP 38 ng/dl. Blood chromosome analysis showed: 45,X/47,X,inv dup(Y)x2. FISH studies revealed that the abnormal Y was a dicentric Yp isochromosome, with two copies of SRY, one at each end and loss of distal Yq. Hence karyotype was 45,X,/47,X,pseu idic(Y)(pter->q11.2:q11.2->pter). At 5.5 months exploratory laparoscopy showed a right dysgenetic gonad in the inguinal region, attached to a cystic structure and a left scrotal testicle. Pathology showed immature testicular tissue in both gonads, with fibrotic stroma in the right gonad. Chromosome analysis of gonadal biopsies showed similar findings to that in peripheral blood. Interphase FISH on skin fibroblasts with SRY and DXZ1 probes showed 45,X[91.9%]/47,X,abn(Y)x2[8%]/46,X,abn(Y)[1.9%]. The conception was therefore likely to have originated as 46,X,pseu idic(Y)with subsequent mitotic nondisjunction resulting in 45,X and 47,XYY cell lines, with pseu idic Ys. Father had normal 46,XY chromosomes. The phenotype of patients with 45,X/47,XYY ranges from almost normal male to mixed gonadal dysgenesis or Turner syndrome. The presence of a Y isochromosome, Yp/Yq breakpoints and the degree of mosaicism are the variables responsible for phenotype. Information on SRY status is not available in most previous reports; in our case the pseu idic(Y) was detected by the position and number of SRY signals. Accurate characterization of a structurally abnormal Y chromosome, with or without a 45,X cell line, requires molecular cytogenetic testing.

The effects of di(2-ethylhexyl)phthalate (DEHP) on reproduction and reproductive fitness. *S. Gupta, B. Bongiovanni, L.R. Adkison* Genetics, Mercer University School of Medicine, Macon, GA.

This study analyzes the effects of di(2-ethylhexyl) phthalate (DEHP), a plasticizer commonly used to make PVC more flexible and durable, on reproductive fitness and reproduction fitness as measured by viability and fertility, respectively. Using Drosophila melanogaster, the number of offspring and the number of eggs laid were determined when adults and larvae were raised on medium containing the phthalate. Drosophila larvae were raised on medium with different dilutions of DEHP (1:50,000 to 1:1 billion) and compared to Drosophila raised on control medium. F1 and F2 progeny were analyzed and represented over 20,000 offspring. Data were collected for each dilution by gender. Egg-laying experiments were conducted to determine differed in larvae raised on DEHP when compared to controls. Each experiment used either a males or females raised on DEHP crossed with normal males or females, respectively. Over 5,000 eggs laid on a smooth medium were counted.
Multiple, corrected t-test analysis were performed to determine the effects of phthalate in each experiment, comparing each dilution group to each other and the control. DEHP had little effect on progeny counts, whereas it caused a significant reduction in the number of eggs produced. These results demonstrate DEHP-treated F1 larvae and the F2 offspring of these matured larvae are equally viable as controls at eclosion. However, the fertility of DEHP-treated parentals is reduced suggesting the viability of eggs from DEHP-treats parentals may be greater for unknown reasons. These data support a complex interaction of genes and the need for more analyses of how specific genes cause the affects observed.
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A common epigenetic signature distinguishes human embryonic stem cells from differentiated cell populations.

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Human embryonic stem (hES) cells are unique among all human cells in their ability to self-renew and to differentiate into multiple cell types. The pluripotence of hES cells is rooted in their origin from an early stage of embryogenesis, during an active period of epigenetic remodeling of the genome. To begin to understand the epigenetic state of hES cells, we used a new microarray method to measure DNA methylation at 1536 specific CpG sites in the promoters of 371 genes in fourteen independently isolated hES cell lines collected from laboratories around the world at various times in culture. For comparison we also determined the DNA methylation status of a pluripotent human embryonal carcinoma cell line (NTERA2), two human neuronal and two bone marrow stem cell lines, four lymphoblastoid cell lines, five human tissues and 24 cancer cell lines. We found that the undifferentiated hES cells had a methylation profile distinct from those of other cell types, and that this profile was relatively stable during prolonged cell culture. This epigenetic signature is likely to be linked to ES cell-specific properties such as self renewal and pluripotence. From the global methylation profiles, we selected 49 CpG sites from 40 genes that contribute most strongly to the distinct signatures of hES cells, somatic stem cells, fully differentiated cells and cancer cells. We also identified 25 CpG sites from 23 genes that clearly distinguish hES cells from other normal cell types; these should be useful biomarkers for monitoring hES cells for undifferentiated self-renewal and during induced differentiation.

Altered expression of trophoblast-specific genes in preeclampsia. *L. Avila[1], M. Pearson[4], P. vonDadelszen[2], S. Langlois[1], W.P. Robinson[1, 3]* 1) Dept. of Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 2) Dept. of Obstetrics and Gynecology, UBC, Vancouver, BC, Canada; 3) Reproductive Health Research Program, Child & Family Research Institute, Vancouver, BC, Canada; 4) Dept. of Medical Genetics, Alberta Children's Hospital, Calgary, AB, Canada.

Successful placentation is crucial for optimal fetal growth and development. Disruption of this process could result in pregnancy complication and poor perinatal outcome. Investigation of altered gene expression in the placenta of such pregnancies can provide insight into the relationship between placentation and disease. Because of the inherent cellular heterogeneity of placentae we initially evaluated normal site-to-site variability in gene expression and devised a sampling strategy useful for clinical studies. To do this we evaluated the expression patterns of two genes involved in trophoblast differentiation and invasion control: E-cadherin and Kiss1, both expressed specifically in the cytotrophoblast. Healthy placentae (N=4) were sampled at 3 sites at 4 depths, and after reverse transcribed to cDNA, mRNA levels were quantified by real time PCR on an ABI 7000. Although intra-placental variability was observed, inter-placental differences were significantly greater than intra-placental variation for both E-cad and Kiss1 ($p<0.0001$; $p=0.019$, respectively). The least intra-placental variability was observed at sites located halfway between cord and placental periphery and at an intermediate depth. This sampling location was used in our subsequent studies. Plac1, a gene expressed in the syncitiotrophoblast and involved in trophoblast differentiation, and Kiss1 expression were then assayed in control (N=4), preeclamptic (N=4) and abnormal maternal serum screen (MSS) (N=4) placentae. Expression of both genes was significantly decreased in the preeclamptic placentae (t-test, Kiss1 $p=0.006$; Plac1 $p=0.003$), but no difference was observed between the abnormal MSS group and control. We are currently evaluating E-cad and Cad11, and will extend these studies to additional trophoblast-specific genes and additional samples. Our goal is to identify molecular markers useful in diagnostic and maintenance of complicated pregnancies.

Development and Use of a Genetic Literacy Concept Inventory for Undergraduates. *B.V. Bowling, C.A. Huether, E.E. Acra* University of Cincinnati, Cincinnati, OH.

There is continued emphasis on increasing and improving genetics education at all levels from grades kindergarten-12 to medical professionals to the general public. Perhaps the most accessible audience with the greatest potential for future impact is that of undergraduate students in introductory biology and genetics courses. However, there has been little effort to assess these students understanding of genetics concepts and more specifically the students level of genetic literacy (i.e. genetics knowledge as it relates to their lives) after completing an introductory course. This project consists of the development and use of a new survey instrument to assess the genetic literacy of undergraduate students taking introductory biology or genetics courses. The Genetic Literacy Concept Inventory is a 35-item multiple choice test that addresses 17 concepts identified as important for genetic literacy. The items were selected and modified based upon reviews by over 25 professional geneticists, genetic counselors, and genetics instructors. The inventory is undergoing additional review for validity and reliability, including student focus group interviews and administration of the test to a control group of students. It is currently being employed as a pre-course and post-course inventory in several introductory biology and genetics courses at 4 colleges/universities in the Cincinnati area. The factors of pedagogy and course content are also being considered for their potential effect on inventory scores. Most significantly, this study directly enhances genetics education research by providing a valid and reliable instrument for assessing genetic literacy in undergraduate students.

Identification of candidate genes specific to asthma and atopy using microarray data. *A. Chamberland*[1,2], *J. Chakir*[2,3], *M-C. Bernier*[3], *M. Laviolette*[2,3], *C. Laprise*[1,4] 1) University of Montreal Community Genomic Medicine Centre, Saguenay, Canada; 2) Laval University, Quebec, Canada; 3) Institut universitaire de cardiologie et de pneumologie de l'Université Laval, Quebec, Canada; 4) Université du Québec à Chicoutimi, Saguenay, Canada.

Background: Allergic asthma and rhinitis share a common inflammatory etiology mediated by different molecular and cellular components. Although a genetic predisposition for atopy, an important determinant factor for these two phenotype expressions, is documented we need to identify specific genetic markers for these complex traits. **Aims:** 1) To compare thousands of gene expression patterns of healthy control, allergic rhinitis, allergic asthmatic and nonallergic asthmatic subjects; 2) To target genes that are specific to asthma and atopy. **Methods:** Bronchial biopsies were performed on 4 healthy control (HC), 4 allergic rhinitis (AR), 4 allergic asthmatic (AA) and 4 nonallergic asthmatic (NA) subjects paired for age. RNA was extracted using the Qiagen kit and microarray expression studies used Affymetrix HG-U133A GeneChips. Candidate genes were selected when they were at least differently expressed in two pairwise comparisons of microarray results and on the basis of their biological functions. **Results:** So far, 3 pairwise comparisons have been completed (HC/AR, HC/AA and AR/AA). Respectively, 140, 69 and 241 transcripts were differently expressed. Among those, 6 are immune signalling molecules and 12 are inflammatory molecules. Definitive candidate genes will be selected when all pairwise comparisons will be completed. **Perspectives:** The expression of 5 candidate genes of asthma and 5 of atopy will be validated by real-time RT-PCR and characterize by immunohistochemistry. Association studies between asthma, atopy and variants that are present in candidate genes will be performed too. Thus, this study should allow targeting biological elements that are specific to asthma and atopy.

Genetic Similarity Matching for Genome-wide Association Studies. *W. Guan, L. Liang, M. Boehnke, G.R. Abecasis*
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Recently, genome-wide association studies have drawn great interest as a promising tool to dissect complex diseases such as hypertension, diabetes, and bipolar disorder. Although case-control association tests are generally more powerful than family-based association tests, population stratification that can lead to spurious disease-marker association or mask a true association remains a major concern. Several methods have been proposed to match cases and controls prior to genotyping or using genotype data for a modest number of genetic markers. Here, we describe a method for efficient matching of cases and controls after genotyping a large number of genetic markers in a genome-wide association study or large-scale candidate gene association study. Our method is comprised of three steps: 1) calculating similarity scores using the genotype data; 2) conducting optimal matching based on the scores so that matched cases and controls have similar genetic background; 3) using conditional logistic regression to perform association tests. Through simulations we show that out strategy can correctly control false positive rates and improve power to detect true disease predisposing variants. For example, in a simulation in which 300 cases and 300 controls are randomly sampled from two equally sized populations (Han Chinese and Japanese) and assuming a single-locus disease model (prevalence K=6.5% and 13%, $_s$=1.03 and 1.03, in the two subpopulations, respectively), the type I error rate using our method drops from .091 to .052, and the power increases from .23 to .98, compared to a standard chi-square test for contingency tables. We also show that the optimal many-to-one matching has substantial advantages over one-to-one matching. We will illustrate our method with data from the Finland-United States Investigation of NIDDM Genetics (FUSION) genome-wide association study.

SURPRISES AT PRENATAL DIAGNOSIS. *D. Burkhardt, A. Hajianpour, F. Rashidian, C. Lee, L. Drugan, L. Dong, R. Habibian* Cytogenetics, Genzyme Genetics, Monrovia, CA.

The detection of monosomy X in a chorionic villi specimen is not always an accurate indicator of the fetal karyotype, especially in the absence of features common to Turner syndrome observed on ultrasound examination. Only when both the direct preparation and long-term tissue cultures show aneuploidy in almost 100% of the cells can the fetal genotype be determined with any degree of certainty. We present a case of mosaic chromosome aneuploidy detected by FISH performed on a direct CVS preparation (of the nuclei examined, 70% showed one signal for the X chromosome and 30% showed two signals for the X chromosome). Cytogenetic analysis of the long-term tissue culture revealed a 45,X chromosome complement in 29 out of 30 cells examined, with only one cell having a 46,XX chromosome complement. An interphase FISH study from a long-term culture showed 65% of the nuclei with a single signal for the X chromosome and 35% of the nuclei with two signals for the X chromosome. In view of the absence of ultrasound findings and mosaic chromosome and FISH results, a follow-up amniocentesis was performed. Interphase FISH studies showed 97% of the cells to have two X chromosome signals (within our reportable range for a normal result). Cytogenetic analysis revealed a 46,XX chromosome complement in the first 15 colonies examined. Only when additional colonies from three independent cultures were examined 5 out of 30 colonies showed a 45,X chromosome complement. At this stage of the pregnancy the fetus had developed a cystic hygroma. This case illustrates that even after half a century of prenatal diagnosis, there are still surprises. Considering that examination of 15 colonies from amniotic fluid in situ cultures is the universal practice, without CVS examination this case would have been reported as normal. In order to ensure accurate diagnoses in cases where mosaicism may be present, i.e., abnormal fetal ultrasound findings, it is suggested to examine additional cells from all available cultures in addition to the standard analysis.

Alternative dosing regimens of agalsidase alfa (Replagal) in Fabry Disease. *J. Clarke[1], M. West[2], R. Schiffmann[3], J. Bultas[4], M. Askari[3], R.O. Brady[3]* 1) Hospital for Sick Children, Toronto, ON, Canada; 2) Queen Elizabeth II Hospital, Halifax, NS, Canada; 3) National Institutes of Health, Bethesda, MD, US; 4) Charles University Hospital, Prague, Czech Republic.

Agalsidase alfa is used for enzyme replacement therapy (ERT) in Fabry disease at a dose of 0.2 mg/kg infused over 40 minutes every other week (EOW). Agalsidase alfa is produced in a human cell line, and using the approved dosing regimen, reduces plasma globotriaosylceramide (Gb_3) levels in patients with Fabry disease. This study was performed to determine the effect of alternative doses and/or dosing frequencies on plasma Gb_3 levels, a marker of a metabolic effect of agalsidase alfa. In this open-label pharmacodynamic study, 18 ERT-naïve adult males with Fabry disease were randomized to 1 of 5 dosing regimens for 10 weeks: 0.1 mg/kg weekly (n=4), 0.2 mg/kg EOW (n=4), 0.2 mg/kg weekly (n=4), 0.4 mg/kg EOW (n=3), and 0.4 mg/kg weekly (n=3). Plasma Gb_3 concentrations were the primary pharmacodynamic endpoint. Pharmacokinetics of each dose were evaluated by measuring enzyme activity in serum at intervals for 24 hours. Following the initial infusion, the area under the enzyme activity-time curves (AUC) was proportional to dose. Plasma half-life of infused agalsidase alfa was approximately 1 hr (range, 56-76 min) and was independent of dose. Baseline plasma Gb_3 levels averaged 9.12 0.62 nmol/mL (mean SEM). After 10 weeks of treatment, mean plasma Gb_3 had declined by about 50% with all 5 dosing regimens (48-51%, *P*=NS between regimens). This magnitude of reduction is comparable to recently published values in patients treated with agalsidase beta infused at a dose of 1 mg/kg EOW (Wilcox WR, et al. Am J Hum Genet. 2004;75:65-74). In conclusion, mean reduction in plasma Gb_3 levels was similar for all of the agalsidase alfa dosing regimens tested. Based on these observations, the currently recommended dose of agalsidase alfa, 0.2 mg/kg infused EOW, is sufficient to produce maximal reduction of plasma Gb_3 in adult male patients with Fabry disease.

Low bone mineral density (BMD) scores are associated with osteoporotic type fractures in adults with Neurofibromatosis type 1 (NF1). *L. Armstrong[1], P. Birch[1], H. Cheng[2], J.M. Friedman[1], D.A. Hanley[2], T.Y. Kydland[1], D.L. Kendler[3]* 1) Dept Medical Genetics, UBC, Vancouver, BC, Canada; 2) Dept of Medicine, University of Calgary, Alb; 3) Dept of Medicine, University of BC, Vancouver, BC.

Purpose-- Low BMD is known to predict increased bone fracture risk in the general population. Recent studies have shown that people with NF1 often have low BMD measurements. The basis of the low BMD scores in NF1 is a topic of ongoing research. It is unclear whether a low BMD measurement in the context of NF1 is a risk factor for fracture. We studied whether there is an association between site specific BMD and fracture in adults with NF1. **Methods**-- Fifteen individuals with NF1, and over 50 years old, were surveyed for history of fracture since their 40th birthday, occult fracture on spine radiograph, and BMD at the spine, hip, and forearm. Also measured were other determinants of bone health including medical history, medication use, and vitamin D status. **Results**-- Based on BMD scores 5/15 individuals were osteoporotic, and a 6/15 were osteopenic. BMD T score was -2.5 (the WHO definition of osteoporosis) in 5/15 at the forearm, in 1/15 at the hip, and in 0/14 at the spine (scoliosis precluded spine BMD measurement in 1). 2/15 had history of 2 forearm fractures each, 1/15 had had a hip fracture, and no spine fractures were reported or found on the films of the 14 who consented to radiographs. All 5 described fractures occurred in individuals where BMD T score was -2.5 at the fracture site. **Conclusions**-- All fractures were seen in the context of BMD T score -2.5. A larger prospective study is required to confirm this correlation. The fractures and low BMD scores were disproportionately seen in the forearm. This is consistent with a previous report which found that BMD is particularly decreased in non-weightbearing regions in NF1.

Mucolipidosis II and III: Clinical and Molecular Characterization. *M. Friez, S. Cathey, K. Draper, J. Leroy*
Greenwood Genetic Center, Greenwood, SC.

Background Mucolipidosis II (I-Cell disease) and mucolipidosis III (pseudo-Hurler polydystrophy) are disorders of lysosomal enzyme targeting. Both are due to deficiency of UDP N-acetylglucosamine-phosphotransferase (GlcNAc-phosphotransferase), the initial enzyme involved in the formation of the mannose 6-phosphate recognition marker which is essential for the uptake of lysosomal enzymes into lysosomes. GlcNAc-phosphotransferase is an $_{222}$ hexamer encoded by two separate genes. The and subunits are encoded by the *GNPTA* gene and the subunit is encoded by the *GNPTG* gene. Prior to molecular testing, ML II was distinguished from ML III by the severity of the clinical course. The goals of this project are to genotype individuals with clinical ML II or ML III and make genotype-phenotype correlations. **Methods** Molecular analyses, which include sequencing the 21 exons of *GNPTA* in ML II and ML III probands and targeted sequencing in parents, have been completed in 29 of 34 participating families. Pathogenic changes have been found in *GNPTA* in every family. The mutations are found in 15 of the 21 exons. No mutations have been found in *GNPTG*. Clinical data on all patients are being obtained from medical records and questionnaire completed by the parents of affected children. When feasible, patients are examined by Greenwood Genetic Center clinicians. **Results** The patients with the most severe phenotype and clinical ML II have homozygous frameshifts, two different frameshifts, or one frameshift and one nonsense mutation. These patients have evidence of growth retardation at birth and dysmorphic features detected at birth or shortly thereafter. Intellectual development and lifespan are compromised. Individuals with clinical ML III have one missense or splice mutation with one frameshift. These individuals may have no abnormalities noted for the first several years of life. Joint manifestations, particularly limited mobility, are often the first problems appreciated. These individuals also have short stature, but not to the extreme seen in children with ML II. Individuals with the clinical diagnosis of ML II/III have one severe mutation and one milder mutation. They have mixed phenotypes.

Evidence for association of the IL-10 receptor 1 gene with childhood-onset mood disorders. *L. Gomez[1], V.L. Misener[1], K.G. Wigg[1], P. Luca[1], N. King[2], A. Vetro[3], E. Kiss[3], Z. Tamas[4], J.L. Kennedy[2], M. Kovacs[5], C.L. Barr[1,6], The International Consortium for Childhood-Onset Mood Disorders* 1) Toronto Western Research Institute, Toronto, ON, Canada; 2) Neurogenetics Section, Centre for Addiction and Mental Health, Toronto, ON, Canada; 3) Department for Child and Adolescent Psychiatry, Szeged University, Szeged, Hungary; 4) Vadaskert Hospital, Budapest, Hungary; 5) School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA; 6) Hospital for Sick Children, Toronto, ON, Canada.

Inflammatory cytokines induce a syndrome of "sickness behaviour", resembling symptoms typically seen in depression. This has led to the hypothesis that cytokine activity contributes to depressive disorders. A logical extension of this hypothesis is that some variations in cytokine system genes confer risk for depression. To test this, we are investigating cytokine network genes in childhood-onset mood disorders (COMD). Childhood-onset depression is highly heritable, making this a useful approach for identifying genes underlying depression. To test for associations with COMD, we have used the transmission/disequilibrium test (TDT), analysing 386 families with children diagnosed with depression before age 14. Twenty-five polymorphisms in 7 genes have been investigated. These include the genes for 4 inflammatory cytokines, IL-1, IL-1, IL-6 and TNF-, and for 3 anti-inflammatory mediators, IL-10, IL-10 receptor 1 (IL-10R1), and IL-1Ra. TDT analyses showed no significant evidence for association with the polymorphisms, individually (*p*-values>0.05). However, TDT analyses of haplotypes showed significant evidence for association with a 2-marker haplotype (rs4252272[A]/rs2229113[A]) of IL-10R1 (^{2}=5.03; *p*=0.02). Each of the variants comprising this haplotype changes the amino acid (aa) sequence of the IL-10R1 protein (aa138:Ser/Gly and aa330:Gly/Arg, respectively). Moreover, functional evidence for a proinflammatory effect of the 330Arg variant, and the possibility of altered ligand binding due to the Ser138Gly change, have been reported. Evidence for association of these IL-10R1 variants with COMD suggests their involvement in risk for depression, and warrants further investigation.

Utilizing array comparative genomic hybridization (aCGH) to characterize chromosomal stability in microsatellite stable (MSS) young onset rectal cancer. *L. Boardman[1,2], R. Johnson[1,3], K. Hafner[1,3], A. Oberg[1,4], B. Morlan[1,4], R. Jenkins[1,3], S. Thibodeau[1,3]* 1) Mayo Clinic College of Medicine, Rochester, MN; 2) Division of Gastroenterology and Hepatology; 3) Division of Laboratory Medicine and Pathology; 4) Cancer Center Statistics.

Approximately 80% of CRC is MSS. MSS CRC has been considered to have chromosomal instability (CIN), as opposed to microsatellite instability (MSI). Tumor phenotyping studies have depicted a portion of MSS CRC as having little or no genetic changes, being chromosomally stable (CIN-), while others exhibited large gains/ losses of genetic material, being CIN+. We selected a group of MSS rectal cancers from people 50 years old with stage II or Stage III disease to assess DNA copy number changes using the Spectral Genomics aCGH which contains nearly 3000 BACs. In all cases, DNA was extracted from chemoradiotherapy naïve rectal cancer. Eight tumors were diploid, 3 were tetraploid and 9 were aneuploid by flow cytometry. Seven tumors (35%) had low levels of chromosomal disruption with < 1% to 17% of the clones having DNA copy number changes and were classified as CIN-. Five tumors (25%) had > 20 % and 30% < of the clones showing DNA alterations, being intermediate CIN. Eight tumors (40%) had a high frequency of alterations in 30-49% of clones and were classified as CIN+. Copy number gains in >40% of tumors were found in chromosome arms 7p/ q, 13q and 20 p/ q and shared smaller consensus regions of gain. 18 p/ q losses were noted in all CIN + tumors but no CIN- tumors, and 17p was lost in all but 1 CIN+ tumor but only 1 CIN-CRC Consensus regions of loss were shared for 17p/ 18 p/q for all CRC showing loss on these arms. Arms more uniformly affected in CIN+ tumors than CIN- CRC included 4p and/or 4q loss in 75%; 7p and/or q gain in 75%; gain of 13q and 20q in 88% of CIN + CRC. Conclusions: These results substantiate that a subset of MSS CRC will have no or little evidence of CIN and represent the molecular subtype of MSS CIN- CRC. Certain chromosomal arms alterations important in CIN+ do not appear to be affected in CIN - CRC, suggesting that the genes involved in CIN- CRC may differ from those in CIN+ CRC.

Analysis of the interactome of RPS19, mutated in Diamond Blackfan Anemia. *I. Dianzani[1], A. Aspesi[1], S. Orrù[2], M. Armiraglio[1], M. Caterino[2], F. Loreni[3], M. Ruoppolo[2], C. Santoro[1]* 1) Dept Medical Sciences, Univ Piemonte Orientale, Novara; 2) CEINGE Advanced Biotechnologies, scarl, Napoli; 3) Dept of Biology,Univ.Tor Vergata, Roma.

RPS19 is a component of the ribosomal 40S subunit. Its mutations are responsible for Diamond Blackfan Anemia (DBA, MIM 205900), a disease characterized by defective erythroid progenitor maturation and malformations. Dysregulation of RPS19 has been implicated in this defective erythropoiesis, though the link is unclear. Two not mutually exclusive hypotheses have been proposed: altered protein synthesis, and loss of unknown functions not directly connected with RPS19s structural role in the ribosome. A role in rRNA processing has been surmised for the yeast ortholog. Four proteins are known to interact with RPS19: FGF2, C5R1, RPS19BP, PIM1. We have used a proteomic strategy to look RPS19 proteins interactors in order to determine its functions. Proteins were isolated by affinity purification with a GST-RPS19 recombinant protein and identified using LCMS/MS analysis coupled to bioinformatics tools. We identified 159 proteins from the following GO categories: NTPases (ATP- and GTPases; 5 proteins), hydrolase/helicases (19 proteins), isomerases (2 proteins), kinases (3 proteins), splicing factors (5 proteins), structural costituents of ribosome (29 proteins), transcription factors (11 proteins), transferases (5 proteins), transporters (9 proteins), DNA/RNA-binding protein species (53 proteins), other (1 dehydrogenase protein, 1 ligase protein, 1 peptidase protein, 1 receptor protein, 1 translation elongation factor) and 13 proteins of unknown function. Results were validated by affinity purification and western blotting. Interactions were further confirmed by co-immunoprecipitation using a monoclonal RPS19 antibody. Many interactors are nucleolar proteins, thus expected to take part in the RPS19 interactome; however, some proteins suggest additional functional roles for RPS19. It is intriguing that among the direct or indirect RPS19 interactors we found proteins involved in pathologies with phenotypes similar to DBA (i.e.DKC1, RPL24, TCOF1, SBDBS). This suggests a common pathogenetic mechanism.

Analysis of ancestral origins of three populations using *structure*. *C.A. Brandon[1], T. Goldstein McHenry[1], M.E. Cooper[1], B.S. Maher[1], K.M. Bardi[1], J.R. Avila[2], J.C. Murray[2], A.R. Vieira[1], M.L. Marazita[1]* 1) Dept Craniofacial & Dental Gen, Univ Pittsburgh, Pittsburgh, PA; 2) Dept Pediatrics, Univ Iowa, IA.

Assessing ancestral origin is important for human genetic studies due to varying phenotypic prevalences and allelic frequencies. Ancestral origins and ethnicity are often elicited from study subject self-reports which can be problematic in cases where individuals identify with their national affiliation rather than ethnicity, for example in Guatemala which is part of our ongoing studies of Oral-Facial Cleft (OFC) families. In such cases, DNA based methods may be more informative. Therefore, we explored a method using the program *structure* to assign origins in unrelated individuals from 3 OFC studies: Pittsburgh, PA (US Caucasian, n=102), Madrid, Spain (European Caucasian, n=78), and Guatemala (Mixed, n=38). We utilized genotypes of 31 SNPs, chosen for OFC candidate gene studies. Founder data for these 31 SNPs was obtained from the International HapMap Project [Chinese (CHB) + Japanese (JHP): n=90; Yoruba (YRI): n=60; CEPH people from Utah (CEU): n=60] as prior information to assist in clustering the OFC populations. Each of the 3 OFC populations were added individually and run at K=3. Pittsburgh and Madrid populations clustered closest to the CEU population as expected (93%). The Guatemala population did not cluster with any of the 3 HapMap groups. This population is of interest due to the mixed ancestry primarily from Spain and indigenous Mayan. Substituting the Madrid population for the CEU population showed a shift of the Guatemala population towards the Madrid cluster (86%). Birth prevalence of OFC in the Guatemalan indigenous population is similar to the increased prevalence seen in East Asian populations, but our results do not support similar ancestral origins between the Guatemalan and the HapMap East Asian populations. East Asian populations that were more migratory (i.e. Mongolians) may be a better reference group. Our results suggest that we can successfully generate individual ancestral information in admixed groups by using relatively small, non-random genotyping data. Supported by NIH Grants P50-DE016215, R01-DE016148, R21-DE016930.

A comparison of robust methods for QTL mapping in nuclear families. *S. Bhattacharjee[1], C. Kuo[2], N. Mukhopadhyay[1], D.E. Weeks[1,2], E. Feingold[1,2]* 1) Dept of Human Genetics, GSPH, Univ of Pittsburgh, Pittsburgh, PA; 2) Dept of Biostatistics, GSPH, Univ of Pittsburgh, Pittsburgh, PA.

Recently, a number of new methods have been developed for quantitative trait locus (QTL) mapping in humans using small pedigrees. Most of the new methods are based on score statistics or linear regression statistics, and thus they are at least potentially robust to non-normality of the trait distribution and also to selected sampling. Whereas the theoretical development of these statistics is more or less complete, some practical issues concerning their implementation still need to be addressed. Here we study some of these issues, such as choice of denominator variance estimates, weighting of pedigrees, parameter misspecification and non-normality of the trait distribution. We consider a number of recently-developed statistics, primarily score statistics, including some variations of our own. We investigate the differences in power and robustness among the statistics analytically, and then verify those properties with extensive simulation studies using nuclear families. We give particular attention to appropriateness of the statistics for highly-selected samples such as sibships containing a discordant or affected pair.

Candidate genes for congenital diaphragmatic hernia from animal models: Mutation screening of FOG2, SIX1 and PDGFRA in 96 patients reveals rare variants. *S.B. Bleyl[1], A. Moshrefi[2], G. Shaw[3], Y. Saijoh[1], G.C. Schoenwolf[1], L. Pennacchio[4], A.M. Slavotinek[2]* 1) Dept Pediatrics, Dept Neurbiol Anat Univ Utah, Salt Lake City, UT; 2) Dept Pediatrics, UCSF, San Francisco, CA; 3) California Birth Defects Monitoring Program, Berkely, CA; 4) Genomics Division, Lawrence Berkley National Laboratory, Berkley, CA.

Congenital diaphragmatic hernia (CDH) is a life threatening birth defect that can be isolated or occur with other anomalies. Although isolated CDH is usually sporadic, recent reports have implicated chromosome deletions at 15q26, 8p23.1, 4p16.3-4pter, and 1q41-1q42.1 in CDH patients, but no causative genes have been identified from these studies. We selected three candidate genes involved in diaphragm formation from animal models - *FOG2, SIX1* and *PDGFRA* - and hypothesized that mutations in these genes could cause CDH in humans. Mice homozygous for a hypomorphic *fog2* allele have diaphragmatic eventration, a defect related to CDH. Homozygous null mice for *Six1* have selective loss of muscles including the diaphragm and limbs. Null and conditionally inactivated alleles of *Pdgfra* in mice result in diaphragmatic defects and other anomalies resembling Fryns syndrome. We therefore sequenced *FOG2*, *SIX1* and *PDGFRA* in 96 patients with CDH of which 53 (55.2%) had isolated CDH, 36 (37.5%) had CDH and additional anomalies and 7 (7.3%) had CDH and known chromosome aberrations. For *FOG2*, we identified novel sequence alterations predicting p.M703L and p.T843A that were absent in more than 100 control chromosomes in two patients with isolated CDH. These altered amino acids are highly conserved, but we were not able to determine if they were de novo. For *PDGFRA*, we found an alteration predicting p.L221F in two patients, one with isolated CDH and the other with CDH and preauricular tags. *SIX1* sequencing did not reveal any novel alterations. In conclusion, our data found no evidence that CDH *commonly* results from mutations in these three candidate genes. We did identify rare variants of unknown significance in both *FOG2* and *PDGFRA* that could play a role in the pathogenesis of isolated CDH and further evaluation of these variants is proceeding.

IL-10 and TNF are associated with airflow obstruction in severe Alpha 1-Antitrypsin Deficiency. *D.L. DeMeo[1], E.J. Campbell[2], A.F. Barker[3], M.L. Brantly[4], E. Eden[5], N.G. McElvaney[6], R.A. Sandhaus[7], J.M. Stocks[8], J.K. Stoller[9], C. Strange[10], G. Turino[5], E.K. Silverman[1]* 1) Channing Laboratory, Brigham & Women's Hosp, Boston, MA; 2) Univeristy of Utah; 3) Oregon Health and Science University; 4) University of Florida; 5) St. Luke's/Roosevelt; 6) Beaumont Hospital, Dublin; 7) National Jewish Medical and Research Center; 8) University of Texas; 9) Cleveland Clinic; 10) Medical University of South Carolina.

Severe alpha 1-antitrypsin (AAT) deficiency is a proven genetic risk factor for chronic obstructive pulmonary disease(COPD). There is marked variability in the development of lung disease in individuals homozygous(PI ZZ) for this recessive Mendelian condition, suggesting that modifier genes are important.We hypothesized that asthma and other COPD genetic determinants may be modifier genes of lung function in individuals with severe AAT deficiency.In 378 PI ZZ individuals in the AAT Genetic Modifier Study,we observed that a clinical history of asthma is an important predictor of the presence and severity of lung disease.To identify modifier genes, we have performed association analyses for 10 asthma and/or COPD candidate genes as follows:IL10, TNF, GSTP1, NOS1, SERPINA3, SERPINE2, SFTPB, TGFB1, XRCC5 and EPHX1.All analyses were performed using the pedigree-based association test (PBAT) with covariates for cigarette 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 of which are key intermediate phenotypes of COPD. IL10 and TNF demonstrated the most significant associations among the 10 candidate genes; 5 of 11 SNPs in IL10 were associated with FEV1 and FEV1/FVC phenotypes (p=0.0008-0.03) and 3 of 5 SNPs in TNF were associated (p=0.006-0.05). We conclude that IL10 and TNF, two candidate genes frequently associated with risk for asthma, are important modifier genes for the development of lung disease in subjects with severe AAT deficiency. These genetic association findings provide biological support for the observation that features of asthma are important modifiers of COPD in AAT deficiency. Funding:HL072918,HL68926,ALA.

TRMU related to tRNA modification is a nuclear modifier gene for the phenotypic expression of the deafness-associated mitochondrial 12S rRNA mutation. *M. Guan[1], Q. Yan[1], X. Li[1], Y. Bykhovskaya[2], I. del Castillo[3], P. Hajek[4], T. Suzuki[5], M. Shohat[6], X. Estivill[7], J. Lu[8], N. Fischel-Ghodsian[2]* 1) Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 2) Steven Spielberg Pediatric Research Center, Cedars-Sinai Medical Center, Los Angeles, CA; 3) Unida de Genetica Molecular, Hospital Ramon y Cajal, Madrid, Spain; 4) Division of Biology, California Institute of Technology, Pasadena, CA; 5) Department of Integrated Biosciences, Graduate School of Frontier Sciences, University of Tokyo, Chiba, Japan; 6) Department of Pediatrics and Medical Genetics, Basil and Gerald Felsenstein Medical Research Center, Tel Aviv University Medical School, Petah Tikva, Israel; 7) Genes and Disease Program, Center for Genomics Regulation, Barcelona Biomedical Research Park, Barcelona, Spain; 8) School of Life Sciences, Wenzhou Medical College, Wenzhou, China.

Nuclear modifier genes have been proposed to modulate the phenotypic manifestation of the deafness-associated mitochondrial 12S rRNA mutations. We identified the nuclear modifier gene TRMU encoding a highly conserved mitochondrial protein related to tRNA modification. Genotyping analysis of TRMU in 613 subjects of 242 Arab-Israeli, Spanish, Italian and Chinese pedigrees with nonsyndromic deafness revealed a missense mutation (A10S) in evolutionarily conserved N-terminal region of Trmu protein. All eighteteen Arab-Israeli/Italian/Spanish matrilineal relatives carrying bothTRMU A10S and A1555G mutations exhibited congenital profound deafness. Functional analysis showed that this mutation did not affect importing of Trmu precursors into mitochondria. However, the homozygous A10S mutation cause a failure in mitochondrial tRNA metabolisms, specifically reducing the steady-state levels of mt tRNAs. As a consequence, these defects contribute to the impairment of mitochondrial protein synthesis. Resultant biochemical defects aggravate the mitochondrial dysfunction associated with the A1555G mutation, exceeding the threshold for expressing the deafness phenotype. These indicate that the mutated TRMU modulates the phenotypic manifestation of the deafness-associated mitochondrial 12S rRNA mutations.

An association study of a glutamate receptor gene (GRIN2A) in obsessive-compulsive disorder. *P.D. Arnold[1], T. Sicard[1], E. Burroughs[1], G.L. Hanna[2], M. Pato[3], C. Pato[3], M.A. Richter[1], J.L. Kennedy[1]* 1) Dept Psychiatry, CAMH-Univ Toronto, Toronto, ON, Canada; 2) University of Michigan at Ann Arbor, MI; 3) University of Southern California.

Purpose: Recent investigations suggest a role for glutamate in the pathogenesis of obsessive-compulsive disorder (OCD), and our group has previously reported a preliminary positive association with a glutamatergic N-Methyl-D-Aspartate (NMDA) receptor gene in OCD (GRIN2B). The purpose of the study was to perform candidate gene testing of the glutamate receptor ionotropic NMDA 2A (GRIN2A) subunit gene in OCD. Methods: We genotyped 255 nuclear families of adult (n=191) or child (n=64) probands collected from 3 North American centres. Five GRIN2A variants were genotyped including one GT repeat polymorphism and 4 single nucleotide polymorphisms. The analysis for genetic association was conducted using the Family Based Association Test (FBAT) under the additive model of inheritance. Results: A statistically significant association was identified with the intronic SNP rs4782053, with the more common T allele over-transmitted to OCD-affected individuals (z=2.00, p=0.04). This association was also found in the subset of families ascertained through a proband with an early onset (less than 15 years old) of OCD. These results were not corrected for multiple comparisons. Significantly increased transmission of the minor C allele of rs727605 (z=2.04, p=0.04) was identified in transmissions to males with OCD but not in the total sample or in transmissions to females. Conclusions: Overall, these findings provide preliminary evidence that variation in GRIN2A may be associated with OCD. Further study of additional variants in GRIN2A and other glutamate genes in larger samples is needed.

XIST-dependent silencing following expression of an inducible human XIST transgene in human somatic cells.
C.J. Brown, J.C. Chow, S. Sidhu Dept Medical Genetics, Univ British Columbia, Vancouver, BC, Canada.

XIST is a large alternatively spliced nuclear RNA involved in the initiation of X chromosome inactivation (XCI) in female mammals. Ectopic expression of Xist in mice will induce silencing of flanking genes when expressed during an early developmental window. In order to model the events occurring in human XCI we have generated single copy integrations of an inducible human XIST cDNA construct in male (HT1080) and female (293) transformed human cell lines. Localization of the induced RNA is observed by RNA FISH, and silencing has been demonstrated by three alternative approaches. We first demonstrate global silencing by the loss of CoT1 hybridization to heteronuclear RNA in the region of the XIST RNA. Second, to characterize expression of individual genes we analysed cSNPs in autosomal genes on the same chromosome as the integration sites. Reduction in biallelic expression was observed for a subset of these genes. Third, we have integrated an EGFP reporter gene adjacent to the inducible XIST gene and observed loss of GFP expression upon induction of XIST. This silencing is XIST-dependent as removal of XIST expression results in re-expression of the GFP gene. While GFP is reduced to ~20% within 4 days of XIST expression, ongoing XIST expression continues to reduce the level of fluorescence, suggestive of the gradual acquisition of chromatin marks, an observation supported by chromatin immunoprecipitation at the GFP promoter. We observed rapid loss of histone acetylation, slower acquisition of H4K20m1 and HP-1 and slow loss of H3K4m2. Unlike the Xi, ectopic XIST expression in these cells does not lead to H3K9/27 methylation, and no observable focus of H3K27m3 or macroH2A was detected. In conclusion, this model system allows us to examine the events involved in human XIST-induced silencing. XIST expression in human somatic cells can induce transcriptional inactivation and recruit chromatin modifications, but not factors required for stable maintenance of silencing. By examining different integration sites and genes that escape XIST-induced silencing this system also allows us to examine the role of cis-acting DNA elements in the spread of X inactivation.

BRCA2 mutations in Utah high-risk prostate cancer pedigrees. *K. Allen-Brady[1], J.M. Farnham[1], N.J. Camp[1], E.A. Ostrander[2], L.A. Cannon-Albright[1]* 1) Dept Genetic Epidemiology, Univ of Utah, Salt Lake City, UT; 2) National Human Genome Research Institute, Bethesda, MD.

Germline mutations in the BRCA2 gene have been suggested to account for about 5% of familial prostate cancer; mutations have been reported in 2% of early onset (i.e., 55 years) prostate cancer cases and a founder mutation has been identified in Iceland (999del5). However, the role of BRCA2 in high risk prostate cancer pedigrees remains unclear. We examined the potential involvement of BRCA2 in Utah prostate cancer pedigrees. Using the Utah Population Database, we identified 59 high-risk prostate cancer pedigrees (N = 464 cases and an additional 1,261 unaffected relatives), in which all prostate cases were no more distantly related than two meioses from another case, and the resulting cluster contained at least four prostate cancer cases [see Prostate 2005 65(4):365-74]. We genotyped these pedigrees using a panel of 404 fluorescent microsatellite markers spaced ~10 cM apart across the genome. We performed linkage analyses using MCLINK and the Smith et al prostate cancer inheritance model [Science 1996 274: 1371-1374]. We identified five pedigrees with at least nominally significant linkage evidence (LOD score 0.588, $p < 0.05$) within 10 cM of the BRCA2 gene locus on chromosome 13. The LOD scores ranged between 0.95 ($p=0.018$) and 1.42 ($p=0.005$). The number of prostate cancer cases in each of these pedigrees ranged from 5 to 12 and the earliest age at diagnosis ranged from 52 to 60 years. None of the 5 linked pedigrees had a significant excess of breast cancer cases among the descendants of the founder, nor any known male breast cancer cases. There are no special identifying prostate cancer characteristics evident in these pedigrees. We are performing BRCA2 mutation screening in the youngest prostate cancer cases who carry the segregating chromosome 13 haplotype in each pedigree. If BRCA2 germline mutations are found to explain the linkage evidence in these pedigrees, the BRCA2 gene may account for up to 8% (5/59) of Utah high-risk prostate cancer pedigrees.

Strong selection pressures have acted in the evolution of forkhead gene family members. *C.D. Fetterman, B. Rannala, M. Walter* Dept Medical Genetics, Univ Alberta, Edmonton, AB, Canada.

Members of the forkhead gene family act as transcription factors in biological processes including development and metabolism, and have been associated with a variety of human diseases. Forkhead genes contain a highly conserved DNA binding domain originally termed 'forkhead' due to the physical appearance of *Drosophila fork head* mutants. *In silico* methods were used to examine selection pressures acting on the entire coding sequence of six multi-species FOX protein clusters. Of the six clusters analyzed, only one, FoxC, was found to be under positive selection. A single amino acid site, within the inhibitory domain of FOXC1, was under positive selection in the FoxC cluster. A positively selected site may be key to protein function and/or differentiating among species. The remaining five clusters analyzed, FoxA, FoxD, FoxI, FoxO and FoxP were under neutral and negative selection. Neutral selection of an amino acid site suggests that the composition of amino acids at that site is unimportant for protein function whereas negative selection of a site suggests that the amino acid composition that occurs at that site is highly important for protein function. These results have allowed predictions to be made regarding potential functional and non-functional amino acids in FOX proteins. This analysis has also provided insight into forkhead gene family evolution. Features that differentiate different species lineages have been identified in the FoxA and FoxC clusters while features that differentiate paralogs were identified in the FoxO cluster. Analysis of the FoxD cluster suggested that the FOXD proteins are either not related outside of the forkhead domain or are still diverging from one another.

Investigation of Subjective Social Status in orofacial cleft Case families versus Controls. *K.M. Bardi[1], M.E. Cooper[1], D.E. Polk[2], C.A. Brandon[1], M.L. Marazita[1]* 1) Ctr for Craniofacial & Dental Genetics, Univ of Pittsburgh, Pittsburgh, PA; 2) Dept of Dental Public Health, Univ of Pittsburgh, Pittsburgh, PA.

Cleft lip with or without cleft palate (CL/P) is a physical birth defect that involves many factors influencing physical and psychological health. Strong evidence indicates that subjective social status (SSS) is determined in part by objective criteria including education, income, and occupation; other findings suggest that higher SSS may be associated with better physical and psychological health. We hypothesized that parents of CL/P affected children would have lower SSS than controls due to psychological and emotional stresses associated with having a child with CL/P. We used the MacArthur Scale of SSS to assess participants drawn from the Pittsburgh Oral-Facial Cleft (POFC) study of CL/P. In the MacArthur Scale, subjects are asked to place themselves on one of 10 rungs on a diagram of a ladder. In addition, education and income data was acquired during the POFC demographic interview. A total of 185 families participated in this study. The study subjects for these analyses were drawn from 77 control families and 49 case families; each case family was ascertained because there were two or more individuals with CL/P (i.e. each family was multiplex). From those families, we analyzed data from 82 parents of children with CL/P and 91 proportionately matched controls. We used multilevel regression nesting spouses within families and controlling for age and marital status. We regressed SSS on education, income, gender, and case status simultaneously and including interaction terms. Although of borderline statistical significance (p=0.055), the model that best predicted SSS included case status, gender and the interaction of case and gender, therefore supporting our hypothesis that parents of individuals with CL/P have different SSS than controls. Supported by NIH Grants P50-DE016215 and R01-DE016148.

De novo direct duplication of 9(p12p24): a case presentation, review of literature, and possible mechanisms of duplication. *P.C. Cosper, A.J. Carroll, M. Descartes, L. Messiaen* Genetics, University of Alabama at Birmingham, Birmingham, AL.

Since the first reported case in 1970, duplications of 9p have been described in a number of patients. Most of these patients have had a partial duplication of 9p as a result of adjacent I segregation of a balanced translocation carried by one of the parents. There have been very few patients reported to have de novo direct duplications of all or almost all of the 9p. We report a patient who has a de novo duplication of 9(p12p24) including duplication of the subtelomere region of 9p; 46,XY,dup(9)(p12p24).ish dup(9p)(pter++). Trisomy 9p is associated with developmental delay, mental retardation, short stature, prominent bulbous nose, downward-slanting palpebral fissures, downturned corners of the mouth, micrognathia, short stature, abnormal low set ears, abnormal philtrum, clinodactyly, abnormal fingers and toes with abnormal nails, and syndactyly. Clinical findings in our patient that are associated with trisomy 9p include developmental delay, mental retardation, lowset bulbous nose, epicanthic folds, micrognathia, and cup shaped, prominent ears. His hand abnormalities include clinched position, short fifth finger with clinodactyly and hypoplasia of the middle phalanx; the nails are mildly dysplastic. Previously reported cases report similar findings. Studies to determine the parental origin of the duplication are planned. The most likely mechanism of these duplications is unequal homologous or sister chromatid exchange. This mechanism will be discussed.

CFH and LOC387715 genes and susceptibility to Age-Related Maculopathay: AREDS cohort and a meta-analysis. *Y. Conley[1, 2], J. Jakobsdottir[3], T. Mah[4], D. Weeks[2, 3], R. Ferrell[2], M. Gorin[2, 4]* 1) Department of Health Promotion & Development, Univ Pittsburgh, Pittsburgh, PA; 2) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA; 3) Department of Biostatistics, University of Pittsburgh, Pittsburgh, PA; 4) Department of Ophthalmology, University of Pittsburgh, Pittsburgh, PA.

Age-related maculopathy (ARM) is an important cause of visual impairment in the elderly population. It is of crucial importance to identify genetic factors and their interactions with environmental exposures for this disorder. This study was aimed at investigating the CFH and LOC387715 genes, previously reported in ARM susceptibility, in an independent cohort as well as conducting a meta-analysis. The study used a case-control design with subjects originally recruited through the Age-Related Eye Disease Study (AREDS). The meta-analysis encompassed nine reports for CFH and four reports for LOC387715, both of which included the AREDS population presented. CFH was significantly associated with ARM in the AREDS cohort (P 0.00001). A meta-analysis confirmed that the risk allele in the heterozygous or homozygous state (OR, 2.5 and 6.4; 95% CI, 2.2-2.8 and 5.5-7.4, respectively) confers susceptibility. LOC387715 was also significantly associated with ARM in the AREDS cohort (P 0.00001) and a meta-analysis confirmed that the risk allele in the heterozygous and homozygous state (OR, 2.7 and 8.0; 95% CI, 2.3-3.1 and 6.1-10.5, respectively) confers susceptibility. Both CFH and LOC387715 showed an allele-dose effect on the ARM risk, homozygotes at either locus were at more then twofold risk compared to heterozygotes. Joint action of CFH and LOC387715 was best described by independent multiplicative effect without significant interaction in the AREDS cohort. Interaction of both genes with cigarette smoking was insignificant in the AREDS cohort. This study provides additional support for the CFH and LOC387715 genes in ARM susceptibility via the evaluation of the AREDS cohort and through the meta-analyses.

TCF7L2 polymorphisms are associated with type 2 diabetes(T2DM) and reduced insulin sensitivity in U.S. Caucasians. *S.K. Das[1,2], W.S. Chu[1,2], S.C. Elbein[1,2]* 1) Department of Internal Medicine and Research Service, Central Arkansas Veterans Healthcare System, Little Rock,AR; 2) University of Arkansas for Medical Sciences, Little Rock, AR.

Recent studies demonstrated an association of both single nucleotide polymorphisms (SNPs) and a microsatellite in introns of the TCF7L2 gene on chromosome 10q with T2DM in Caucasian populations from Iceland, Denmark, and the United States. The combined relative risk was 1.56, but the mechanism by which the noncoding variants in TCF7L2 increased T2DM risk were not explored. We sought to confirm the association in a population of 183 controls and 190 cases of Northern European Ancestry and in which most cases were from families with no linkage to chromosome 10. We also examined 126 nondiabetic members of Utah T2DM families linked to chromosome 1q, and 177 unrelated normoglyemic controls from Arkansas who had undergone frequently sampled intravenous glucose tolerance tests with minimal model analysis of insulin sensitivity (SI). Both SNPs rs12255372 and rs7903146 were associated (MAF 0.32 and 0.35 in cases, 0.25 and 0.24 in controls, p=0.023 and 0.001, respectively) with relative risks of 1.46 and 1.72 for the T allele. Association of these SNPs was not replicated in a cohort of 369 African American cases and 185 controls. Among nondiabetic Utah family members, SI tended to be lower among TT homozygotes, but neither SNP reached significance except in interaction with body mass index (BMI; p=0.02). In contrast, both SNPs were strongly associated with a reduction in SI in euglycemic, unrelated Arkansas Caucasians with means that suggested a recessive inheritance (rs12255372 p=0.002; rs7903146 p=0.008; means 3.36 in nonrisk homozygotes, 2.93 heterozygotes, 1.41 in TT homozygotes). Beta cell compensation for insulin resistance (SI * acute insulin response) was also reduced (p=0.025), but measures of insulin secretion (AIRglucose and maximal secretory response, AIRmax) did not differ by genotype. Our results confirm the reports of TCF7L2 association with T2DM with similar relative risk in Caucasians, and demonstrate a strong association with reduced insulin action but less impact on insulin secretion.

PedGenie 2.0: Meta genetic association testing in mixed family and case-control designs. *K. Curtin, J. Wong, K. Allen-Brady, N.J. Camp* Biomedical Informatics, University of Utah Genetic Epidemiology Division, Salt Lake City, UT.

In the study of common diseases and genes with modest effects, large consortium and multi-center efforts hold the promise of increased power to detect associations, but present analysis challenges. Studies differ geographically and ethnically, and considerable differences in case-control ascertainment and pedigree structures between resources are likely. Currently, no software package exists that allows association testing in mixtures of family-based and independent resources, between or within studies. PedGenie 2.0 (beta-version) extends the functionality currently available in PedGenie 1.2 (Allen-Brady et al. 2006) by incorporating meta statistics for combined analysis of multi-study resources, along with Monte Carlo significance testing which allows for a mixture of pedigree members and independent individuals. Briefly, study-specific allele or haplotype frequencies for markers of interest are used within studies. A Mendelian gene-drop simulation is performed independent of trait; each possible null genotype configuration is used to create an empirical null distribution for the significance testing of meta statistics. The currently incorporated meta statistics for genotype, composite genotype or haplotype analysis across studies are based on Cochran-Mantel-Haenszel (CMH) techniques to calculate odds ratios, chi-squared test of independence, and chi-squared test of trend. Future efforts will include meta-extensions for other quantitative and transmission statistics, such as the transmission-disequilibrium test (TDT). PedGenie is a flexible, easily implemented analysis tool that is enhanced significantly in beta version 2.0 by the incorporation of meta-statistics to allow valid combined analysis of multiple studies in the detection of genetic association with common disease. Reference: Allen-Brady K, Wong J, Camp NJ (2006) PedGenie: an analysis approach for genetic association testing in extended pedigrees and genealogies of arbitrary size. BMC Bioinformatics 7:209.

Quantifying the distribution of selective effects among newly arising mutations in the human genome. *C.D. Bustamante[1], A.R. Boyko[1], S. Williamson[1], K. Lohmueller[1], R. Hernandez[1], A. Indap[1], J. Pillardy[1], A. Fledel-Alon[2], R. Nielsen[3], A.G. Clark[1,4]* 1) Dept Biol Stats & Comp Bio, Cornell Univ, Ithaca, NY; 2) Dept Human Genetics, Univ of Chicago, Chicago, IL; 3) Univ of Copenhagen, Copenhagen, Denmark; 4) Dept of Mol Biol & Genetics, Cornell Univ, Ithaca, NY.

Quantifying the amount of human genetic variation that is moderate to slightly deleterious is an important problem in both evolutionary and medical genomics. Here we report a novel statistical approach for addressing this issue and apply the method to a data set of 30,000 coding SNPs identified by direct resequencing of over 11,000 known human genes in 39 individuals (19 African Americans and 20 European Americans). Specifically, we will present a method for modeling SNP frequencies, the number of invariant sites in the sample as well as the number of fixed differences between humans and chimpanzees based on a population genetic model with natural selection that can jointly handle population growth, bottlenecks, and migration. We investigate several models for the distribution of selective effects among newly arising mutations (Normal, Gamma, mixture of Normals, etc.) and investigate the relationship between physicochemical measures of amino acid distance and evolutionary exchangeability. By using the draft build of the Macaque genome we are also able to polarize fixed differences along the human and chimpanzee lineages and assess the selective impact of specific types of changes. For example, we can quantify the average selective effect of substituting a small for a large amino acid and vice versa. We also demonstrate that various structural features such as solvent accessibility, breaking of hydrogen or disulfide bonds, location in secondary structure, and location in a known domain strongly impact the distribution of selective effects. Lastly, we demonstrate that there is significant variation in the distribution of deleterious mutations among different individuals and hypothesize how bottlenecks in the distant past may have contributed to present-day patterns of deleterious variation.

Behavioral phenotype of Cornelia de Lange Syndrome (CdLS). *B.W. Clark[1], C. Landy[2], G. Jabbar[3], A.D. Kline[4], M.A. Grados[5]* 1) Dept. of Behavioral Psychology, Kennedy Krieger Institute, Baltimore, MD; 2) Genetic Counseling, University of Maryland, Baltimore, MD; 3) Dept. of Psychology, Notre Dame University of Maryland, Baltimore, MD; 4) Harvey Institute of Human Genetics, Greater Baltimore Medical Center, Baltimore, MD; 5) Dept. of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD.

Background: The behavioral phenotype of CdLS is poorly defined and variable. Given the discovery of genes for CdLS, a defined behavioral phenotype would be useful for genotype-phenotype correlation studies. **Methods**: 31 children and adolescents with CdLS underwent parental self-report on physical and behavioral measures. The Aberrant Behavior Checklist (ABC) for maladaptive behaviors, the CdLS Diagnostic Checklist (DC) for physical features of CdLS, the Childhood Autism Rating Scale (CARS) for autism, the Childrens Yale-Brown Obsessive-Compulsive Scale for Pervasive Developmental Disorders (CY-BOCS PDD) for compulsive behaviors, and the Dunn Sensory Profile for abnormal sensory patterns were completed. **Results**: The ABC suggests a bimodal distribution of irritability, aggression, self-injury, stereotypies and hyperactivity. 2,3 toe syndactyly is associated with higher irritability-aggression-SIB and hyperactivity ($p < 0.001$), more touch hypersensitivity ($p < 0.01$) (Dunn Profile) and higher CARS autism scores ($p < 0.02$). 2,3 toe syndactyly may correspond to homeobox gene alterations associated with the CdLS NIPB-L gene function. Autism scores are correlated with the CY-BOCS PDD compulsions scores (Pearson correlation = 0.38, $p < 0.05$), suggesting that a subgroup has both autism features and compulsive behaviors. Greater abnormal auditory and touch sensitivity are also present with increasing autism and compulsive scores ($p < 0.001$). Approximately 30% of the sample had high autism, compulsive, touch sensitivity scores and more frequent 2,3 toe syndactyly. **Conclusions**: Behavioral and physical markers identify a subgroup of children with CdLS with 2,3 toe syndactyly, high maladaptive behaviors, autistic features, compulsive behaviors and sensory abnormalities. These measures may be useful in studying genotype-phenotype correlations.

***VLDLR* common genetic variation is associated with very low-density lipoprotein (VLDL), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) particle density.** *D.C. Crawford[1], A.S. Nord[2], M.D. Badzioch[2], J. Ranchalis[2], M. Ahearn[1], C. Bertucci[1], E.M. Wijsman[2], M.J. Rieder[1], D.A. Nickerson[1], G.P. Jarvik[2]* 1) Department of Genome Sciences, Univ Washington, Seattle, WA; 2) Division of Medical Genetics, Univ Washington Medical Center, Seattle, WA.

Carotid artery disease (CAAD) is a major risk factor for stroke. Those with CAAD also suffer from coronary heart disease and hyperlipidemia. A 10-cM genome scan of LDL particle size conducted on a family with familial combined hyperlipidemia yielded evidence for linkage on chromosome 9p24 in the region of very low-density lipoprotein receptor (*VLDLR*). To identify SNPs associated with CAAD and hyperlipidemia, *VLDLR* (37kb) was re-sequenced in 46 U.S. Caucasian male military veterans who had >80% internal bilateral or unilateral carotid artery stenosis or had a carotid endarterectomy. These patients, who are a subset of a larger CAAD study, were selected to represent the extremes of the LDL particle density (Rf) distribution: 23 with low LDL-Rf (cases) and 23 not on lipid lowering medication with high LDL-Rf (controls). 157 SNPs were annotated in *VLDLR*, and 66% were shared between cases and controls. Twenty tagSNPs (r^2>0.64; minor allele frequency >10%) were identified among controls, and univariate analyses were performed to identify tagSNPs associated with case status. Eight tagSNPs were associated with case status at p <0.0025, of which five had a p-value <0.001. To replicate the pilot study findings, we genotyped *VLDLR* tagSNPs in an additional 81 cases (>80% internal bilateral or unilateral carotid artery stenosis or had a carotid endarterectomy) and 320 age-matched controls (<15% internal, bilateral carotid artery stenosis on carotid duplex ultrasound). Nine tagSNPs were evaluated using models adjusting for sex, age, and triglycerides (LDL-Rf model only). Results suggest several *VLDLR* tagSNPs demonstrate strong evidence of association with VLDL (R^2_{adj}=0.04; p=0.001), HDL (R^2_{adj}=0.16; p<0.0001), cholesterol (R^2_{adj}=0.06; p=0.0005), and LDL-Rf (AIC=308.64) among Caucasians not on lipid lowering medication. We are genotyping an additional 11 tagSNPs to better define these associations.

MC1R gene mutations in Jewish malignant melanoma patients. *E. Friedman[1], G. Galore[1], E. Azizi[2], A. Scope[2], F. Pavlotsky[2], E. Yacobson[3]* 1) Oncogenetics Unit, Chaim Sheba Medical Ctr (CSMC), Tel Hashomer, Israel; 2) Department of Dermatology, CSMC, Tel-Hashomer, Israel; 3) Laboratory for Molecular Cell Biology, Sheba Medical center, CSCM, Tel-Hashomer, Israel.

To assess the putative role of Melanocrtin 1 receptor (MC1R) gene sequence variants in conferring malignant melanoma (MM) risk, we genotyped 79 Jewish Israeli MM patients for germline sequence variants by direct sequencing of the gene, and compared the rates of sequence variants with those of other populations. Two MM patients, an Ashkenazi male with two MM and non-Ashkenazi female with familial MM harbored an identical truncating mutation - Y152X. Haplotype analysis showed that both patients shared an identical haplotype using 8 intra and flanking MC1R markers spanning 400 Kb of genomic DNA. A missense mutation (Val60Leu) was detected in 24 of the 79 patients (30.3%), a rate that far exceeds the rate of this mutation in Italian, Dutch and French MM patients - 10-18%. Whether this is sequence variant is more prevalent in MM patients than in the general Jewish population is being tested. We conclude that rare truncating mutations occur in the MC1R gene and confer MM risk and that a single missense mutation is the same gene is more prevalent among Jewish MM patients than among non Jewish MM patients.

VLCFA in patients with different clinical forms of X-linked adrenoleukodystrophy treated wiht Lorenzos oil. *M. Deon[1,3], A.G. Barschak[1,3], A. Sitta[1,3], M.P. Garcia[2], G.O. Schmit[1], T.F. Brizolari[1], M.H. Oliveira[1], D.M. Coelho[1], J. De Mari[1], M. Wajner[1,3], R. Giugliani[1,3], C.R. Vargas[1,2,3]* 1) Medical Genetics Service, HCPA, Porto Alegre, Brazil; 2) Dep. of Clinical Analysis, Pharmacy Faculty, UFRGS, Porto Alegre, Brazil; 3) Dep. of Biochemistry, ICBS, UFRGS, Porto Alegre, Brazil.

The X-linked adrenoleukodystrophy (X-ALD) is an inherited disease biochemically characterized by the accumulation of VLCFA (very long chain fatty acids) in tissues and biological fluids, particularly hexacosanoic (C26:0) and tetracosanoic (C24:0) acids. This disorder present predominantly central and peripheral demyelination in addition to adrenocortical insufficiency and hypogonadism. At least seven phenotypes can be made out in this illness. The most common clinical forms are the childhood cerebral (cALD) and adrenomyeloneuropathy (AMN). The recommended therapy consists of the use of the glyceroltrioleate/glyceroltrierucate (GTO/GTE) mixture known as Lorenzos Oil (LO), combined with a VLCFA-poor diet. In the present work it was studied the biochemical profile of VLCFA in 15 patients with different clinical forms of X-ALD (5 AMN, 5 cALD and 5 asymptomatic) in the diagnosis and during treatment with LO. It was verified that C26:0 plasma levels were statistically different between the three clinical forms studied during the treatment. Asymptomatic patients were the only ones who had normal plasma levels of C26:0, during treatment. Thus, our results confirm the main biochemical effect of LO and reinforce that this therapy is efficacious in asymptomatic patients.

Proteomic Analysis of Retinoic Acid-induced Clubfoot-like Deformity in Rat Fetuses. *W.N. Fu[1], K.L. Sun[1], Z.G. Li[1], H. Ji[1], H.T. Zhou[1], S.J. Ji[2], Y.Y. Zhao[1], C.L. Jin[1]* 1) Department of Medical Genetics, Basic Medical College, China Medical University, Shenyang 110001, P.R. China; 2) Department of Pediatric Surgery, the Second Affiliated Hospital of China Medical University, Shenyang, 110003, P. R. China.

Proteomic Analysis of Retinoic Acid-induced Clubfoot-like Deformity in Rat Fetuses Wei-Neng Fu1, Kai-Lai Sun1, Zeng-Gang Li1, Hong Ji1, Hai-Tao Zhou1, Shi-Jun Ji2, Yan-Yan Zhao1, Chun-Lian Jin1 1. Department of Medical Genetics, Basic Medical College, China Medical University, Shenyang 110001, P.R. China; 2. Department of Pediatric Surgery, the Second Affiliated Hospital of China Medical University, Shenyang, 110003, P. R. China. To explore the expression profiling of clubfoot proteome and the clubfoot-related genes in the development of clubfoot. Clubfoot-like deformity model in rat fetuses were established with ATRA (135mg/kg) in E10 pregnant Wister rats. 2-DE was applied to separate the total proteins of related tissues of the animal models. The peptide mass fingerprints of the interesting protein spots were identified based on MALDI-TOF-MS and database searching. Apoptosis study was performed by terminal deoxynucleotidyl transferase nick end labeling. Some clubfoot-related genes were analyssed by RT-PCR. The expression profiling of clubfoot proteome from spinal cord, tibia-fibulae musculature, ankle joint tissue and ankle joint bone of the animal models were presented, in which the peptide mass fingerprint of 23 protein spots differential expressed in the model fetuses were obtained and analyzed. RT-PCR results showed that XIAP, TNNT1 and Col21 were confirmed to be significantly down-regulated in correspondence to the results of 2-DE.The rates of the apoptosis in the spinal, vertebra and muscle from model fetuses were 5.4, 10 and 3.7 times higher than those from the normal fetuses, respectively. The result implies that apoptosis and certain differentially expressed proteins identified in this study might play a role in the development of Clubfoot.

Efficient Analysis of Associations between Haplotypes and Quantitative Traits in Family Studies. *G. Diao, D.Y. Lin* Dept Biostatistics, Univ North Carolina, Chapel Hill, NC.

The associations between haplotypes and quantitative traits provide valuable information about the genetic basis of complex human diseases. Two major challenges arise in haplotype association analysis in family studies. First, the haplotype phases may not be determined with certainty from the genotype data. Second, the trait values within a family tend to be correlated because of common genetic and environmental factors. We present an efficient likelihood-based approach to investigating the associations between haplotypes and quantitative traits. This approach accounts for within-family trait correlations and can handle arbitrary pedigrees with missing genotypes. We characterize the effects of haplotypes on quantitative traits by regression models with random effects and derive appropriate likelihood functions. We develop efficient numerical algorithms to implement the corresponding likelihood-based methods. Extensive simulation studies showed that the proposed methods perform well in realistic situations. A real example is provided. A computer program is freely available.

FFU complex associated with agenesis of kidney, a case report. *G. Contreras[1], J.C. Prieto[1,2]* 1) Instituto de Genetica Humana, Universidad Javeriana, Bogota, Cundinamarca, Colombia; 2) Departamento de Genetica, Hospital la Victoria, Bogota, Colombia.

The original description the femur-fibular-ulna complex mentioned the absence of the proximal part of the femur, absence of the fibula and malfomations of the ulnar side on the upper limb. Later, cases of bilateral femur and fibula defects with normal arms were included en the same category. The main reason for summarizing defects of femur, fibula and ulna is their tendency to occur together in the same patient much more frequently than could be expected on the basis of chance combinations of the single defects. This malformation syndrome that usually occurs sporadically. It has been described aplasia of ulna and fibula with renal malformation without femur anomalies. We present a new case of a three day-old boy born in Bogotá (Colombia) of non consanguineous parents product of the mother's first term 37-week pregnancy and cesarean delivery. The birth height: 49 cm., and weight: 2660 gr. Prenatal ultrasonographic study showed amelia of right upper limb right and hemimelia of right lower limb. The positive findings found on the physical examination were: short neck, amelia of right upper limb, the rhizomesomelic shortening of right lower limb, varo feet, hypoplasia of the hallux. X-ray studies report: absence of structures the right upper limb, hypoplasia of femur and absence of fibula right with hypoplasia of tibia. In column we observed sacral left hemivertebral. Postnatal ultrasonographic showed associated agenesis of right kidney. Here we report a case with additional phenotypic manifestations mainly in kidney and limbs.

A strong signature of balancing selection on Succinate Dehydrogenase Subunit A gene (*SDHA*) in the African-American population. *B.E. Baysal[1], E.C. Lawrence[2], R.E. Ferrell[2]* 1) OB/GYN & Reproductive Sci, Magee-Womens Research Inst, Pittsburgh, PA; 2) Dept. Human Genetics, University of Pittsburgh, Pittsburgh, PA.

Mitochondrial complex II (succinate dehydrogenase; SDH) is an essential enzyme complex in the Krebs cycle and in the electron transport chain. Mutations in *SDHB*, *SDHC* and *SDHD* subunit genes cause hereditary paraganglioma (PGL), whereas those in *SDHA* lead to neuronal and muscular pathologies. 3-Nitropropionic acid (3-NP), a widely distributed plant and fungal neurotoxin, inhibits the *SDHA* gene product and can cause severe central nervous system defects and death. To gain further insights into mitochondrial complex II function, we analyzed variation patterns in its subunit genes by sequencing a total of 10.8 kb coding and non-coding DNAs in 24 African-American and 24 European-American samples.

In total, 3,828 coding and 7,013 non-coding nucleotides were sequenced for each sample and a total of 52 polymorphisms were detected. Increased sequence diversity distinguished *SDHA* from the PGL genes and refuted the previous suggestions that *SDHA* missense variants originate from two different genetic loci. The *SDHA* variation pattern showed statistically significant deviations from neutral expectations in both racial groups. The elevated values of the nucleotide diversity (% = 0.231) and the Tajimas statistics (D= 1.954) in the *SDHA* gene were comparable to the most prominent cases for balancing selection in the African-American population. *SDHA* nucleotide diversity was higher than 274 ($p<0.011$) and Tajimas D statistics was higher than 276 ($p<0.0036$) of the 277 fully-sequenced genes in the African-American ppopulation in the Seattle SNP database. Two distinct *SDHA* haplotype clusters were present in intermediate frequencies in both racial groups. The unexpectedly high sequence diversity in *SDHA* has revealed one of the most outstanding cases for balancing selection in the African-American population. These findings suggest that a balancing selection mechanism, such as 3-NP toxicity or an infectious agent, has shaped the *SDHA* variation pattern in Africa and that PGL and *SDHA* gene products are under distinct functional constraints.

Gene-Gene Interaction Analysis with Models Based on Entropy Method. *C. Dong[1, 2, 4], Y. Li[1, 4], X. Chu[2], Y. Wang[2], T. Shi[1, 4], Y. Wang[3], L. Jin[3], W. Huang[2]* 1) Bioinformatics Center, SIBS, CAS, Shanghai, China; 2) Chinese National Human Genome Center at Shanghai, Shanghai, China; 3) Fudan University, Shanghai, China; 4) Shanghai Center for Bioinformation Technology, Shanghai, China.

Gene-gene interaction may play important roles in complex disease studies, in which, however, individual genes cant be detected through association analysis. The current approaches including statistical or epidemiological methods and data mining rarely consider the effect of genetic interaction. In this study, we develop an entropy-based method to explore such interaction. This method includes a model-free option and a model-based one. The former aims to explore the joint effect of two SNPs and the latter fits the data to a potential interaction model. Simulation results show that this method is effective to detect gene-gene interaction and furthermore, fit the interaction to various models. Furthermore, the application of the method to malaria data revealed negative epistatic effect between HbAA and $^{+}$-thalassemia against malaria.

Neonatal Asymmetric Crying Facies: phenotype in monozygotic twins associated with preaxial polydactyly, a case report. *C. Duran[1], G. Contreras[1], J.C. Prieto[1,2]* 1) Inst de Genetica Humana, Univ Javeriana, Bogota Cundinamar, Colombia; 2) Departamento de Genetica, Hospital la Victoria, Bogota, Colombia.

Unilateral neonatal facial asymmetry, an age-old problem, is a generic heading for several clinical phenotypes. The neonatal asymmetric crying facies (NACF) indicates that the condition is present at birth, and it allows for multiple etiologies. The clinical hallmark of NACF is a symmetric appearance of the oral aperture and lips at rest, but significant depression of one side of the lower lip with crying. One etiology is due to congenital hypoplasia of the depressor anguli oris muscle and reported other associated anomalies, it has an autosomal dominant pattern of inheritance. The effect is asymmetry of the lower lip, especially evident in smiling or crying. In 70%, children with hypoplasia of the depressor anguli oris muscle found accompanying anomalies. These included anomalies of the head and neck (48%), heart (44%), skeleton (22%), genitourinary tract (24%), central nervous system (10%), gastrointestinal tract (6%), and miscellaneous minor anomalies (8%). Deletion of 22q11.2 was observed in cases fitting into the spectrum of Cayler syndrome and has considered to be part of the CATCH22 phenotype. Monozygotic twins who were concordant for 22q11.2 deletion and Cayler syndrome described. Both had tetralogy of Fallot, as well as hypoplasia of the depressor anguli oris muscle, bifid uvula, and T-cell anomalies. These twins were diamniotic and dichorionic. In monochorionic twins, discordant malformation may be related to the twinning process itself. We report monozygotic twins 14 months old girls born in Bogotá (Colombia) of nonconsanguineos parents, the patients were the product of the mother's first unterm 34-week pregnancy and cesarean delivery. No teratogenic exposure. The birth height: 43 cm., and weight: 1600 gr., with asymmetric crying facies. Face was symmetrical except for marked depression of left lower lip with crying, associated in one the twins preaxial polydactyly of the right hand. To the best of our knowledge, this association has not been previously published.

A novel chimeric gene formed by exon-shuffling and retrotransposition in the hominoid lineage. *D.V. Babushok[1], K. Ohshima[2], X. Chen[3], Y. Wang[3], E.M. Ostertag[1,4], N. Okada[5], C.S. Abrams[3], H.H. Kazazian[1]* 1) Dept Genet, Univ PA, Philadelphia, PA; 2) Nagahama Inst Bio-Sci Tech, Nagahama, Japan; 3) Dept Medicine, Univ PA, Philadelphia, PA; 4) Dept Path Lab Medicine, Univ PA, Philadelphia, PA; 5) Tokyo Inst Tech, Yokohama, Japan.

The structure of most cellular genes, with exons often containing separate domains, has long led to speculation on the role of exon (and domain) shuffling in new gene formation. Such exon shuffling was observed in cases of illegitimate recombination, and can also occur by retrotransposon-mediated transductions of host sequences. Recent studies of intergenic RNA splicing have suggested another important way to form new genes, whereby genes can undergo exon-shuffling at the RNA level by splicing from one gene to another, followed by reverse-transcription and insertion into a new location by the LINE-1 (L1) retrotransposon. To further study this novel mechanism of gene formation, we analyzed the structural features and evolutionary history of the PIP5K1A-PSMD4 chimeric gene. We found that PIP5K1A-PSMD4 chimera was formed roughly 17 MYA in the hominoid lineage by L1-mediated retrotransposition of a rare intergenically-spliced transcript between the phosphatidylinositol-4-phosphate 5-kinase (PIP5K1A) gene and its neighbor, the 26S proteasome subunit 4 (PSMD4) gene. This retrogene has testes-specific transcription starting within the 5UTR of PIP5K1A, contains a moderate Kozak consensus, and is likely translated to make an 862 aa protein. The novel protein is localized predominantly in the cytoplasm, with an apparent loss of membrane localization of PIP5K1A protein and proteasome interaction of PSMD4 protein. The chimera is undergoing rapid evolution in its PSMD4 region, and has functionally diverged from parental proteins. It lacks significant PIP5 kinase activity, but is able bind to endogenous ubiquitinated proteins through its PSMD4 region. Future studies will determine if this evolutionarily young gene is important in ubiquitin signaling in the testis. Novel gene formation by retrotransposition of unusually spiced transcripts likely represents a significant mechanism for exon shuffling.

Partial tandem duplication of *GRIA3* in a male with mental retardation. *T. Chiyonobu[1,2], S. Hayashi[3], M. Morimoto[2], Y. Miyanomae[4], A. Nishimura[2], A. Nishimoto[1], C. Ito[1], I. Imoto[3], T. Sugimoto[2], Z. Jia[5], J. Inazawa[3], T. Toda[1]* 1) Division of Clinical Genetics, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; 2) Department of Pediatrics, Kyoto Prefectural University of Medicine, Kyoto, Japan; 3) Department of Molecular Cyotogenetics, Tokyo Medical and Dental University, Tokyo, Japan; 4) Department of Pediatrics, Kyoto City Child Wellbeing Center, Kyoto, Japan; 5) Program in Brain and Behavior, the Hospital for Sick Children, Toronto, Canada.

The genetic factors underlying mental retardation (MR) are very heterogeneous. Recent studies have identified a number of genes involved in MR, several of which lie on the X chromosome, 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 genes. Using array-based comparative genomic hybridization analysis, we identified a small copy number gain at Xq25, which was undetectable by conventional G-band analysis, in a boy with unexplained MR. Further characterization revealed a partial tandem duplication of *GRIA3*, an alteration also present on one allele in his mother. RT-PCR analysis of lymphoblastoid cell RNA revealed remarkably reduced *GRIA3* transcript levels in the patient. The mother, whose cognitive level is normal, also demonstrated remarkably reduced *GRIA3* transcript levels in lymphoblastoid cells, and X-chromosome inactivation (XCI) was completely skewed in her peripheral lymphocytes. It is possible that XCI in the brain is not completely skewed and that *GRIA3* expression from the normal allele may account for the mothers normal cognitive function. Taken together with previous findings of *GRIA3* disruption by a balanced translocation and severely reduced *GRIA3* transcript levels in a female with MR, our study strengthens the idea that *GRIA3* is a candidate gene for X-linked MR and that severely reduced *GRIA3* expression results in MR.

A hybrid approach to genetic studies - Genome wide association study and comprehensive candidate gene analysis of nicotine dependence. *L. Bierut[1], P. Madden[1], N. Breslau[2], E. Johnson[3], D. Hatsukami[4], O. Pomerleau[5], G. Swan[6], J. Rutter[7], A. Goate[1], A. Hinrichs[1], K. Konvicka[8], N. Martin[9], G. Montgomery[9], N. Saccone[1], S. Saccone[1], J. Wang[1], G. Chase[10], J. Rice[1], D. Ballinger[8], NICSNP Consortium* 1) Washington University School of Medicine, St Louis, MO; 2) Michigan State University, East Lansing, MI; 3) Research Triangle Institute International, Research Triangle Park, NC; 4) University of Minnesota, Minneapolis, MN; 5) University of Michigan, Ann Arbor, MI; 6) SRI International, Menlo Park, CA; 7) National Institute on Drug Abuse, Rockville, MD; 8) Queensland Institute of Medical Research, Herston QLD Australia; 9) Perlegen Sciences, Mountain View, CA; 10) Penn State College of Medicine, Hershey, PA.

Smoking is the leading source of preventable death in the U. S., and twin studies consistently demonstrate strong genetic contributions to smoking. The NICSNP Project is a hybrid study of a genome wide association (GWA) study in tandem with the systematic coverage of biologically relevant candidate genes. The sample consisted of 1050 nicotine dependent cases and 879 non-dependent smokers as controls. All participants were selected from two community-based studies, the Collaborative Genetics Study of Nicotine Dependence (U.S.) and the Nicotine Addiction Genetics Project (Australia). The GWA study performed pooled genotyping of 2.4 million single nucleotide polymorphism (SNPs) followed by individual genotyping of the top 40,000 signals. The second arm of the study was a comprehensive candidate gene study, where individual genotyping was conducted in over 300 genes chosen for their biological significance by experts in the field of addiction. There were convergent findings in these complementary approaches. Common variants in candidate genes exhibited the highest level of statistical significance and are among the top 25 signals from the GWA study. In addition, the GWA study results identified novel loci not previously associated with the risk for nicotine dependence. These findings are a major advance in the genetics of complex human disease, and data will be available upon publication for further analyses by the scientific community.

Deficiency of ^{1}-pyrroline-5-carboxylate synthase (P5CS) in a consanguineous New Zealand family. *L.S. Bicknell[1], J. Pitt[2], M. Maw[3], R. Ramadas[4], C. Wilson[5], S. Aftimos[5], S.P. Robertson[1]* 1) Dept of Paediatrics & Child Health, University of Otago, Dunedin, New Zealand; 2) Genetic Health Services Victoria, Melbourne, Australia; 3) Dept of Biochemistry, University of Otago, Dunedin, New Zealand; 4) Whakatane Hospital, New Zealand; 5) Northern Regional Genetics Service, Auckland, New Zealand.

We have characterised a consanguineous family segregating a connective tissue disorder (joint dislocations, lax skin) associated with severe global developmental delay, and choreoathetosis. An MRI scan showed underdevelopment of the white matter. Metabolic testing demonstrated no abnormalites of urea cycle intermediates, amino acids or organic acids. We performed a 400 microsatellite genome screen under an assumption of homozygosity for a common ancestral disease allele. Multipoint analysis identified a 50 cM region (~47 Mb) at 10q23.1-23.4 with a peak LOD of 2.99. Fine mapping further refined this interval to a 12 Mb critical region which demonstrated homozygosity by descent with a maximal LOD of 3.25. Of the 85 genes located within this interval, one gene *ALDH18A1,* encoding ^{1}-pyyroline-5-carboxylate synthase (P5CS), was identified as a candidate since a missense mutation had previously been shown to cause progressive neurodegeneration, cataracts, skin laxity, joint dislocations and metabolic derangement in a consanguineous Algerian family. A mutation in *ALDH18A1*, 2350C>T, predicting the substitution H784Y was identified at a residue conserved across all phyla. P5CS is a catalytic bifunctional enzyme that converts glutamate to proline and ornithine prior to ornithine incorporation in the urea cycle. Proline, a major constituent of connective tissue, has also been implicated in neurotransmission. Impaired synthesis could therefore account for many features of this phenotype, however, *in vivo* enzymatic assays suggest proline biosynthesis is not perturbed in this family, consistent with normal results from clinical metabolic testing. Moonlighting roles, the evolutionary adoption by an enzyme of an unrelated function, have been found for other metabolic enzymes, and thus P5CS may possess additional uncharacterised functions within the cell.

Polymorphisms in the Activating Transcription factor 6 (ATF6) a candidate gene are associated with Type 2 Diabetes(T2DM) in Utah Caucasians. *W.S. Chu[1,2], S.K. Das[1,2], S.C. Elbein[1,2], International Type 2 Diabetes Chromosome 1q Consortium* 1) Dept Medicine, Univ Arkansas Medical Sci, Little Rock, AR; 2) Research Service, Central Arkansas Veterans Healthcare System, Little Rock, AR.

ATF6 is located under the well replicated linkage peak for T2DM on chromosome1q21-q23, and is a key activator of the endoplasmic reticulum (ER) stress response. ER stress in turn has been implicated in hepatic and peripheral insulin resistance and in impaired insulin secretion in animal models. We evaluated 78 SNPs over 213 kb, from 8 kb upstream through the 3 flanking region. We used linkage disequilibrium (LD) in our population to select 39 SNPs for r2>0.9 to type in 189 unrelated, euglycemic Caucasian controls and 191 Caucasians with T2DM and a T2DM family history. Additionally, 44SNPs were typed by the Chromosome 1q Consortium in a subset of 191 cases and 165 controls. A nonsynonymous coding SNP rs1058405 (M67V) in exon 3 (MAF 0.316 in case, 0.228 in control) and SNP rs1027700 in intron 4 (MAF 0.321 in case, 0.241 in control) were significantly associated with T2DM (p=0.007 and 0.016 respectively). A second nonsynonymous SNP (S157P, exon 5) in the same LD block also showed a trend to association, and 3 other SNPs in the 3 flanking region of ATF6 gene showed a significant association (p<0.05), but no other SNPs were associated. The significantly associated SNPs were not in Hardy Weinberg equilibrium despite assay redesign. Association of the coding or intronic SNPs was not replicated in a cohort of 370 African American cases and 185 controls, nor did any coding SNP significantly effect insulin sensitivity (SI), secretion (AIRg), or disposition index (DI) among two Caucasian populations (n=125 and n=199) that underwent intravenous glucose tolerance tests. Consortium typing of 44 SNPs in Amish, French and UK Caucasian populations, as well as in Pima and Chinese populations failed to find any significant association of ATF6 variants with T2DM, but did not test SNP M67V. Our association data and deviation from HWE suggest a role of for M67V as a causative variant in one population, but the lack of confirmation suggests that additional studies are needed to assess the significance of ATF6 in T2DM.

Genome-wide linkage analysis of components of the metabolic syndrome in the Beaver Dam Eye Study. *C.Y. Cheng[1,2], K.E. Lee[3], P. Duggal[1], R. Klein[3], E.L. Moore[3], J.E. Bailey-Wilson[1], B.E. Klein[3]* 1) Inherited Disease Research Branch, NHGRI/NIH, Baltimore, MD; 2) Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 3) Department of Ophthalmology and Visual Sciences, University of Wisconsin Medical School, Madison, WI.

The metabolic syndrome (MS), characterized by obesity, dyslipidemia, hyperglycemia, and hypertension, has become one of the major public-health challenges worldwide. We sought to identify genetic loci with potential influence on components of the MS in the Beaver Dam Eye Study (BDES), a large cohort study of adults in Beaver Dam, Wisconsin. Components of the MS were derived using principal component analysis on 7 MS-related traits, including body mass index (BMI), systolic and diastolic blood pressure, blood glucose, glycosylated hemoglobin, high-density lipoprotein (HDL) cholesterol, and uric acid level. Three component factors were identified and represented glycemia, blood pressure and combined (BMI, HDL and uric acid) factors, respectively. A genome-wide scan with 385 microsatellite marker was conducted on 1,524 sipships from 486 families. Model-free single- and multipoint linkage analyses of the factor scores were performed using Haseman-Elston regression. Estimates of heritability of the MS factors ranged from 40% for the combined factor to 17% for the blood pressure factor. Genome-wide suggestive evidence for linkage of the glycemia factor was found on chromosome 22 at 32cM (LOD = 2.99, P = 0.0001, near marker D22S658). Regions linked to the combined factors were on chromosome 1 at 102cM (LOD = 2.26, P = 0.00063, near marker D1S1665) and chromosome 17 at 82cM (LOD = 2.52, P = 0.00033, near marker D17S1290). Several regions showed suggestive linkage to the blood pressure factors: chromosome 1 at 188cM (LOD = 2.74, P = 0.00019, marker D1S1619), chromosome 10 at 149 cM (LOD = 2.77, P = 0.00018, marker D10S1656), and chromosome 16 at 54cM (LOD = 2.74, P = 0.00019, between marker D16S769 and D16S540). The linkage signals on the three factors did not overlap with each other. Our findings suggest that the MS factors are influenced by multiple distinct loci across the genome, suggesting its complex pathogenesis.

Familial Thoracic Aortic Aneurysms and Dissection: Rapid Identification of Causative Variants in TGFBR3 through Direct Sequencing of Genes in the TGF- Pathway. *N. Avidan[1], J. Kao[1], D. Divecha[1], H. Pannu[1], D.C. Guo[1], V.T. Tran-Fadulu[1], D.H. Kim[3], S.E. Scherer[2], R. Gibbs[2], D.M. Milewicz[1]* 1) Dept Medical Genetics, Univ Texas Medical Sch, Houston, TX; 2) Human Genome Center, BCM, Houston, TX; 3) Department of Neurosurgery, Brigham and Women's Hospital, Boston, MA.

Familial thoracic aortic aneurysms and dissections (FTAAD) is inherited in an autosomal dominant manner. Mapping studies indicate significant genetic heterogeneity for FTAAD, with 5 loci mapped to date. Mutations in type II transforming growth factor receptor (*TGFBR2*) have been identified as causing disease at the TAAD2 locus. Moreover, the TGF- signaling pathway was activated in smooth muscle cells explanted from TGFBR2 mutation negative FTAAD patients. To test the hypothesis that we could identify defective genes causing familial TAAD more rapidly through direct sequencing of genes in the TGF- pathway, 12 genes encoding proteins in the pathway were sequenced from 200 unrelated FTAAD families. Four novel non-synonymous mutations that segregated with disease in 6 unrelated families and were not present in 384 control chromosomes were identified in type III TGF- receptor (*TGFBR3*). TGFBR3 presents the TGF-2 ligand to TGFBR2 on the cell surface, and enhances TGF- signaling. One of the alterations, Ile790Pro, found in three unrelated families, was predicted to disrupt a cleavage site responsible for shedding the receptor from the cell surface and to increase TGF- signaling. Immunoblot analysis of TGFBR3 using mutant fibroblasts from 2 patients revealed increased receptor in the cell membrane and decreased receptor in the media when compared to control fibroblasts, suggesting the mutation prevented proteolytic shedding of the receptor. Furthermore, when stimulated with TGF-2, the patients fibroblasts had enhanced phosphorylation of Smad2 when compared with controls. The results indicate that the Ile790Phe substitution has functional consequences, including increased TGFBR3 on the cell surface and enhanced TGF-2 signaling. FTAAD is the first phenotype genetically and functionally linked to mutations in TGFBR3 and may account for 3% of affected familial cases.

Replication of candidate regions for familial idiopathic scoliosis with model-independent linkage analysis. *D. Behneman[1], C. Justice[1], D. Fallin[2], T. Beaty[2], B. Marosy[3], N.H. Miller[3], A.F. Wilson[1]* 1) Genometrics Section, Inherited Disease Research Branch, NHGRI/NIH, Baltimore, MD; 2) Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 3) Department of Orthopaedic Surgery, Johns Hopkins University, Baltimore, MD.

Idiopathic scoliosis (IS) is a structural lateral curvature of the spine that develops in late juvenile or adolescent period in otherwise normal individuals. Linkage analysis has been used to identify candidate regions on chromosomes: 6, 9, 10, 16, 17, 18, 19, and X [Wise et al. 2000; Chan et al. 2002; Salehi et al. 2002; Justice et al. 2003; Miller et al. 2005]. In this study, an independent sample of families was used in an attempt to replicate these regions. Seventy-one families with at least 2 affected (lateral curvature 10º) individuals, were ascertained and clinically characterized. A genomic screen with 385 STRPs was performed on 306 individuals. Scoliosis was analyzed as a qualitative trait, with varying thresholds of curvature for affectation status (10, 21, 30 and 40) and as a quantitative trait (the observed degree of lateral curvature). Single-point model-independent sib-pair linkage analysis was carried out with SIBPAL [S.A.G.E. v4.5, 2001]. Regions with 2 or more markers significant at the 0.1 level in the Miller et al. [2005] study were combined with the p-value at the corresponding marker in this replication sample using Fishers combined probability test [Whitlock, 2005]. Replicated regions were defined as markers for which the Fishers combined p-value < 0.01. Five regions corresponded to those reported by Miller et al. [2005] and can be considered true replications: D1S2845 to D1S2660, D1S1660, F13A1 to D6S2434, D8S262 to D8S1130, and D9S938 to D9S1826.

Migraine Linkage Mapping using the Norfolk Island Genetic Isolate. *L.R. Griffiths[1], C. Bellis[1], R.M. Hughes[1], K.N. Begley[1], S. Quinlan[1], R.A. Lea[1], S.C. Heath[2], J. Blangero[3]* 1) Genomics Res Ctr, Sch Med Sci, Griffith Univ Gold Coast, Southport, Australia; 2) Centre National de Génotypage, 2 Rue Gaston Cremieux, Evry, France; 3) Southwest Foundation for Biomedical Research, San Antonio, Texas, USA.

Migraine is a common and debilitating neurological disorder with a significant genetic component. Genetic linkage studies in our laboratory have so far revealed migraine susceptibility regions on chromosome 19p13, Xq28, 1q31 and 18p. To aid in migraine gene mapping and identification, we are currently undertaking linkage studies using a unique genetic isolate. The Norfolk Island community is a population of ~1200 permanent residents, the majority of whom are direct descendents of 18th century English Bounty mutineers and Polynesian women who relocated to Norfolk Island from Pitcairn Island in the 1850s. As a genomic study population, Norfolk is of interest because there are strong family groupings and well-documented family histories and the population initially grew in isolation from other communities. We have recruited individuals from this isolate to investigate the genes involved in migraine. DNA samples from two-thirds of the Islands adult permanent population have been prepared for these studies and individuals phenotyped for the disorder. 602 individuals have been collected with information relating to migraine, eg age of onset, severity, triggers and medication response obtained, and affected individuals diagnosed as MA or MO using IHS guidelines. The population has a high prevalence of migraine with 26% affected by the disorder. Most of these individuals fit within a single large, 12-generation (~6500) pedigree extending back to the original founders. Heritability and power estimates have been determined with these studies indicating that the pedigree should provide a unique and powerful resource for disease gene mapping. We are currently investigating migraine implicated loci and a full genome scan is being undertaken. Results implicating vascular related migraine susceptibility variants in the MTHFR gene and investigating the Xq24-28 genomic region in this genetic isolate will be presented.

Strong evidence for gene-gene interactions in rheumatoid arthritis (RA). *L.F. Barcellos[1], P.P. Ramsay[1], E. Madden[1], P.K. Gregersen[2], L.A. Criswell[3]* 1) University of California, Berkeley, CA; 2) North Shore LIJ Institute for Medical Research, Manhasset, NY; 3) University of CA, San Francisco, CA.

Association between MHC genes and RA is well established, and accounts for ~30% of the genetic component in RA. Multiple HLA-DRB1 (shared epitope, SE) alleles largely explain this association; however, exact mechanism(s) by which MHC genes operate in RA are not known. Genome screens have been performed to identify non-MHC loci, but little overlap has been observed between studies, suggesting that the existence of genes with strong individual effects is not likely. Non-parametric tree-based classification methods that accommodate large marker datasets and allow for genetic heterogeneity and interactions among variables are useful for RA studies. Theoretical work has shown that prediction accuracy of classification trees is significantly improved by building a collection of trees, or Random Forests. We analyzed whole-genome screen data (381 markers) for 583 Caucasian RA affected sib pairs from the North American Rheumatoid Arthritis Consortium using a Random Forest approach, to identify evidence for non-MHC loci associated with important RA outcome variables. Genetic sharing (mean IBD) at non-MHC genomic regions, gender distribution, and exposure to smoking within each sib pair (total=383 predictors) were used to predict concordance (both sibs either positive or negative) for the following outcomes: (1) SE status, (2) DRB1*0401 status, (3) carrier status for the PTPN22 R620W variant, (4) RF status, (5) CCP status, (6) age of onset, and (7) exposure to tobacco smoke. In addition, sharing of SE was included as a predictor for CCP status. Several non-MHC regions were identified by Random Forest as predictive of age of onset (chr. 1, 7, 12, 14), CCP status (chr. 1, 4, 10), and RF status (chr. 7 and 9). Evidence for gene-gene interactions was also observed; chr. 3, 4, 10, 14 were predictive of PTPN22 status, chr. 7 was predictive of DRB1*0401 status, and chr. 4, 11 were predictive of SE status. Gender and SE status were predictive of CCP status, but were not important for other outcomes. Results suggest that multiple non-MHC loci influence clinical and HLA-associated risk for RA.

Common deletions in Iranian patients with -thalassemia. *F. Esteghamat[1, 2], H. Imanian[1], A. Azarkeivan[1], N. Almadani[1], H. Najmabadi[1, 2]* 1) Kariminejad-Najmabadi Pathology and Genetics Center, Tehran, Iran; 2) Genetics Research Center, University of Social Welfare and Rehabilitation Sciences,Tehran, Iran.

Backgrounds: Hereditary Persistence of Fetal Hemoglobin (HPFH) and -thalassemia are heterogeneous disorders, characterized by increased level of fetal hemoglobin (HbF) in adult life. A considerable number of deletions of variable size and position that involve the -globin gene cluster on chromosome 11 are associated with the clinical entities of HPFH and -thalassemia. Here, we studied the most eight common deletions involved in HPFH and -thalassemia in Iranian patients. Materials and Methods: We included 32 patients from 19 families who have referred to our private genetic center since last 3 years with elevated levels of HbF, and low MCV. After obtaining the informed consent, DNA was extracted from whole blood by salting-out method. Detection of 8 deletions including HPFH-1, HPFH-3, Spanish, Sicilian, Chinese, Asian-Indian inversion-deletion G(A)0, and the Turkish form of inversion-deletion ()0 thalassemia, Hb-Lepore was based on PCR method described previously by Craig et al. Results: We found Sicillian, Hb lepore and Asian-Indian inversion-deletion G(A)0 deletions causing -thalassemia in 10(52.62%), 3(15.78%) and 5(26.31%) of patients, respectively. We could not find any of eight deletions in one of the patients. Conclusion: Taken together, this is the first study of deletions involved in HPFH and -thalassemia in Iranian patients with -thalassemia that highlight the heterogeneity of the genetic background of Iranian population and importance of screening this deletions in prenatal diagnosis.

TMEM16A is amplified and overexpressed in head and neck cancer. *U. Duvvuri[1, 2], B.J. Henson[2], X. Huang[2], J.S. White[2], R.A. Parikh[2], R. Seethala[3], A. El-Naggar[5], J.R. Grandis[1, 4], S.M. Gollin[1,2,3,4]* 1) Otolaryngology, University of Pittsburgh, Pittsburgh, PA.,; 2) Department of Human Genetics Graduate School of Public Health; 3) Pathology; 4) University of Pittsburgh Cancer Institute; 5) Pathology, MD Anderson Cancer Center.

TMEM16a is a novel gene that we recently identified by high-resolution physical mapping of chromosomal band 11q13 in squamous cell carcinoma of the head and neck (SCCHN). We prepared and validated a polyclonal antibody against TMEM16A. Immunoflourescence demonstrated the presence of TMEM16a in the cytoplasm, plasma membrane, nuclear membrane and nucleoplasm of SCCHN cell lines. The expression of TMEM16A was evaluated in paired samples of SCCHN and normal adjacent mucosa by immunoblotting. TMEM16A was overexpressed by 500% in 80% of tumors, but not in normal mucosa ($p<0.001$). QuMA was used to determine the gene copy number in SCCHN cell lines. qRT-PCR was used to determine transcript levels. Gene copy number, transcript and protein levels were not directly correlated in cell lines that do/ do not overexpress the 11q13 amplicon. PCR was used to map the genomic sequence of *TMEM16a* in 16 primary tumor samples. No mutations were identified in these tumors. TMEM16a expression in SCCHN was further investigated by immunohistochemical staining of a SCCHN tissue array. Two distinct tumor populations distinguished by differences in the nuclear and cytoplasmic staining were identified. Tumors with a high nuclear to cytoplasmic ratio were more poorly differentiated than those with a lower ratio. *TMEM16a* is a novel gene that is amplified in SCCHN with 11q13 amplification. This gene encodes a protein with an undiscovered function that is predicted to have eight transmembrane domains (TMDs). TMEM16A was found in the nucleoplasm in cell lines and tumor samples, despite the presence of TMDs. The lack of correlation between the gene, mRNA and protein levels suggests that factors other than overexpression at the DNA level may regulate TMEM16a expression. The overexpression of TMEM16A observed in tumors versus normal tissue, when coupled with the lack of mutations suggest that TMEM16a overexpression may be related to carcinogenesis.

Ulcerative colitis and sensorineural hearing loss. Mother and daughter in a Mexican family. *L.Hdez Gomez[1], S.G. Juarez Garcia[2], D.O. Gomez Torres[3], G. Garcia Sanchez[3], M.R. Izquierdo Ortiz[4], L. Acosta Ramos[5]* 1) Servicio de Audiologia, Instituto Nacional de Rehabilitacion Mexico D.F; 2) Servicio de Neuropsicologia infantil, Instituto Nacional de Rehabilitacion Mexico D.F; 3) Depto. Investigacion, Instituto Nacional Rehabilitacion, Mexico, DF, Mexico; 4) Servicio de Otoneurologia, Instituto Nacional de Rehabilitacion Mexico D.F; 5) Servicio de Genetica, Instituto Nacional de Rehabilitacion Mexcio D.F.

Ulcerative colitis (UC) is a polygenic disorder that gives rise to multiple clinical subgroups within UC and Crohn disease (CD) . Genome-wide searches have shown disease-associated loci on chromosomes 16, 12, 7, 5, 3, and 1. The major symptoms of UC are diarrhea, rectal bleeding, tenesmus, passage of mucus, and crampy abdominal pain. The severity of symptoms correlates with the extent of disease. Although UC can present acutely, symptoms usually have been present for weeks to months. Occasionally, diarrhea and bleeding are so intermittent and mild that the patient does not seek medical attention; hearing loss (SHL) was found in 46%; associated with UC, frist described by McCabe in 1973. We report a Mexican family with 2 affected members with UC and SHL in 2 generations, in which mother and her daughter are affected. CASE 1: female patient 79-year-old, mother, hearing loss and UC onset at 40-year-old. Audiometric test showed sensorineural bilateral middle hearing loss. Speech audiometric test showed sensorineural deficit. Timpanogram: curves A of Jerger bilateral. Stapedial reflex: absent bilateral. ABR performed reported response at 50 dB ear right, left at 60 dB. Trascient evoked otoacoustic emissions absent bilateral. Vestibular response to caloric is markedly reduced. Case 2: female patient 54 year old, hearing loss and Ulcerative Colitis onset at 40 year old. Audiometric test showed mild sensorineural hearing loss. Speech audiometric test showed sensorineural deficit. Timpanogram: curves A of Jerger bilateral Stapedial reflex: absent bilateral ABR performed reported bilateral response at 50 dB. Trascient evoked otoacoustic emissions absent bilateral. Vestibular response to caloric is reduced and posturographic testing is normal.

A putatively expressed, imprinted and functional mouse pseudogene proposed to regulate its source gene and cause neonatally lethal disease is actually an inactive relic. *T.A. Gray[1], R.D. Nicholls[2]* 1) Wadsworth Ctr, New York State Dept Health, Albany, NY; 2) Dept of Pediatrics, Children's Hospital of Pittsburgh, Pittsburgh, PA.

A recently promoted genome evolution model posits that mammalian pseudogenes can regulate their founding source genes, and it thereby ascribes an important function to junk DNA, an idea that has also gained significant support by the intelligent design community. This model arose from analysis of a serendipitous mouse mutant in which a transgene insertion/deletion caused severe polycystic kidney disease and osteogenesis imperfecta with ~80% neonatal lethality, when inherited paternally (Hirotsune et al. *Nature* 423:91-96, 2003). The authors concluded that the transgene reduced the expression of a nearby robustly transcribed and imprinted pseudogene named *Mkrn1-p1*. This reduction in chromosome 5 imprinted *Mkrn1-p1* transcripts was proposed to destabilize the cognate chromosome 6 *Mkrn1* source gene mRNA, with a partial reduction in one of two full-length *Mkrn1* isoforms leading to the imprinted phenotype. In contrast to this and two ensuing evolutionary claims (Podlaha & Zhang, *Mol Biol Evol* 21, 2202-9, 2004; Kaneko et al. *Genetics* 172, 2421-9, 2006), we find that 5 *Mkrn1-p1* is fully methylated on both alleles, a pattern indicative of silenced chromatin, and that *Mkrn1-p1* is not transcribed and therefore cannot stabilize *Mkrn1* transcripts in *trans*. Moreover, the short mRNA erroneously attributed to *Mkrn1-p1* is a *Mkrn1* isoform. Additionally, *Mkrn1* is not imprinted and the 5 promoter is fully unmethylated. Finally, mice that produce 50% or 0% of normal *Mkrn1* mRNA amounts due to a gene-trap mutation show none of the phenotypes claimed for mice having ~ 75% normal levels of *Mkrn1* mRNA. These data annul previous assertions that *Mkrn1-p1* is imprinted and that either it or its source *Mkrn1* gene relates to the original imprinted transgene phenotype. We conclude that the *Mkrn1-p1* pseudogene is a typical evolutionary relic, negating the pseudogene *trans* regulation model.

Beckwith-Wiedemann syndrome: report of a new case. *G.Juarez Garcia[1], L. Hernandez Gomez[2], L. Acosta Ramos[3], D.O. Gomez Torres[4], T. Gomez Torres[4]* 1) Neuropsicologia Infantil, Instituto Nacional de Rehabilitacion, Mexico, DF; 2) Servicio de Audiologia, Instituto Nacional de Rehabilitacion, Mexico, DF; 3) Servicio de Genetica, Instituto Nacional de Rehabilitacion, Mexico, DF; 4) Depto Investigación, Instituto Nacional de Rehabilitacion, Mexico, DF.

Beckwith-Wiedemann syndrome (BWS) is a common genetic overgrowth syndrome that is associated with visceromegaly, macroglossia, abdominal wall defects, pre and postnatal overgrowth, neonatal hypoglycemia, exomphalos, macroglossia, and gigantism, are considered the characteristic diagnostic triad of findings; due to this it , earlobe creases and pits,facial nevus flammeus,and prominent eyes with infraorbital creases,is also known as EMG-syndrome. Recognition of BWS is important because of the associated risk for development of embryonal neoplasms affecting abdominal organs, the need for prompt treatment of neonatal hypoglycemia, and for purposes of genetic counseling. Overall, intelligence is usually normal, although BWS has previously been reported in association with mild to moderate mental deficiency (due to hypoglycemic episodes or to subtle cytogenetic alterations) Although most cases appear to be sporadic (85%). Present autosomal dominant inheritance of BWS, with reduced penetrance and variable expressivity that may relate to the effects of genomic imprinting. Families with more than one relative affected, have been reported, and linkage studies in these familial cases have located the gene for BWS on the chromosome 11 (11p15.5). It is estimated the BWS occurs in between one in 14 000 births, with approximately equal incidence in males and females. We present a new case of Beckwith-Wiedemann in a female, mexican 2 years old child, that presents inconvenience of language, characterized by being found to level of dissyllables, basing its communication with signs and carrying al object desired. Two maternal cousins with congenital tumors. Geste product I, both parents of 20 years old, obtained pretermino. She presented onfalocele, macroglosia, visceromegaly, palate cleft and cardiac puff. EEG normal. ABR performed reported response at 30 dB ear right, left at 40 dB. Two years average mental age.

Screening ARX Gene in Iranian Families with X-linked Mental Retardation. *S. S. Abedini[1], K. Kahrizi[1], S. Esmaeeli Nieh1[1], F. Behjati[1], A. Aghajani[1], S. Ghasemi Firoozabadi1[1], L. Abbasi[2], S. Banihashemi[1], A. Tzchach[2], H.H. Ropers[2], H. Najmabadi[1]* 1) University of Social Welfare and Rehabilitation Sciences , Genetics Research Center, Tehran, Tehran, Iran; 2) Max Planck Institute for Molecular Genetics, Berlin, Germany.

The recently identified gene ARX (Aristalles-Related Homeobox), codes the ARX protein, an important transcription factor that belongs to one of the three largest classes of homeoproteins, the paired (Prd) class. Several mutations have been identified in ARX gene, which are responsible for a wide spectrum of phenotypes, including both nonsyndromic X-linked mental retardation (MRX), and syndromic (MRXS) forms such as X-linked lissencephaly with abnormal genitalia (XLAG), Partington syndrome and X-linked infantile spasm syndrome. The most common mutation in ARX gene identified is a duplication 24 bp (24 bp dup) in exon 2. This duplication leads to an expansion of the second polyalanine tract of ARX protein. The aim of this study is to obtain the relative prevalence of ARX mutations in the Iranian population. We have collected 65 probands from 65 families with two or more individuals with X-linked mental retardation (XLMR) inheritance pattern after obtaining informed consent form from the parents of probands. Each propband was subjected for molecular FMR1 gene mutation and karyotyping .The result of these tests for all probands were negative. In the first step these families were screened for the most common form of mutation, 24 bp duplication and if the result of each sample was negative, SSCP (Single Strand Conformation Polymorphisms) analysis performed. Consequently, we identified one family with 24 bp dup. We have also identified numbers of shifts in our families and DNA sequencing is on the way. Hence, we suggest that the molecular analysis of ARX mutations as the second cause of MR should be considered for any male which is negative FMR1 mutation as a part routine diagnostic test.

Kawasaki disease is strongly associated with common ancestral haplotypes of the major histocompatability complex in Australian Caucasoids. *D. Burgner[1], B. Edel[2], M. Odam[1], C. Witt[2], D. Sayer[2], S. Davila[3], M. Hibberd[3], F. Christiansen[2]* 1) School of Paediatrics, University of Western Australia; 2) Dept. of Clinical Immunology and Biochemical, Royal Perth Hospital, Australia; 3) Infectious Disease, Genome Institute of Singapore, Singapore.

BACKGROUND: Kawasaki disease (KD) is the commonest cause of pediatric acquired heart disease and a potential paradigm for identifying common mechanisms in atherosclerosis. Epidemiologic data supports a substantial genetic contribution to KD susceptibility. The Major Histocompatability Complex (MHC) contains over 30 immune genes, including The Human Leucocyte Antigens (HLA) and is a prime candidate region for inflammatory and infectious diseases. HLA data in KD suggest an association with class I, but not class II, regions, but studies are underpowered, admixed and may reflect inaccuracies with older HLA typing methodologies. We analysed the MHC in a carefully defined ethnically-matched Australian population using state-of-the-art molecular methods. METHODS: 70 Australian Caucasoid KD cases and 120 Australian Caucasoid population controls were typed for HLA A, B, C and DR loci using sequence-based methods. Recombinant mapping, using published and private markers within the central MHC, defined the associated genetic regions. RESULTS: KD susceptibility was associated with HLA-B*702 and HLA-DRB1*1501 (OR = 3.2; P=0.007), which lie on the 7.1 ancestral haplotype (AH). Fine-mapping defined a central MHC susceptibility region centromeric to the complement genes and telomeric to HLA-B (OR=9.5; P=0.0016). Protection from KD was associated with HLA-B*4403 and HLA-C*1601, which lie the 44.2 AH (OR = 0.2; P =0.01). DISCUSSION: Susceptibility and protective loci for KD are located within the MHC and are likely to be in linkage disequilibrium (LD) with markers defining common ancestral haplotypes. Further mapping, using genome-wide studies and both case-control and family-based methods, and with trans-racial mapping in areas of high LD, are on-going. Significantly associated KD variants will be assessed in large atherosclerosis cohorts to identify common determinants.

No effect of folic acid and methionine on D4Z4 methylation in facioscapulohumeral muscular dystrophy. *J.C. de Greef[1], E.L. van der Kooi[2], M. Wohlgemuth[2], R.R. Frants[1], R.J.G.P. van Asseldonk[2], H.J. Blom[3], B.G.M. van Engelen[2], S.M. van der Maarel[1], G.W. Padberg[1]* 1) Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands; 2) Neuromuscular Center Nijmegen, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands; 3) Laboratory of Pediatrics and Neurology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands.

Facioscapulohumeral muscular dystrophy (FSHD) is associated with contraction of the polymorphic D4Z4 repeat on chromosome 4qter. In healthy individuals, D4Z4 consists of 11-100 units on both chromosomes, whereas FSHD patients carry one 4q array of 1-10 units. This contraction is associated with significant hypomethylation of the shortened D4Z4 allele. In phenotypic FSHD cases in which patients do not have a contraction of D4Z4 on chromosome 4q, both their D4Z4 alleles show hypomethylation. These findings suggest a central role of D4Z4 hypomethylation in the pathogenesis of FSHD. As DNA methylation and demethylation are reversible processes, DNA methylation levels can potentially be influenced. Folic acid is essential for the synthesis of methione and S-adenosyl methionine (SAM), the common methyl donor required for DNA methylation maintenance. Intervention studies in humans taking folic acid supplements show that DNA hypomethylation, chromosome breaks, and uracil misincorporation are minimized when serum concentration of folate is higher than 34 nmol/l. We therefore performed a pilot study to evaluate the effect of supplemental folic acid and methionine on the methylation level of D4Z4 alleles on chromosome 4qter in peripheral blood lymphocytes (PBLs) of FSHD patients, including phenotypic FSHD patients, and healthy controls in order to decide if a larger clinical trial is warranted. Despite the fact that the recommended serum folate level to minimize DNA hypomethylation was reached in all subjects and total DNA methylation levels increased in the majority of subjects, D4Z4 methylation levels did not change in PBLs of FSHD patients and controls. At present, there is insufficient ground for a larger clinical study on folic acid supplements in FSHD.

SCNN1G variants are associated with systolic blood pressure in the Victorian Family Heart Study. *C.J. Büsst, K.J. Scurrah, J.A. Ellis, S.B. Harrap* Department of Physiology, University of Melbourne, Melbourne , Australia.

The -subunit of the epithelial sodium channel, encoded by SCNN1G, has been implicated in the Mendelian blood pressure diseases Liddles syndrome and pseudohypoaldosteronism type 1 - severe. SCNN1G is located on a region of chromosome 16 that has been linked to systolic blood pressure (SBP) by a number of independent studies, including our Victorian Family Heart Study (VFHS). To assess the impact of SCNN1G variation on SBP in the Caucasian VFHS population, 26 single nucleotide polymorphisms (SNPs) throughout SCNN1G were genotyped in 190 unrelated VFHS subjects selected for high SBP (n=96, SBP: 160.5, SD: 12.7) or low SBP (n=94, SBP: 98.4mmHg, SD: 5.2). Genotyping was carried out using a combination of single nucleotide primer extension and sequencing on the MegaBACE DNA Analysis Platform. Association of individual SNPs was assessed by logistic regression analysis in the statistical package R, with adjustments made for the covariates age, sex and body mass index. Haplotype estimation and association testing was performed within the Haplo.Stats software package. Four intronic SNPs displayed modest individual associations with SBP (P between 0.01 & 0.03), three of which formed a haplotype with evidence of strong association to SBP (global p-value = 0.00006). In summary, we have found evidence that common variants within SCNN1G are associated with SBP in the Victorian population. In order to validate the role of SCNN1G in SBP variation in the general population, it will be necessary to confirm these results in a larger population and to identify the causative functional variant.

Molecular analysis of Tumor Suppressor genes in synchronous and metachronous secondary carcinomas of the upper aerodigestive tract. *R. Birkenhager, W. Maier, G. Ridder, R. Laszig, J. Schipper* Otorhinolaryngology H&N Surg, Univ Freiburg, Freiburg, GermanyDep. ORL&HN Surg, Medical School, University of Freiburg, Killianstrasse 5, D-79106 Freiburg, Germany.

Genomic instability, reflecting the propensity and the susceptibility of the genome to acquire multiple alterations, might be considered a driving force behind multiple carcinogenesis. Head-and-neck cancer (HNC) patients have a risk of 10-15 % of developing second tumors of the upper aerodigestive tract. Due to the identical histological tumor entity of secondary carcinomas, their appearance cannot be explained exclusively by somatic mutations. Therefore an additional genomic predisposition for the development of synchronous and metachronous secondary carcinomas of the upper aerodigestive tract by this patient collective must be postulated. The genetic reason for this appearance of this special type of secondary carcinomas on genomic level has not been analyzed and investigated up to now. This patient collective demonstrates an inimitably homogenous gene pool for the analysis for such genomic predisposition. In our project we investigated a group of clinically consistently characterized patients who developed synchronous or metachronous secondary carcinomas of the mucosa in the upper aerodigestive tract with a SNP array-based analysis. By utilizing the Affymetrix GeneChip Chromosome Copy Number Tool, the allelic imbalance profiles on genomic level of these patients with secondary tumors were generated based on 10 K and 100 K XbaI/HindIII SNP mapping array. With this method it was possible to identify different loss of heterozygosity (LOH) areas specially on chromosome one and six. The aim of our project is to analyze the whole genomic background of these patients for identification of genomic alterations loss of LOH's areas and suppressor genes, responsible for the development of this special kind of secondary tumors in the upper aerodigestive tract.

***POLG1* GENE MUTATIONS ARE NOT A COMMON CAUSE OF SPORADIC ATAXIA IN ITALY.** *C. Cagnoli[1], A. Brussino[1], E. Di Gregorio[1], E. Dragone[2], M. Ferrone[1], S. Padovan[1], N. Migone[1, 2], A. Brusco[1, 2]* 1) Dept Genetics, Biol & Biochem, Univ Torino, Torino, Italy; 2) San Giovanni Battista Hospital, Medical Genetics Unit, Turin, Italy.

Mutations in the *POLG1* gene, encoding the catalytic subunit of the mitochondrial DNA polymerase , have recently been reported in autosomal recessive and sporadic cases with syndromic ataxia. The frequency of the Trp748Ser allele seems to be high among Finnish (1:125), and, due to a common haplotype of ancient origin, it has been suggested to be an important cause of ataxia also in other populations of European descent. We studied 257 consecutive Italian probands referred to our diagnostic center for a SCA testing (227 sporadic, and 30 with a possible familiarity). The screening for Trp748Ser and four other mutations reported in ataxic patients (Ala467Thr, Arg627Trp, His932Tyr, and Gly1051Arg) did not identify any carrier. Our findings suggest that *POLG1* mutations do not represent a major determinant for the pathogenesis of cerebellar ataxias in our population.

Effect of deferiprone on cerebellar iron accumulatio in Friedreich's ataxia. *N. Boddaert[1], K.H. LE QUAN SANG[2], A. Rotig[3], A. LEROY-WILLIG[4], S. GALLET[5], F. BRUNELLE[1], D. SIDI[6], J.C. THALABARD[2], I.Z. CABANTCHIK[7], A. MUNNICH[3]* 1) Dept Radiology, Hosp Necker, Paris, France; 2) Clinical Research Unit, Hôpital Necker-Enfants Malades, 75015 Paris France; 3) Medical Genetic Clinic and Research Unit INSERM 781. Hôpital Necker-Enfants Malades, 75015 Paris France; 4) U2R2M, CNRS UMR 8081, Université Paris Sud, 91405 Orsay, France; 5) 3. Pediatric Unit, Hôpital de Montluçon, 03113 Montluçon Cedex, France; 6) Pediatric Cardiology Unit, Hôpital Necker-Enfants Malades, 75015 Paris France; 7) 4. Department of Biological Chemistry and Charles E. Smith Laboratory of Psychobiology, Institute of Life Sciences, Hebrew University, Jerusalem 91904 Israel.

Friedreichs ataxia results from deficient frataxin levels causing reduced iron-sulphur-cluster formation, respiratory chain deficiency, mitochondrial iron accumulation and ensuing oxidative damage. We aimed at dissipating the allegedly toxic iron in the brain by using an orally active chelator that removes labile cell iron in clinical iron overload. An efficacy-toxicity phase I-II open trial was conducted with deferiprone on 11 adolescents who were already on the antioxidant Idebenone. Compared to age-matched controls, brain magnetic resonance imaging revealed relatively small and irregularly shaped dentate nuclei and significant ($p<0.027$) increase in iron content reflected in proton relaxation rates R2-star. Intake of 20-30mg/kg/d deferiprone for 6 months led to a progressive decrease in R2-star from 18.31.6msec-1 to 15.70.7msec-1 linearly related to the initial iron content ($r=0.90$). The chelation treatment was substantially milder than used for hemosiderosis and seemingly safe in the study period. Patients showed cessation of constipation and incontinence and improvements in manipulative dexterity and speech fluency and reduced signs of neuropathy and ataxic gait in the youngest treated patients. This is the first clinical demonstration of removal of labile iron accumulated in brain areas implicated in neurodegenerative disorders. The modest neurological improvements attained by chelation warrant future controlled prospective studies.

Splicing Sequences Finder: a bioinformatics resource to identify sequences involved in splicing. *C. Beroud, D. Hamroun, S. Tuffery-Giraud, O. Leroy, G. Collod-Beroud, M. Claustres* Laboratoire de génétique moléculaire, IURC, Institut Universitaire de Recherche Clinique UFR Médecine Site NORD UPM/IURC Montpellier, France.

Today thousands of intronic variations are identified yearly in molecular diagnostic laboratories. To assess the pathogenicity of these mutations, their impact on the well-characterized motifs acceptor and donor splice sites is usually evaluated. It is also important to identify branch point sequences (BP) whose key role in splicing can also explain the pathogenicity of some intronic mutations. Parallel to these motifs, which allow the cellular machinery to recognize an exon, it has recently been shown that exonic and intronic sequences are able to reinforce or inhibit these signals. These sequences are named ESE and ESS (exonic splicing enhancer or silencer), ISE and ISS (intronic splicing enhancer or silencer). Their identification was mainly based on bioinformatics and functional analyses. Therefore, many matrices are today available to predict these auxiliary-splicing sequences and to evaluate the pathogenic impact of not only intronic but also exonic missense mutations. The diversity of bioinformatics resources necessary to an exhaustive evaluation of these various elements as well as the need for new algorithms led us to develop the Splicing Sequences Finder (SSF) tool, which allows the identification of the various splicing elements from a crude sequence. To identify enhancer and silencer motifs various matrices (Cartegni and Krainer, Fairbrother and Burge, Zang and Chasing, Sironi and Pozzoli and Wang and Burge) were used. We also developed a specific algorithm to identify BP sequences. It was confronted to all mutations known to involve a BP sequence and was found reliable in all cases. The SSF tool could give valuable information for molecular diagnostic laboratories to evaluate the pathogenicity of a mutation. It can also be used to identify potential targets for antisens oligonucleotides to silence ESE or BP and therefore lead to exon skipping. This tool is freely accessible at: http://www.umd.be/SSF.

Mutations in the ND5 subunit of complex I of the mtDNA are a frequent cause of OXPHOS disease. *M.J. Blok[1], L. Spruijt[2], I.F.M. DeCoo[3], K. Schoonderwoerd[3], A. Hendrickx[1], H.J.M. Smeets[1,2]* 1) Clinical genetics, Academic Hospital Maastricht, Maastricht, Netherlands; 2) Department of Genetics and Cell Biology and Research Institute Growth & Development (GROW), University of Maastricht, the Netherlands; 3) Department of Child Neurology, Department of Clinical Genetics, Erasmus Medical Centre Rotterdam, the Netherlands.

Objective: To investigate if regions in the mtDNA are preferentially mutated in patients with OXPHOS disease. Background: Mutation detection in the mitochondrial genome is usually limited to common mutations and the tRNA genes. However, mutations in other mtDNA regions can be an important cause of OXPHOS disease as well. **Methods**: Screening of the mtDNA for heteroplasmic mutations was performed by DHPLC-analysis of 116 patients with OXPHOS disease but without the common mtDNA mutations. Heteroplasmy levels in different tissues were determined using PCR with fluorescently labeled primers and mutation specific restriction enzymes. **Results**: A mtDNA mutation was detected in 15 patients, five of which were present in the ND5 gene. Two mutations were new and two were known, one of which was found twice. The novel mtDNA point mutation, 12622G>A, was identified in three brothers, all with infantile encephalopathy (Leigh syndrome) fatal within the first 15 days of life. The other novel point mutation, 13511A>T, occurred in a patient with a Leigh-like syndrome. The known 13513G>A mutation, associated with MELAS and MELAS/Leigh/Leber hereditary optic neuropathy overlap syndrome, was found in a relative low percentage in two patients from two different families, one with a MELAS/Leigh and one with a MELAS/CPEO phenotype. The 13042G>A mutation, once detected in a patient with a MELAS/MERRF phenotype, was now found in a patient with a Leigh-like phenotype. **Conclusions**: Mutation screening of the ND5 gene is advised for routine diagnostics of patients with OXPHOS disease, especially MELAS- and Leigh(-like) patients.

Clinic and genetic study in a family with a clinical picture of pantothenate kinase-associated neurodegeneration.
I.C. Ciro Candiano, F. Annesi, S. Carrideo, E.V. De Marco, D. Civitelli, P. Tarantino, F.E. Rocca, M. Caracciolo, G. Provenzano, G. Annesi Inst Neurological Sciences, National Research Council, Mangone, Cosenza, Italy.

Pantothenate kinase-associated neurodegeneration (PKAN) is a disorder characterized by dystonia, parkinsonism, and iron accumulation in the brain. Many patients with PKAN have mutations in the gene encoding pantothenate kinase2 (PANK2) and a specific MRI pattern of hyperintensity within the hypointense medial globus pallidus (eyes of the tiger). Abnormal accumulation of iron in the brain is detected also in other neurodegenerative diseases such as neuroferritinopathy, characterized by extrapyramidal symptoms and low serum ferritin levels. Neuroferritinopathy is associated with mutations in the ferritin light polypeptide (FTL) gene whereas mutations in the ferritin heavy polypeptide (FTH1) gene are associated with a disorder presenting with iron overload. In this study, we performed a molecular study of the PANK2, FTL and FTH genes in a family with PKAN classic phenotype. The patient originated from Southern Italy. Her parents were healthy and consanguineous. Progression of disease was severe, with appearance of progressive dystonia, rigidity and spasticity of the four limbs. Brain MRI study revealed the presence of the eyes of the tiger. Serum ferritin was 16 ng/mL (N= 20 to 300). The exons of the PANK2, FTL and FTH genes were sequenced on ABI 3130xl. All members of the family were genotyped for 7 microsatellite markers from the PANK2 region (20p12.3-p13); linkage analysis was performed by GeneMapper software. Sequencing analysis of the PANK2, FTL and FTH genes excluded presence of mutations. Microsatellite analysis excluded linkage with PANK2 locus. On the basis of the clinical and neuroimaging features, a diagnosis of classic PKAN was made in our patient but the molecular analysis excluded linkage with this locus. Considering the abnormally low levels of ferritin in the serum of the patient, we supposed a ferritin-related neurodegeneration but the sequences of the FTH1 and FTL genes were found to be normal. These findings suggest a probable involvement of other iron regulatory proteins.

EURExpress, a web-based transcriptome atlas of the developing mouse embryo. *G. Diez-Roux, EURExpress Consortium* Telethon Institute of Genetics and Medicine, Naples, Italy.

Genome-wide expression analyses have a crucial role in functional genomics. RNA in situ hybridization (ISH) provides an accurate spatio-temporal description of the distribution of transcripts at cellular resolution. The EU-funded EURExpress consortium is generating a transcriptome-wide acquisition of expression patterns by means of ISH with non-radioactive probes and using this data to establish a web-linked, interactive digital transcriptome atlas (www.eurexpress.org). An initial phase of the project led to the generation of the Human Chromosome 21 Atlas (Nature 420, 582-586 (2002)). The goal of this 4 year project is to build on this success and generate the expression data of > 20,000 genes on sagittal sections from E14.5 wild type murine embryos. Since its establishment in 2005, EURExpress has generated over 3500 expression patterns, which have been thoroughly annotated using a special interface for high-throughput annotation. This interface includes 1420 anatomical structures and correlative trees regarding ontological (embryological) and topological relations allowing advanced queries. The analysis of the data produced so far has determined that 30-40% of genes show a specific/restricted pattern of expression at E14.5. Interestingly, 20% of these are uncharacterized genes of which a large percentage show restricted expression patterns in organs such as the central nervous system, ear, eye, skin, liver, skeletal muscle, mesenchyme and salivary glands. Among the high priority genes, we analyzed the expression pattern of 150 murine counterparts of genes located within the ENCODE regions, which will allow determining potential expression clustering and /or long distance tissue specific enhancers or promoters. To gain maximum leverage from the transcriptome atlas, the database will be linked to other bioinformatics resources. Our future plans include adding other developmental stages and adult tissues as well as analyzing the mutant mice resources being generated by the mutagenesis projects in USA and Europe.

Whole genome scanning for inversion polymorphisms based on population substructural patterns. *L. Deng[1], J. Kang[2], T. Liu[3], Y. Zhang[1], Q. Wang[4], H. Yang[1], C. Zeng[1], BGI HapMap Group* 1) Beijing Genomics Institute, Beijing, Beijing, China; 2) Department of Mathematical Sciences, Tsinghua University, Beijing, China; 3) Institute of Biophysics, Chinese Academy of Sciences, Beijing, China; 4) College of Mathematics, Beijing Normal University, Beijing, China.

Submicroscopic inversion is usually hard to detect, as it is commonly very large at the genomic level and gene structures are often left undisturbed. However, recent reports suggest that such inversions might be widespread in the human genome. Based on the unusual population substructure patterns generated from HapMap data, we developed an algorithm to discover stuctural polymorphisms. Here we present the application of this statistical method in the identification of inversion polymorphisms in human genome and the distinctive genetic structures of the rearrangement regions. Scanning of HapMap genotyping data on autosomes revealed numerous candidates of large scale inversion polymorphisms, covering more than 1% of human genome. Among them, several previously reported inversions also shown as strong signals of polymorphism. Further population genetic analysis revealed great differentiation and non-equilibrium haplotype distributions among geographic populations (African, Asian and European) in these candidate regions. Particularly, at our previously identified three hyplotype clades in the 3.8 Mb inversion region of 8p23, allele sharing distance (ASD) analysis showed that most of YRI haplotypes located in Clade_1 and most CHB and JPT haplotypes were in Clade_3. In CEU samples, however, divergent population subgroups were resolved whose haplotypes distributed in both Clade_2 and Clade_3. As determined by our statistical computation and confirmed by fluorescent in situ hybridization (FISH), Clade_2 are non-inverted chromosomal alleles, and Clade_3 corresponds to inverted structure. Taken together, our results provide a practical method to resolve structural polymorphisms on human genome and a panoramic view on haplotype structural and features of the candidate regions, including the inversion at 8p23.

Maternal serum screening in Abruzzo using triple test. *P. Guanciali Franchi[1,2], I. Iezzi[1], C. Palka[1], G. Calabrese[1,2,3], E. Morizio[2], M. Di Zio[2], G. Castriota[2], C. Nuzzi[1], G. Palka[1,2,3]* 1) Sci Biomed, Univ Chieti, Chieti, Chieti, Italy; 2) U.O. Genetica Umana, Pescara Hospital, Pescara, Italy; 3) CESI, Center for Aging, D'Annunzio Foundation, Chieti, Italy.

From January 1995 to December 2005, 17909 pregnant women were submitted to triple test screen in Abruzzo, a region in the middle of Italy. Women with age 35 yrs were 3617 (21%) while those ageing <35 yrs were 14302 (79%). Mean age was 30.6 yrs. Complessively 1295 women had a positive triple test for Down syndrome (DS) (7.2%) and 211 for neural tube defects (NTD) (1.18%). After genetic counseling, all the women with positive triple test for DS underwent to amniocentesis while those positive for NTD were screened by ultrasonographic scan at 20 weeks of gestation. Sixty three pregnant women had a fetus with abnormal karyotype consisting in 32 DS, 12 trisomy 18, 8 Turner syndromes, one triploidy, one partial trisomy 10p, one i(18p), one complex karyotype with inv(12) and der(16)t(12;16)mat, and one 46,XX male resulted from the SRY gene translocation onto a Xp chromosome. Among the women at risk for NTD, ultrasonographic scan showed 5 NTD and one abdominal wall defect. As a whole, the total detection rate for chromosome abnormalities was 88%, specifically for DS 80%, false positive cases were 1232 (6.8%), while false negative 8 (0.04%). The detection rate for NTD was 100%. Finally, 490 women showed a negative triple test and isolated hCG 2.5MoM. Forty nine of these women, all ageing <35yrs, presented a sonographic (soft) marker consisting in pielectasy, short femour, cardiopathy, cystic hygroma, or duodenal atresia. In 9 of these patients (18%), amniocentesis disclosed a chromosome abnormality consisting in 6 DS, and 3 Turner syndromes.

Linkage disequilibrium between polymorphisms at the IRF6 locus and nonsyndromic cleft lip with or without cleft palate in the Italian population. *P. Carinci[1], L. Scapoli[1], A. Palmieri[1], M. Martinelli[1], F. Pezzetti[1], F. Carinci[2]*
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Cleft lip with or without cleft palate (CL/P) is one of the commonest birth defects but its etiology is largely unknown. Very likely both genetic and environmental factors contribute to this malformation. Mutations in interferon regulatory factor 6 (IRF6) gene have been shown to be the cause of Van der Woudes syndrome, a dominant disorder that includes CL/P as a common feature. Recently, it has been reported that genetic polymorphisms at the IRF6 locus are associated with nonsyndromic CL/P with stronger signals in Asian and South American populations. We investigated four markers spanning the IRF6 locus using the transmission disequilibrium test. A sample of 219 patient/parents triads from Italy were enrolled for the study. Strong evidence of linkage disequilibrium was found between markers and disease in both single alleles (P=.002 at rs2235375) and haplotype analyses (P=0.0005). These findings confirm the contribution of IRF6 in the etiology of nonsyndromic CL/P and strongly support its involvement in populations with European ancestry.

Association study of the locus 19q13 in the Cystic Fibrosis liver disease. *S. Gambardella[1], M.R. D'Apice[1], S. Ciacci[1], S. Petrocchi[1], T. Santostasi[2], L. Narzi[3], S. Quattrucci[3], G. Castaldo[4], F. Salvatore[4], C. Colombo[5], G. Novelli[1]*
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Cystic fibrosis (CF; #219700) is a disorder caused by mutations in the CFTR gene that encodes a chloride conducting channel expressed in the apical plasma membrane of the epithelial cells. The disease is characterized by a wide variability of clinical expressions which include pancreatic insufficiency, lung disease, hepatic manifestations, meconium ileus (MI) and male infertility. Even though CF is principally a monogenic disease, its wide phenotypic spectrum is influenced by secondary genetic factors, that can modulate the severity. Previous results through trasmission disequilibrium test in CF sibpairs, demonstrated the presence of a genetic modifier locus, CFM1 (Cystic Fibrosis Modifier 1) for MI on chromosome 19q13. In this study, we performed an association analysis by three microsatellite markers to test the role of this region as possible modifier of the hepatic involvement in Cystic Fibrosis. We genotyped a cohort of 90 CF patients (E) with hepatic involvement and pancreatic insufficiency, carrying two class I mutations, homozygous F508del, or compound heterozigous for a class I allele and F508del. We selected as control subject 75 CF patients with pancreatic insufficiency without hepatic involvement (CFC) and 100 unaffected individulas (C). We scanned the region on 19q13 by microsatellite D19S903- D19S219-D19S112, spanning 1.1 Mb. There was no particular allele or haplotype associated with the hepatic involvement compared with CFC and control subjects. These rules out the possibility that genes mapping in this region act as modifier genes of the CF hepatic manifestation.

Linkage study in three Italian families with autosomal dominant nocturnal frontal lobe epilepsy. *S. Carrideo[1], E.V. De Marco[1], A. Gambardella[2], A. Labate[2], F. Annesi[1], I.C. Cirò Candiano[1], P. Tarantino[1], F.E. Rocca[1], D. Civitelli[1], M. Caracciolo[1], G. Annesi[1]* 1) Inst Neurological Sciences, CNR, Mangone, Cosenza, Italy; 2) Institute of Neurology, University Magna Graecia, Catanzaro, Italy.

Mutations in the genes encoding the 4 and 2 subunits of the neuronal nicotinic acetylcholine receptor (nAChR) play a causative role in autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE). A third ADNFLE locus (15q24) has been identified but mutations linked to the disease have not yet been found. Recently, variations in the promoter of the corticotropic-releasing hormone gene (CRH) were reported in patients with NFLE. However, only a minority of ADNFLE families carry a mutation of these genes. Here, we investigated whether nine brain-expressed genes, encoding distinct nAChR subunits, or CRH gene are associated with the disease in three ADNFLE families from Southern Italy. We performed a linkage study on 11 affected and 29 unaffected individuals of these families with microsatellite markers and RFLPs encompassing the chromosome localization of the nAChR subunits and the CRH genes. We examined the following chromosome regions: 1q21 (CHRNB2), 8p21 (CHRNA2), 8p11.2 (CHRNA6, CHRNB3), 15q14 (CHRNA7), 15q24 (CHRNA5/A3/B4), 20q13.2 (CHRNA4), 8q13 (CRH). Two point and multipoint linkage analysis were performed by LINKAGE and GENEHUNTER. In two families, most markers were not informative for CHRNB2 and CRH, respectively. Mutational analysis of these two genes were therefore performed in the probands. Negative LOD scores values (less than -2) at theta 0 were obtained for all the informative markers related to nAChR subunits or CRH genes. No mutation of CHRNB2 and CRH genes was detected in the probands of the families with non informative markers. The results of this study illustrated no association between nAChR subunits or CRH genes and ADNFLE in our families. It is therefore reasonable to hypothesize that further genes are involved in the disease. These results confirm the considerable genetic heterogeneity of ADNFLE, despite the quite homogeneous clinical picture. Supported by FIRB-MIUR, year 2001 RBNEO1XP4.

Impact of *DNAI1* and *DNAH5* pathogenic mutations in a cohort of unselected PCD patients. *M. Failly[1], A. Saitta[1], M. Alvarez[1], C. Ramos[1], L. Gilbert[1], S.E. Antonarakis[1,2], L. Bartoloni[1], J-L. Blouin[2]* 1) Department of Genetic Medicine and Development, University of Geneva Medical School, Switzerland; 2) Medical Genetics, University Hospitals of Geneva, Switzerland.

Primary Ciliary Dyskinesia (PCD) is a rare congenital disorder (affecting 1/20'000) mainly inherited on autosomal recessive mode and caused by ciliary/flagellar dysmotility due to ultrastructural/functional defects of the axoneme. Symptoms are respiratory infections, with chronic sinusitis, nasal polyps, bronchiectasis, and male subfertility. In addition, 50% of cases show a situs inversus (Kartagener syndrome). To date, 4 genes have been found to be mutated in PCD: *DNAI1*, *DNAH5*, *DNAH11*, and *RPGR* in a X-linked form always associated to Retinis Pigmentosa. In order to determine the impact of the known disease genes, we screened for mutations in 96 unrelated affected individuals without any preselection of phenotype subtype. All exons and splicing sites of *DNAI1* were investigated by DHPLC and sequencing. In the *DNAI1* gene we found 1 novel missense variant (E174K) and 2 previously described mutations (IVS1+3insT, c.1703C>G). The impact of *DNAI1* mutations in our large cohort of unselected patients represents only 4.2%. We have also screened exons with previously reported mutations as well as exons coding for the 4 P-loop domains of gene *DNAH5* (15 of the 79 exons). One published mutation S2264N, in P-loop 2 domain, has been identified in heterozygosity. In addition a new heterozygous non-sense (K1853X) was found in exon 34 in one patient. The mutation search in these *DNAH5* exons among those coding for important functional domains of this heavy chain dynein is not compatible with a large impact of this gene in PCD patients without subphenotype selection. We conclude from our preliminary results that mutations in the *DNAI1* and *DNAH5* genes are not the main contributors of PCD. Additional PCD genes, remain to be characterized.

The Spine and the Craniocervical Junction in Ehlers-Danlos Syndrome. *C.A. Francomano[1,2], R. Razza[3], B. Griswold[2], P. Bolognese[4], T. Milhorat[4], N.B. McDonnell[2]* 1) Greater Baltimore Med Ctr, Harvey Inst Human Gen, Baltimore, MD; 2) National Institute on Aging, Baltimore, MD; 3) Harbor Hospital, Baltimore, MD; 4) The Chiari Institute, NS-LIJHS, NY.

The Ehlers-Danlos syndromes (EDS) are a heterogeneous group of hereditary disorders of connective tissue characterized by joint, skin and vascular abnormalities. Joint laxity and dislocations are commonly recognized musculoskeletal features of EDS. However, the frequency of spinal involvement, including degenerative disc disease and dural ectasia, is unknown. Previous anectodal reports have described spinal abnormalities including spondylolisthesis, lumbar platyspondyly and scoliosis. We assessed images of the lumbar spine by Magnetic Resonance Imaging (MRI) in 58 consecutive patients with a diagnosis of EDS. The cohort included patients with hypermobile, classical and vascular forms of EDS, and the age range was 12-67. The abnormalities observed included degenerative disc disease, disc herniation, facet arthrosis, dural ectasia and dural cysts. The abnormalities were observed in all age groups, including patients as young as 15 years of age. All patients over the age of 45 had significant disc disease. 45/58 patients were found to have some degree of disc disease. 15/58 patients had dural ectasia. Degenerative disc disease was highly correlated with the presence of back pain. During the course of the clinical evaluation, it was noted that twelve patients in the cohort had a previous diagnosis of Arnold Chiari I malformation, or received the diagnosis during longitudinal follow-up. Two patients had a history of previous decompression surgery; four others were operated after enrollment in the study. The remaining patients were treated conservatively. Several additional patients had evidence of a retroflexed odontoid or pannus formation around the odontoid that was felt to contribute to complaints of head and neck pain. The results indicate that pathology in the spine and the craniocervical junction is a frequent cause of morbidity in EDS. The data suggest that it is advisable to pursue appropriate radiological evaluations in the presence of back, head and neck pain in this group of patients.

Association of the *FCRL3* gene with rheumatoid arthritis: a further example of population specificity of disease loci? *A. Barton, S. Eyre, J. Worthington* ARC-EU, The University of Manchester, Manchester, United Kingdom.

Background: Association of a functional promoter polymorphism mapping to the Fc receptor-like 3 (*FCRL3*) gene has recently been reported and replicated with rheumatoid arthritis (RA) in independent Japanese populations, but not in a population from North America.

Aim: To investigate association of the *FCRL3* gene with RA in UK subjects.

Methods: DNA was available from 1065 patients with RA and 2073 population controls from the UK. Four single nucleotide polymorphism (SNP) markers (FCRL3-169C/T (fclr3_3, rs7528684), fclr3_4 (rs11264799), fclr3_5 (rs945635), fclr3_6 (rs3761959)) all previously associated with RA in a Japanese population were genotyped in 761 RA samples and 484 controls. In the remaining samples, only the putative disease causal polymorphism, FCRL3-169C/T, was tested. Genotyping was performed using either the Sequenom MassArray iPlex platform (www.Sequenom.co.uk) or a 5 Allelic discrimination assay (Taqman, ABI).

Results: Extensive linkage disequilibrium was present across the promoter SNPs genotyped (r^2 values = 0.60-0.98). Allele frequencies did not differ between RA cases and controls either for the putative disease causal polymorphism (odds ratio FCRL3-169C allele = 0.97 (0.87-1.07), p = 0.51) or for the other SNPs tested. Similarly, no association was detected with RA when stratification by shared epitope carriage or by presence of rheumatoid factor was undertaken. Of the 16 possible estimated haplotypes, only 3 existed at a population frequency greater than 1% but no difference in their frequencies were observed between cases and controls (p = 0.97).

Conclusion: This study was powered to detect an effect size of 1.24 or greater for the FCRL3-169C/T functional promoter polymorphism but no evidence for association was detected, suggesting that this gene will not have a substantial effect in determining susceptibility to RA in populations of Northern European descent.

Exon resequencing and mutation detection in syndromic oesophageal atresia. *A. Coffey[1], E. Howard[1], K. McLay[1], A. Dunham[1], S. Hunt[1], S. Leonard[1], J. Burton[1], J. Rogers[1], C. Shaw-Smith[2]* 1) Human Genetics, Sanger Institute, Cambridge, United Kingdom; 2) Department of Medical Genetics, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK.

Oesophageal atresia and/or tracheo-oesophageal fistula are common malformations occurring in approximately 1 in 3500 births. In around half of cases (syndromic oesophageal atresia), there are other associated anomalies, with cardiac malformations being the most common. Oesophageal atresia is regarded as a sporadic entity with a low recurrence risk, but there are well-defined instances of this malformation where genetic factors clearly are important. This is highlighted by the recent identification of no fewer than four separate genes with a role in its aetiology. The genes identified to date are the genes for Feingold syndrome (N-MYC), anophthalmia-oesophageal-genital (AEG) syndrome (SOX2), CHARGE syndrome (CHD7) and X-linked Fanconi anaemia (FANCB). We are actively collecting DNA samples from patients with syndromic forms of oesophageal atresia and resequencing all exons from a set of 25 candidate genes using a stratified approach. These genes have been sequenced in a panel of 48 unrelated Caucasian samples and novel single nucleotide polymorphisms identified, approximately 40% of which are rare. In the first phase of the stratified approach to mutation detection, we have sequenced exons from 4 genes in the patient samples collected to date. The results of this analysis will be presented.

Molecular genetic basis of the disease in a Spanish family with Usher syndrome type 1 and non-syndromic deafness. *E. Aller[1,2], T. Jaijo[1], M. Beneyto[1], C. Vilela[3], C. Nájera[2], C. Ayuso[4], J.M. Millán[1]* 1) Unidad de Genética, Hospital La Fe, Valencia, Spain; 2) Departamento de Genética, Universidad de Valencia, Valencia, Spain; 3) Sección de Neurofisiología, Hospital La Fe, Valencia, Spain; 4) Departamento de Genética, Fundación Jiménez Díaz, Madrid, Spain.

INTRODUCTION: Usher syndrome type I (USH1) is an autosomal recessive hereditary disease characterized by the association of retinitis pigmentosa (RP), congenital severe-profound sensorineural deafness and vestibular dysfunction. Up to date, five genes have been identified as disease-causing for USH1, being the MYO7A (corresponding to USH1B), the gene responsible in most of cases (about 50%). Mutations located in this gene have also been shown to be responsible for some non-syndromic deafness cases, in both dominant (DFNA11) and recessive (DFNB2) forms. AIMS: The aim of the present work is to elucidate the genetic basis of the disease in a Spanish family with some affected members displaying USH1 and some others displaying non-syndromic sensorineural deafness. PATIENTS and METHODS: The paternal family showed non-syndromic deafness compatible with an autosomal dominant hereditary pattern. The maternal family displayed USH1. The two affected sibs of this couple suffered from USH1. DNA samples were obtained from all family members. The maternal family and the affected sibs were screened for mutations in MYO7A gene by SSCPs analysis. Coding regions of MYO7A gene were directly sequenced in the father. The father was also screened for the most prevalent deafness-causing mutations in the Spanish population. RESULTS: Affected sibs were compound heterozygous for 2 MYO7A mutations: (p.Q821X/c.9_10dupGATT). The mother was homozygous for p.Q821X. The father was heterozygous for c.9_10insGATT. A second mutation in the father was not found after the direct MYO7A sequencing. None of the deafness-causing mutations studied were detected in the father. DISCUSSION: USH1 in this family is caused by mutations in MYO7A. The genetic basis of deafness in paternal family is still unclear. The haplotype study of paternal family members will be useful to elucidate this question.

Truncation of Cernunnos/XLF in a patient with polymicrogyria. *V. Cantagrel[1], A-M. Lossi[1], S. Lisgo[2], C. Missirian[1,3], A. Borges[1], N. Philip[1,3], C. Fernandez[4], C. Cardoso[1], D. Figarella-Branger[4], A. Moncla[1,3], S. Lindsay[2], W.B. Dobyns[5], L. Villard[1]* 1) INSERM U491, La Timone, Marseille, France; 2) Institute of Human Genetics, International Centre for life, Newcastle, UK; 3) Departement de genetique medicale, La Timone, Marseille, France; 4) Biopathologie nerveuse et musculaire, La Timone, Marseille, France; 5) Departements of human genetics, Neurology and pediatrics, Chicago, IL, USA.

Polymicrogyria (PMG) is a common malformation of the human cerebral cortex for which both acquired and genetic causes are known. Although genetic heterogeneity is documented, only one gene is currently known to cause isolated PMG. In order to clone new genes involved in this type of cerebral malformation, we studied a fetus presenting a defect of cortical organization consisting of a polymicrogyric cortex and neuronal heterotopia within the white matter. Karyotype analysis revealed that the fetus was carrier of a balanced, de novo, chromosomal translocation t(2;7)(q35;p22). Cloning and sequencing of the two translocation breakpoints reveals that the chromosomal rearrangement disrupts the coding region of a single gene, called Cernunnos or XLF, in 2q35. The Cernunnos gene was recently identified as being responsible for autosomal recessive immunodeficiency with microcephaly and as a member of the NHEJ pathway. Using quantitative PCR experiments, we show that a truncated transcript is expressed in the polymicrogyric patient cells suggesting a potential dominant negative effect possibly leading to a different phenotype. We performed in situ hybridization on human embryos and show that the Cernunnos transcript is preferentially expressed in the telencephalic ventricular and subventricular zones, consistent with the phenotype of the affected individual. In the human adult central nervous system, Cernunnos is mainly expressed in the cerebral cortex and in the cerebellum. The association of polymicrogyia with the disruption of its transcript suggests that, in addition to its recently uncovered function in the development and maturation of the adaptative immune system, the Cernunnos protein may also play a role during development of the human cerebral cortex.

Genetic mapping for *RET*-dependent modifiers in Hirschsprung disease (HSCR). *P.Y. Fong[1], M. Garcia-Barcelo[1,2], P. Sham[1,3], P. Ng[1], C. Lau[1], M.T. So[2], W. Mak[1], P. Tam[1,2]* 1) Genome Research Centre, The University of Hong Kong, Hong Kong SAR, China; 2) Department of Surgery, The University of Hong Kong, Hong Kong SAR, China; 3) Institute of Psychiatry, Denmark Hill, London SE5 8AF, UK.

Hirschsprung disease (HSCR, colon aganglionosis) is a non-mendelian, multifactorial, congenital disorder. Mutations have been identified on several genes, and show incomplete penetrance. Although, a high risk common variant has been found in the *RET* gene (main HSCR gene), the low penetrance of this *RET* variant is not sufficient to explain the disorder. This suggests that additional loci are required in the total susceptibility of the disease. A genome scan study in families identified 3p21 and 19q12 loci as *RET*-dependent modifiers. In this study, we conducted a high density region-wide association study on 3p21. To enhance the cost effectiveness of our study, we refined our study to a gene-rich region, which is also in high linkage disequilibrium (LD). In addition, we selected tag SNPs based on a clustering algorithm with data from the International HapMap Project. This tag SNPs were genotyped in 172 cases, 161 parents and 153 unrelated controls. We performed standard allelic and haplotype association analyses of the case-control and trio data, as well as correlational analyses between alleles and the high risk *RET* variant, as a way of examining for epistasis. Our study demonstrates a systematic approach to map susceptibility loci in multifactorial disease.

Elevated levels of Low Density Lipoprotein are frequently seen in patients with Ehlers Danlos Syndromes. *M. Burchett[1], A. Gustafson[1], B. Griswold[1], N.B. McDonnell[1], C.A. Francomano[1,2]* 1) National Inst on Aging, Baltimore, MD; 2) Harvey Inst Hum Genetics, GBMC, Baltimore, MD.

The Ehlers-Danlos syndromes (EDS) are a heterogeneous group of hereditary disorders of connective tissue characterized by joint, skin and vascular abnormalities. There have been no previous reports on abnormalities of cardiovascular risk biomarkers in patients with EDS. Complete lipid profiles as well as fibrinogen and C-Reactive protein (CRP) levels were obtained on 63 consecutive patients, ages 12-61, with a diagnosis of EDS enrolled in the National Institutes of Aging protocol 2003-086 entitled Clinical and Molecular Manifestations of Heritable Disorders of Connective Tissue. There were 50 females and 13 males in the cohort. An elevated low density protein (LDL) was seen in 37/50 females, and in 10/13 males, whereas elevated total cholesterol was seen in 7/50 females and 3/13 males. Only three persons in the cohort had a BMI > 30, suggesting that obesity was not a pervasive cause of dyslipidemia in this group of patients. Elevated triglycerides were seen in 9 subjects. There were 7 females with elevated CRP and 5 with elevated fibrinogen. The female cohort was broken down to age groups in line with National Health and Nutrition Examination Survey (NHANES III) and age specific comparisons were performed using age specific national averages for the LDL profiles. All age groups in the EDS female cohort showed a skewed distribution pattern of LDL values. The skewed pattern was most remarkable in the younger age groups: In the 12-44 age range, 50% of the patients had LDLs in the greater than 75th centile. The mean LDL for females over 20 was 128 mg/dL, which plots at 70% percentile of the national mean. These data suggest that persons with EDS are at higher than population risk for having an elevated LDL. We suggest that patients with the diagnosis be screened with a complete lipid profile starting in teenage years. The etiology and prognosis for isolated elevated LDL without elevated total cholesterol in EDS patients is unclear. Further studies, including pedigree analysis, are necessary to determine if increased cardiovascular morbidity and/or mortality segregate with EDS.

Collection of human genome variation: The Human Variome Project? *R. Cotton, Collaborators* Dir, Genomic Disorders Res Ctr, St. Vincent's Hospital, Fitzroy, Australia.

Mutation is estimated to affect 60% of humans directly in a lifetime, excluding cancer, members of the extended family and carers, representing billions worldwide. However, little formal attention has been given to collecting human variation causing disease and potentially causing disease.

Collection of inherited disease began with Victor McKusick, later collecting mutations in each disease www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM. In parallel, many individuals began collecting mutations in the genes of clinical and research interest. David Cooper began collecting mutations in genes to see which mutational change was predominant www.hgmd.org/.

As there was no systematic or structured effort to collect mutations, a 1994 Montreal meeting, of a group of the worlds prominent geneticists resolved that collection and curation of mutation data is best done by a federation of LSDB curators, each experts in their gene rather than informatics/database experts centrally. This led to the formation of the HUGO-MDI and subsequently, the Human Genome Variation Society (www.hgvs.org) with Human Mutation as its society journal.

Enormous progress has been made by members of the Society and others despite limited funding. Outcomes include recommendations on nomenclature of mutations, quality control of mutation data, entry form for mutation databases, recommended content of mutation databases, software for LSDBs, a collection facility for mutations (the WayStation www.centralmutations.org), twice yearly scientific meetings, comprehensive listing of LSDBs etc. The SNP community has made more progress due to good funding, but there is still much to be done.

The projects nature makes it difficult to obtain funding for this reason, we have attempted to obtain a focus for the effort by initiating The Human Variome Project Meeting obtaining the World Health Organizations co-sponsorship for this meeting in Melbourne, June 2006. Progress, problems, plans and the meeting outcome will be outlined.

Polymorphisms In Cell Cycle Genes And Predisposition To Ovarian Cancer. *S. Gayther[1], H. Song[2], S. Ramus[1], A. Whittemore[3], S. Krüger Kjær[4], P. Pharoah[2], Ovarian Cancer Association Consortium* 1) Dept Gyn. Oncology, Windeyer Inst, London, United Kingdom; 2) Strangeways Research Laboratories, Cambridge, United Kingdom; 3) Stanford University School of Medicine, Stanford, USA; 4) Danish Cancer Society, Copenhagen, Denmark.

High-risk susceptibility genes explain less than 30% of the excess risk of familial ovarian cancer. Other ovarian cancer susceptible gene are likely to exist. We have used a SNP tagging approach to evaluate common variants in 13 genes involved in cell cycle control - CCND1, CCND2, CCND3, CCNE1, CDK2, CDK4, CDK6, CDKN1A, CDKN1B, CDKN2A, CDKN2B, CDKN2C, and CDKN2D - and risks of invasive epithelial ovarian cancer. We used a two-stage, multi-centre, case-control study. SNPs that tag common variation in these genes were initially genotyped in three studies from UK, USA, and Denmark (~1500 cases and 2500 controls). Seven other studies from Australia, Poland, and USA (~2500 cases and ~3500 controls) were used for replication of the most significant results. Genotype frequencies in cases and controls, were compared using a logistic regression model. In the first stage we genotyped 87 SNPs in 13 genes. Eight SNPs in 4 genes were associated with P greater than 0.05. The five most significant SNPs were then genotyped in the second stage. After adjusting for 5 hypotheses tested no SNP was significantly associated with disease (most significant unadjusted P = 0.045 for CDKN2A rs3731257). Using the full dataset, the same SNP was most significant (unadjusted P = .004), but again not significant after adjusting for multiple testing (87 hypotheses). In conclusion, we have found evidence that a SNP in the CDKN2A gene may be associated with increased risk of ovarian cancer. This study highlights the need for multi-centre collaborations in order to validate and refute preliminary data generated by genetic association studies.

Haplotype Analysis In Patients With Chromosome 16q22.1-linked Autosomal Dominant Cerebellar Ataxia. *T. Amino[1], N. Sato[1], T. Ishiguro[1], T. Tsunemi[1], S. Toru[1], T. Toda[2], H. Mizusawa[1], K. Ishikawa[1]* 1) Neurology and Neurological Science, Tokyo Medical and Dental University, Tokyo, Japan; 2) Department of Clinical Genetics, Osaka University Graduate School of Medicine, Suita, Osaka, Japan.

Background, Purpose We have previously mapped mutations in Japanese families with autosomal dominant cerebellar ataxia (ADCA) to Chromosome 16q22.1,which is the gene locus for SCA4. Last year we reported that Chromosome 16q22.1-linked ADCA(16q-ADCA) patients share a common haplotype in the 600kb genomic region, and the heterozygous single nucleotide substitution in the *puratrophin-1* gene is strongly associated with the disease (Ishikawa et al, AJHG 2005). In this study, newly diagnosed families were additionally enrolled and their haplotypes were determined in more detail.

Methods Cerebellar ataxic patients were diagnosed as 16q-ADCA based on the presence of the single nucleotide substitution in the *puratrophin-1* gene. We have identified multiple single nucleotide polymorphisms(SNPs) on the disease allele. Haplotypes were determined for the 16q-ADCA families with these SNPs in addition to microsatellite markers.

Results We found 61 families of 16q-ADCA. They all had the possibility of harboring the disease allele within the 600kb genomic region for the SNPs as well as the microsatellite markers.

Discussion, Conclusion This study confirmed that 16q-ADCA patients may share a common founder haplotype. Detailed haplotype analysis with SNPs can lead to more accurate diagnosis.

Cutaneous Signs in Ehlers-Danlos Syndromes: The role of skin findings in determining the clinical diagnosis and predicting the genotype. *W. Chen[1], J. Yang[1], B. Griswold[1], A. Gustafson[1], M. Burchett[1], C.A. Francomano[1,2], N.B. McDonnell[1]* 1) Lab Clinical Investigation, Nat Institute on Aging/NIH, Baltimore, MD; 2) Harvey Inst Hum Genetics, GBMC, Baltimore, Maryland.

Ehlers-Danlos Syndomes (EDS) are a group of genetically heterogeneous hereditary disorders of connective tissue manifesting with joint hypermobility, tissue and vessel fragility, and skin involvement. Differentiation between subtypes of EDS has proven to be a challenge on a clinical basis. We present a summary of skin findings in 100 consecutive patients with a diagnosis of EDS enrolled in a natural history study at the NIH. Skin findings were notable for intra-familial variability. Presence of atrophic scars and skin hyperextensibility were variable not only between members of a family, but also on different body parts of the same person. Smooth and velvety skin, which is highly subjective, was noted in many patients, however unaffected members of some families were found to have the velvety skin, casting doubt in the clinical utility of this finding. Bruising, translucent skin, subcutenous venous patterns, and poor wound healing were seen to varying extents in patients with vascular EDS, but were also noted in other subtypes. Patients with a clinical history consistent with the hypermobile form were found to have a youthful appearance, a non-quantifiable finding. Molecular studies of COL5A1 and COL5A2 were completed on a research basis on patients who had skin features suggestive of the classical form of EDS (29/100), such as the presence of skin hyperextensibility, widened atrophic scars, and poor wound healing. Five mutations were found in COL5A1 and no mutations were found in COL5A2. By contrast, a sixteen year old girl, and a sixty year old woman, both with joint hypermobility, chronic joint pain and extensive striae similar to that seen in Marfan syndrome were found to have mutations in N-terminal propeptide of COL5A1. Our results suggest that while the presence of a skin abnormality can help to point to the diagnosis of EDS, skin findings from a single affected family member are often not helpful in determining the clinical subtype or predicting the molecular defect involved.

Genotype/Phenotype correlations with Col2A1 and Col11A1 mutations in Stickler Syndrome. *B. Griswold[1], M. Männikko[2], J. Wells[2], M. Majava-Elo[2], K. Mandel[1], J. Tran[1], P. Rose[3], H. Levy[4], R. A. Liberfarb[5], J. Davis[6], B. Rubin[6], Y. Szymko[6], E. Tsilou[6], M. Kaiser[6], A. Griffith[6], L. Ala-Kokko[2], N.B. McDonnell[1], C.A. Francomano[1,7]* 1) National Inst Aging, NIH, Baltimore, MD; 2) Dept Med Biochem Mol Bio, Univ Oulu, Oulu, Finland; 3) Mayo Clinic, Rochester, MN; 4) Johns Hopkins University Sc of Medicine, Baltimore, MD; 5) Mass Gen Hospital, Boston, MA; 6) National Institutes of Health, Bethesda, MD; 7) Harvey Inst Hum Gen, GBMC, MD.

Stickler Syndrome is estimated to be the most common autosomal dominant connective tissue dysplasia in North America. Mutations in procollagen genes COL2A1, COL11A1 and COL11A2 have been implicated in 35-50% of affected families. Mutation analysis for COL2A1 and COL11A1 was performed on 48 probands with Stickler syndrome using Conformation Sensitive Gel Electrophoresis (CSGE) and direct sequencing. Sixteen mutations in COl2A1 and four mutations in COL11A1 were detected. Six mutations in COL2A1 involved an altered splice site, and 9 mutations created premature termination codons, consistent with previous observations that haploinsufficiency is the predominant mechanism of disease. No mutations in COL2A1 involved substitutions of a glycine in the collagen helix with a bulkier amino acid, which is frequently seen in type II collagenopathies that lead to dwarfism. COL11A1 mutations consisted of a glycine substitution, an insertion leading to a frameshift in the helical domain, and two splice site mutations. Genotype/phenotype analysis of 35 affected persons from the 20 families with the COL2A1 or COL11A1 mutations revealed that all patients met the recently developed Stickler diagnostic criteria. Facial characteristics such as malar hypoplasia, broad nasal bridge or flat facial profile were seen in all affected persons. Cleft palate showed inter and intra-familial variability. All patients had ocular manifestations, with vitreous and/or retinal changes. Hearing loss, failure of femoral head development, skeletal manifestations and premature osteoarthritis were variable but common findings. Efforts are underway to determine the underlying mutations in the remaining 28/48 families.

Do abnormalities of extracellular matrix elements lead to autoimmune disorders? Unexpectedly high incidence of rheumatologic disorders in persons with Ehlers Danlos Syndrome. *A. Gustafson[1], B. Griswold[2], M.E. Burchett[2], S.H. Schurman[2], S. Ling[2], C.A. Francomano[2,3], N.B. McDonnell[2]* 1) Brown Univ, Providence, RI; 2) National Institute on Aging, Baltimore, MD; 3) Harvey Inst Hum Genetics, GBMC, Baltimore, MD.

The Ehlers-Danlos syndromes (EDS) are characterized by joint, skin and vascular abnormalities. Non-inflammatory joint pain is a common feature in many patients with EDS. Rheumatoid Arthritis(RA),Systemic Lupus Erythematosus(SLE), and Sjogren's syndrome(SS) are inflammatory acquired rheumatologic conditions. Raynauds phenomenon consists of vasospastic attacks in the digits, and may be found associated with an autoimmune disorder. We have found a high incidence of the above autoimmune disorders in a cohort of 72 consecutive EDS patients enrolled at the National Institute on Aging study 2003-086. In longitudinal follow-up 5/25 adults 40 years or older developed RA. One adult over 40 presented with a diagnosis of EDS and SLE. A 15 year old girl had a positive ANA, joint swelling and unexplained fevers, and later met the diagnostic criteria for SLE. Another young girl, age 16, in whom a COL5A1 mutation was found, presented with Raynauds phenomenon and joint swelling at the time of initial visit, and was later diagnosed with Juvenile Rheumatoid Arthritis (JRA). The total number of patients who exhibited Raynauds phenomenon in our cohort was 7/72. A 39 year old woman was found to have SS, diagnosed by biopsy of the oral mucosa. In total, there were 12/72 patients in our cohort who either presented with a rheumatologic disorder in addition to EDS, or developed one during follow-up. The number of RA patients, 5/72 (7%) in the total cohort, and 5/25 (%20) in patients over 40, far exceeds the predicted co-occurrence of this disorder with EDS. The same is true, albeit to a lesser extent, for the number of patients with Raynauds phenomenon, 7/72 (10%) and SLE, 2/72 (3%). This raises the possibility that abnormalities of the extracellular matrix might contribute to the development of autoimmunity in the presence of other environmental or genetic influences.

Phenotypic Variation of Congenital Diaphragmatic Defects. *K.G. Ackerman[1,2,3], S.O. Vargas[3,4], H.P. Kozakewich[3,4]* 1) Division of Genetics, Brigham and Women's Hospital, Boston, MA; 2) Division of Emergency Medicine, Children's Hospital, Boston, MA; 3) Harvard Medical School, Boston, MA; 4) Department of Pathology, Children's Hospital, Boston, MA.

Congenital diaphragmatic defects are common and serious, yet we do not understand why they occur. Definition of the range of both normal and abnormal diaphragmatic development in humans is critically important for understanding the pathogenesis of developmental diaphragmatic disorders. Diaphragmatic pathology was systematically reviewed from autopsies performed at Childrens Hospital, Boston on 48 patients diagnosed with a diaphragmatic defect between1990-2006 and from 94 patients diagnosed with unrelated conditions. Among these recent autopsy cases (collected during a time period of aggressive therapy and improved outcomes), 58 defects were identified in 48 patients (10 cases had bilateral defects). Of these 58 defects, 24 were posterolateral,11 were diaphragms with complete hemi-aplasia or diffuse unilateral muscularization defects, 11 had primarily lateral defects, 5 had anterior defects, and 7 were not able to be classified to a precise location. Of the anterior defects, one fit the description of a Morgagni hernia, while the other 4 had phenotypes that have not been previously defined. In the 10 cases with bilateral defects, most had a different type of defect (hernia vs. muscularization defect without herniation) in each hemi-diaphragm, but these different phenotypes tended to occur symmetrically. There is significant variation among the congenital diaphragmatic defects presenting in the neonatal period. Many of these defects are lateral or anterolateral with preservation of the posterior muscular rim and do not fit the classic description or proposed mechanism of Bochdalek hernia. A more rigorous anatomic classification system is warranted so that we can better understand the developmental biology of these disorders. We suggest that a new anatomic classification system be established to prospectively evaluate these defects. This fist step would help achieve the ultimate goal of a classification based on embryonic and genetic mechanisms.

Association of systemic lupus and lupus glomerulonephritis and copy number variation in *FCGR3B*: a follow-up study. *M. Fanciulli, E. Petretto, P. Norsworthy, M.K. Kumaran, T. Cook, T. Vyse, T. Aitman* Physiological Genomics and Med, MRC Clinical Sciences Centre , London, United Kingdom.

Systemic lupus erythematosus (SLE) is a chronic multi-system disease with an autoimmune aetiology. Clinical manifestations of SLE include glomerulonephritis, dermatitis, thrombosis, arthritis and CNS involvement. We recently reported an association between *FCGR3B* copy number variation (CNV) and glomerulonephritis in SLE in a small sample of UK nuclear families. *FCGR3B* is one of a number of immunoglobulin G Fc receptors with a key role in the initiation and regulation of the immune response. SNP variants in *FCGR3B* have previously been associated with numerous autoimmune and infectious diseases. Here, we investigated the association of CNV in *FCGR3B* with glomerulonephritis and SLE in a larger and separate sample including 375 SLE cases, 161 SLE patients with glomerulonephritis and 312 non-family controls from the UK 1958 birth cohort. Copy number was estimated by quantitative real-time q-PCR with SYBR Green. Association analyses were carried out by Mann-Whitney and Chi-square tests. Risk estimates were made using multivariate logistic regression models. We confirmed and strengthened our previous association between glomerulonephritis in SLE and CNV at *FCGR3B* (Mann-Whitney U-test P= $1.4*10^{-8}$, Pearson Chi-Square P= $6.2*10^{-7}$). Furthermore, we found that low copy number at *FCGR3B* is independently associated with SLE (Mann-Whitney U-test P= $1.4*10^{-5}$, Pearson Chi-Square P= $1.4*10^{-5}$). Odds Ratios indicated an increased risk for development of both SLE (OR=2.21, 95% CI 1.43-3.40, P=$3*10^{-4}$) and glomerulonephritis (OR= 2.43, 95% CI 1.47-4.03, P=$5*10^{-4}$). However, no significant increased risk of acquiring glomerulonephritis was observed in SLE subjects who possessed low copy number at *FCGR3B*. These data strongly suggest that *FCGR3B* is a susceptibility gene for both SLE and glomerulonephritis, and highlight implication of CNV in the genetics of complex traits.

Hemangioblastoma as a part of Tenascin-X deficiency spectrum. *P. Blanchet[1], C. Coubes[1], J. Puechberty[1], L. Pinson[1], J. Schalkwijk[2], G. Lefort[1], P. Sarda[1]* 1) Medical Genetics Center, Arnaud de Villeneuve Hosp, Montpellier, France; 2) Dept of Skin Biology and Experimental Dermatology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands.

Tenascin-X deficiency leads to an autosomal recessive form of Ehlers-Danlos (ED) syndrome, a connective tissue disorder characterized by hyperextensible skin, hypermobile joints, and tissue fragility. The phenotype of patients with tenascin-X deficiency includes skin hyperextensibility, easy bruising, and joint laxity but in contrast to classic ED, patients have soft skin, no atrophic scarring and no poor wound healing. Also, particular medical problems occur in these patients : diverticular intestinal disease, rectal prolapse, mitral valve prolapse and obstructive airway disease. In a family of 9 sibs, 2 brothers and one sister presented ED syndrome with an autosomal recessive inheritance and typical findings of Tenascin-X deficiency. This diagnosis was confirmed in the serum with a complete deficiency for Tenascin-X protein as determined by ELISA. At the age 44, a brother with Tenascin-X deficiency presented neurological problems due to cervical spine hemangioblastoma. After surgery, diagnosis of hemangioblastoma was confirmed by histology. Tenascins are a family of extracellular matrix glycoproteins. In mouse models, data suggest the role of TNX in the regulation of vasculogenesis and angiogenesis, with an important role in the control of endothelial cell proliferation. Tenascin-C, the most well-known member of this family, is expressed in various tumors including human astrocytomas, carcinomas and gliomas. Tenascin-X is also expressed in glioma cell lines and human astrocytomas but expression is mainly localized in the perivascular stroma around tumor vessels. These findings indicate that Tenascins could be involved in tumoral neovascularization. Considering the observation of hemangioblastoma in our patient and the data on the implication of Tenascin-X in vasculogenesis and angiogenesis, we suggest that hemangioblastoma be included in the spectrum of Tenascin-X deficiency.

Mutational analysis of the presenilin 2 gene in a group of Italian patients with familial Alzheimer's disease. *V. Andreoli, M. Liguori, F. Trecroci, A. La Russa, R. Cittadella* Institute of Neurological Sciences, National Research Council, Piano Lago di Mangone, Cosenza; Italy.

Alzheimer's disease (AD) is a genetically heterogeneous condition characterized by an early onset (EOAD) and a late onset (LOAD). Family history of dementia is the second major risk factor for AD after age and it has been proposed that several genetic determinants influence the development of the pathology. To date, three genes have been implicated: the amyloid precursor protein gene (APP) and the presenilin (PSEN) 1-2 genes. Mutations in these genes cause autosomal dominant familial AD (FAD). At present only eleven mutations have been described in the PSEN2 gene. We initiated the present study in order to identify mutations in the PSEN2 gene in 25 patients with early and late FAD, to determine whether these mutations were present in this group. We did not find pathogenic mutations in the PSEN2 gene but we found a novel and rare single-nucleotide polymorphism (SNPs) in the PSEN2 gene in a patient having familial early-onset AD, consisting of a heterozygous G->A substitution on exon 6. This variation in the coding region results in a Met to Val substitution in the first position of the codon 174 within the predicted transmembrane 3 (TM3) domain of the PSEN2 protein. The patient was a 51-year-old woman suffering from dementia; she presented a 6-year history of mild memory deficit with problems in spatial orientation and word finding difficulty. We have therefore analyzed TM3 domain in a group of age-matched healthy controls and this change was also detected in one of 125 unrelated individuals tested, suggesting that it was a non-pathogenic SNPs. The M174V change reported herein has a very low frequency compared with other reported polymorphisms, since it was detected in only two of the 150 individuals studied (1/125 healthy controls and 1/25 AD patients). On the other hand, the lack of PS2 mutations in the analyzed subjects confirms their rare occurrence in patients with familial AD. The uncommon SNPs should be always taken into account in the genetic testing of AD, because it may have important implications as genetic marker in FAD.

Type 2 diabetes *TCF7L2* risk alleles reduce beta-cell function and increase birth weight. *R.M. Freathy[1], M.N. Weedon[1], M.I. McCarthy[2], M. Walker[3], G.A. Hitman[4], Y. Ben-Shlomo[5], G. Davey Smith[5], S.M. Ring[5], A.T. Hattersley[1], T.M. Frayling[1]* 1) Peninsula Medical School, Exeter, UK; 2) Oxford, UK; 3) Newcastle, UK; 4) London, UK; 5) Bristol, UK.

The transcription factor *TCF7L2* is the most important type 2 diabetes gene found to date. Heterozygotes and minor allele homozygotes for rs12255372, which constitute 41% and 9% of the population, have odds ratios for type 2 diabetes of 1.30 and 1.66 respectively, and this association has been replicated in multiple studies. The mechanism by which variation in *TCF7L2* predisposes to diabetes is unknown. Identifying intermediate traits in the non-diabetic population will help define the mechanism of action but is notoriously difficult. We studied the role of common variation in the *TCF7L2* gene in diabetes-related intermediate traits in the general population. We assessed the role of *TCF7L2* in beta-cell function using 1053 non-diabetic subjects from two studies (Barry Caerphilly Growth Study, n=631, mean age 25; Warren 2 Extension, n=422, mean age 39) with oral glucose tolerance test data available, and in birth weight using 7211 children and 6573 mothers from the ALSPAC study. All subjects were UK whites. Each copy of the type 2 diabetes predisposing allele of rs12255372 (frequency 0.29) decreased beta-cell function (early insulin response) by 0.15 (0.06-0.24) SD (P=0.001). Each copy of the diabetes risk allele in the fetus was associated with a 21g (3g-40g) increase in birth weight (ANOVA P=0.004), while each copy of the diabetes risk allele in the mother was associated with a 32g (12g-52g) increase in offspring birth weight (ANOVA P=0.0006). There was no association with fasting glucose, insulin or BMI (all P>0.05). Our results suggest that prior to the development of type 2 diabetes, common variation in *TCF7L2* is associated with reduced beta-cell function and increased birth weight. Our results indicate that with large sample sizes it is possible to identify subtle effects of established disease risk alleles in the general population.

Analysis of P2RY12 gene H2 haplotype in coronary artery disease and myocardial infarction. *U. Cavallari[1], E. Trabetti[1], M. Biscuola[1], G. Malerba[1], D. Girelli[2], O. Olivieri[2], N. Martinelli[2], R. Corrocher[2], P.F. Pignatti[1]* 1) Department of Mother-Child & Biol-Genetics, Univ Verona, Verona, Italy; 2) Department of Clinical and Experimental Medicine, University of Verona, Verona, Italy.

BACKGROUND: Platelet activation and aggregation are key elements in coronary atherosclerosis. Platelet receptor P2Y12 stimulated by adenosin diphopsaphate (ADP) modulates affinity of glycoprotein IIb/IIIa for fibrinogen, resulting in fibrinogen binding and platelet aggregation. A haplotype of the P2RY12 gene (H2 haplotype) was found to be associated with maximal aggregation response to ADP in the general population and to increase risk of peripheral arterial disease (Fontana P. et al. Circulation 2003;108:2971-2973). Aim of this study was to test the association of the H2 haplotype with coronary atherosclerosis and myocardial infarction. METHODS AND RESULTS: We studied 1422 unrelated consecutive Italian patients of both sexes. They were diagnosed by coronary angiography as affected by coronary artery disease (CAD or cases, n=1033) or not (CAD-free controls, n=389). In the CAD group 616 patients had a history of myocardial infarction (MI). All patients were genotyped for the H1/H2 haplotype tag SNP of the P2RY12 gene by melting curve analysis of fluorescent real time PCR. We observed a significant difference in genotype distribution between CAD and CAD-free groups (p=0.024). H2 haplotype carriers were more frequent in the CAD group than in the CAD-free group (p=0.041, OR=1.35, CI=1.00-1.83). No significant difference in H2 haplotype frequency was observed between MI and MI-free groups within CAD population. CONCLUSIONS: These results suggest an association of the H2 haplotype of the P2RY12 gene with coronary artery disease. No significant association was observed between the H2 haplotype and history of myocardial infarction in the studied population.

Lysosomal stabilization of -glucocerebrosidase and -galactosidase-A by competitive inhibitors: Implications for chaperone therapy. *T. Edmunds, J. Jaworski, Q. Zhou, A. Park, D. Honey, S. VanPatten* Therapeutic Protein Research, Genzyme Corp, Framingham, MA.

Gaucher disease and Fabry disease are caused by mutations in the genes encoding the lysosomal enzymes -glucocerebrosidase (GC) and -galactosidase-A (Agal), respectively. The standard of care is enzyme replacement therapy with recombinant GC or Agal. Recently, a therapeutic strategy has been postulated in which competitive inhibitors chaperone the trafficking of misfolded enzymes from the endoplasmic reticulum (ER) to the lysosome. Since competitive inhibitors are more likely to bind enzymes in the lysosome than in the ER, it is essential to distinguish between their effect on transport to the lysosome and effects within the lysosome. To do this we evaluated their effect on recombinant enzymes delivered directly to the lysosome. Macrophages were incubated with recombinant GC (rGC) in the presence and absence of derivatives of the competitive inhibitor deoxynojirimycin. Uptake of rGC into macrophages does not involve trafficking from the ER, but occurs via receptor-mediated enzyme delivery to the lysosome. In the absence of competitive inhibitors the lysosomal half-life of rGC was ~15 hours. In the presence of competitive inhibitors the lysosomal half-life of rGC was extended up to 3-fold. rGC containing the N370S mutation was also stabilized within the lysosome, but the concentrations of competitive inhibitor required were 10-fold higher than those required to stabilize wild-type rGC. Similar results were obtained with fibroblasts incubated in the presence of recombinant Agal and the competitive inhibitor deoxygalactonojirimycin. These results suggest that the increased level of normal and mutant enzymes observed in the presence of competitive inhibitors is due to decreased lysosomal degradation, rather than increased transport from the ER to the lysosome. Although enzyme levels are elevated in the lysosome, the enzyme will be non-functional since the stabilization is dependent on the binding of the competitive inhibitor to the enzyme. Thus the increased enzyme level seen in the presence of competitive inhibitors is unlikely to provide any therapeutic benefit.

Hominoid lineage specific evolution of low-copy repeats on 22q11.2 (LCR22s) provides mechanisms for genome plasticity. *M. Babcock[1], S. Yatsenko[2], J. Hopkins[3], M. Brenton[3], Q. Cao[4], P. de Jong[4], P. Stankiewicz[2], J.R. Lupski[2], J.M. Sikela[3], B.E. Morrow[1]* 1) Department of Molecular Genetics, Albert Einstein College of Medicine, Bronx, NY; 2) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Department of Pharmacology and Human Medical Genetics Program, University of Colorado Health Sciences Center, Denver, CO; 4) Children's Hospital of Oakland Research Institute, Oakland, CA.

Low-copy repeats (LCRs) or segmental duplications, constitute roughly 5 percent of the human genome and are associated with many human congenital anomaly disorders. The LCRs on chromosome 22q11.2 (LCR22s) mediate chromosomal rearrangements leading to deletions, duplications, and translocations. Four genes, *USP18*, *BCR*, *GGTLA* and *GGT*, map adjacent to the LCR22s and pseudogene copies are located within them. We hypothesize that gene duplication occurred during primate evolution, followed by recombination events, forming pseudogene copies. To test whether gene duplication could be detected in non-human hominoid species, FISH mapping was performed using probes to the four functional gene loci, avoiding LCR22 sequences. A single copy of each was present in humans but one to five additional copies were found in hominoid species. We then compared LCR22 copy number and organization using LCR22 specific FISH probes. Lineage specific LCR22 variation in structure and copy number was detected all hominoid species examined (chimpanzee, bonobo, gorilla, orangutan, gibbon). To independently validate and extend initial findings, array comparative genome hybridization, real time PCR, and physical mapping was performed. The most striking discovery was a dramatic amplification of LCR22 copies in gorilla. Interestingly, they localized to the telomeric bands of many gorilla chromosomes. The most parsimonious explanation is that the LCR22s became amplified by inter-chromosomal recombination between chromosome termini. In summary, our results are consistent with a lineage specific coupling between gene and LCR22 duplication events. This underscores the instability and dynamic nature of LCRs in the genome and provides a model to study genome evolution and variation.

Homozygous silencing of the TBR2 locus by position effect results in severe neuronal proliferation defect and corpus callosum agenesis. *L. Baala[1,2], S. Briault[3], A. Assermouh[4], F. Laumonnier[3], T. Attie-Bittach[1], F. Encha-Razavi[1], M. Clément-Ziza[1], S. Romana[1], J. Amiel[1], G. Goudefroye[1], A. Sbiti[2], H. Natiq[2], A. Munnich[1], A. Sefiani[2], S. Lyonnet[1]* 1) Génétique INSERM U781, Necker Hospital, Paris 15, France; 2) INH,Rabat, Morocco; 3) INSERM U619, Université Rabelais, Tours, France; 4) Hôpital d'Enfants, Rabat, Morocco.

In accordance with the importance of the regulation of cell division during neuronal proliferation, all genes known to be mutated in human primary microcephaly so far, encode proteins involved in mitosis and, mostly, centrosome formation. We report an autosomal recessive primary microcephaly syndrome co-segregating with a balanced translocation between chromosomes 3p and 10q in a large inbred family. The translocation was found at the homozygous status in all affected individuals (46,XY,t(3;10)(p24;q23)2x), while unaffected parents were heterozygous. Accordingly, homozygosity linkage analysis between the disease trait and polymorphic markers of chromosomes 3p and 10q defined 2 homozygous regions of 27 and 11.4 Mb respectively. We established a physical and characterized the BACs that encompassed the breakpoints on 3p24 and 10q23 (BAC RP11-9a14 and RP11-104H24). Interestingly, neither of the two translocation breakpoints disrupted a known or predicted gene coding sequence. However, we showed that position effect at the breakpoint on chromosome 3, located 200 kb upstream, silences the TBR2 locus with monoallelic expression in heterozygous parents. These data support the involvement of TBR2, a transcription factor of the T-box family, in neuronal proliferation and in the severe syndromic microcephaly observed in this family, in agreement with our data on its expression pattern in early developing human telencephalon. Our findings provide evidence that not only mitotic and apoptotic proteins but transcription factor mutation also, may be responsible for severe proliferation defect of the human brain.

Phelan-McDermid Syndrome: Deletion 22q13 characterized by comparative genomic hybridization microarray analysis and FISH with locus specific probes. *P.I. Bader[1], S.C. Newman[1], F.J. Bader[1], S.-H.L. Kang[2], S.W. Cheung[2]*
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Phelan-McDermid Syndrome (PMS) is characterized by normal to increased growth, severe mental retardation (MR), severe speech deficiency, hypotonia, mouthing/chewing objects, dolicocephaly, prominent/dysplastic external ears, pointed chin, large and fleshy hands, and dysplastic toenails. PMS is caused by deletion 22q13. SHANK3 or PROSAP2 (Proline-rich synapse associated protein 2) which codes for a structural protein found in the postsynaptic density is deleted, and this may lead to neurological abnormality in PMS. We report a 29-year-old female with MR, little speech, autistic features, seizures, keloid formation, hypotelorism, long lashes, inner epicanthal folds, abnormal philtrum without markings, high palate, prominent nose, ears with prominent anthelix, fleshy and large hands, low-set and broad thumbs, clinodactyly of 5th fingers, variant palmar creases including extra creases on thenar and antithenar eminences, edematous feet with dysplastic toenails, cutis marmarata, hirsuitism, hyperextensible joints, poor balance. Height, weight, and OFC were at the 50%ile. Evaluations and chromosome analysis at (IU medical center) at age 1 and 22 resulted in no diagnosis for a specific genetic syndrome. Head MRI was normal. Re-evaluation by FISH analysis for DiGeorge syndrome showed absent control signal at band 22q13.

Chromosomal microarray analysis designed to interrogate clinically relevant genomic regions revealed haploinsuffiency at 22q13.3 with the following clones: RP11-93F4, RP11-66M5, RP5-92517, RP3-402G11, RP11-164E23, GS-99K24 encompassing approximately 5 Mb of the subtelomeric region. This deletion was subsequently confirmed by high resolution banding showing a subtle deletion at band 22q13.3. Although PMS is believed to be caused by deletion of SHANK3/PROSAP2, haploinsuffiency for other contiguous candidate genes in the area may explain the variation seen in the syndrome and could lead to determination of a critical region of deletion overlap.

Detection of an interstitial deletion of 3q21 by Comparative genomic hybridization in a child with multiple congenital abnormalities and pancytopenia with myelodysplasia. *P. Callier, M. Nathalie, J. Guy, C. Thauvin-Robinet, AL. Mosca, F. Huet, L. Faivre, F. Mugneret* Cytogenetics Laboratory, CHU Le Bocage, Dijon, France.

Comparative genomic hybridization (CGH) is a rapid molecular cytogenetic technique, which can detect chromosomal abnormalities in the range of 3-20Mb. This method have been performed in 44 patients with multiple congenital abnormalities and mental retardation with normal standard, high resolution and subtelomeric FISH cytogenetic analyses. We present here the first child of healthy non-consanguineous parents presenting with features overlapping with Townes-Brocks and Baller-Gerold syndrome and an interstitial deletion 3q detected by CGH studies. She had a healthy sister. Examination at birth revealed a length of 43.5 cm, dysmorphism with cranial and facial asymmetry, unilateral right hypoplastic and low-set ear, left preauricular tags and duplication of the right thumb. A right coronal craniosynostosis was evidenced and surgically treated at 6 months of life. She also had posterior agenesis of the corpus callosum, persistent ductus arteriosus, unilateral deafness secondary to external ear canal stenosis and chronic constipation. Follow-up revealed severe mental retardation with hyperactivity and abnormal behaviour, precocious puberty at the age of 8 years with a height at -2SD and pancytopenia with myelodysplasy at the age of 11 years with normal chromosomal breakage studies. CGH revealed an interstitial deletion 3q21 and FISH study with BAC RP11-205A6 (3q21.2) confirmed the deletion. Molecular cytogenetics studies with BACs located in the 3q21 region are in progress to better define the size of the deletion. The clinical features did not overlap with the other rare cases with interstitial 3q deletion including the q21 band. This deletion does not comprise the Townes-Brocks and Baller-Gerold syndromes loci. This study demonstrates the importance of CGH technology to detect interstitial deletions. Interestingly, the RPN1 (Ribophorin 1) gene implicate in myelodysplasia is locate in the 3q21 region.

Familial amniotic band sequence with mutation of FOXC2 gene. *C. Coubes[1], P. Blanchet[1], J. Puechberty[1], L. Pinson[1], S. Jeffery[2], G. Lefort[1], P. Sarda[1]* 1) Dept of Medical Genetics, Arnaud de Villeneuve Hospital, Montpellier, Herault, France; 2) Medical Genetics Unit, St. George's Hospital Medical School. London United Kingdom.

Amniotic band sequence is a common anomaly with an incidence estimated between 1/1200 and 1/15000. It is considered to be an isolated and sporadic defect due to early rupture of the amnion. However, cases with additional anomalies such as cleft lip and palate, congenital heart defects or renal anomalies have been reported. Rarely, familial amniotic band sequence has been described, sometimes associated with connective tissue disorders such as Ehlers-Danlos syndrome type IV or osteogenesis imperfecta. We report a familial case of amniotic band sequence in a mother and her son. The two patients had digital amputations and constriction rings. The mother also presented microretrognathia, duplex kidney, bicornuate uterus, lymphedema and distichiasis. The boy, 12-year-old, had dysmorphic facial traits, bilateral rocker bottom feet, spinal arachnoid cyst and distichiasis. Mutation 1061ins GCCC in the FOXC2 gene was found in the two patients. FOXC2 is a transcription factor gene expressed in many embryonic tissues such as brain, kidney, cardiovascular and skeletal systems. To date, we have no data on the expression of FOXC2 in placenta and amnion. The observation of a family with cosegregation of amniotic band sequence and distichiasis-lymphedema syndome suggests that amniotic band sequence is probably a part of the large spectrum of syndromes due to mutations of the FOXC2 gene.

Epistatic Effects of Polymorphisms in Genes from the Renin-Angiotensin, Bradykinin, and Fibrinolytic Systems on Plasma t-PA and PAI-1 Levels. *F.W. Asselbergs[1], S.M. Williams[2], P.R. Hebert[3], C.S. Coffey[4], H.L. Hillege[1], G. Navis[1], D.E. Vaughan[2], W.H. van Gilst[1], J.H. Moore[5]* 1) University of Groningen, Groningen, Netherlands; 2) Vanderbilt University, Nashville, TN; 3) Yale University, New Haven, CT; 4) University of Alabama Birmingham, Birmingham, AL; 5) Dartmouth Medical School, Lebanon, NH.

Tissue plasminogen activator (t-PA) and plasminogen activator inhibitor 1 (PAI-1) directly influence thrombus formation and degradation and thereby risk for arterial thrombosis. Activation of the renin-angiotensin system has been linked to the production of PAI-1 expression via the angiotensin II type 1 receptor (AT1R). In addition, inhibition of the angiotensin converting enzyme (ACE) augments bradykinin that can induce the acute release of t-PA from the endothelium through a B2 receptor mechanism. In the present study, we investigated the epistatic or synergistic effects of polymorphisms in genes from the renin-angiotensin, bradykinin and fibrinolytic systems on plasma t-PA and PAI-1 levels. We used two-way ANOVA to model epistatis between polymorphisms in the *ACE*, *AT1R*, *bradykinin B2 receptor*, and *PAI-1* genes and their effects on plasma levels of t-PA and PAI-1 in a large population-based sample from the PREVEND study in Groningen, The Netherlands (n=2,527). After correction for multiple testing using a permutation distribution, we demonstrated strong interactions between *bradykinin B2* polymorphisms (p=0.002) and between *ACE* and *bradykinin B2* polymorphisms (p=0.003) that predict plasma t-PA levels in females. In males, polymorphisms in the *bradykinin B2* and *AT1R* genes showed the strongest interactive effects on t-PA levels (p=0.006). In both females and males, the *bradykinin B2* polymorphisms interacted with the *AT1R* polymorphism on plasma PAI-1 levels (p=0.026 and p=0.039, respectively). In addition, we found a borderline significant interaction between *PAI 4G5G* and *ACE I/D* on plasma t-PA and PAI-1 levels. These results support the idea that the relationship between the genome and t-PA and PAI-1 levels is complex involving synergistic interactions among genes from multiple biochemical systems.

Modeling of twin growth during gestation and quantification of the genetic and environmental factors involved.

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Structural equation modeling of birth weight has given inconsistent results with heritability estimates between 10 and 40%. One explanation could be that the heritability changes during gestational age or that different covariates explain different parts of the variance covariance structure. The aim of this study was to model prenatal growth as well as to quantify the genetic and environmental components to explain the effects of several covariates. Perinatal data were obtained from 4232 live-born twin pairs from the East Flanders Prospective Twin Survey, Belgium. Heritability of birth weights at different gestational ages was estimated using a non-linear multivariate Gaussian regression with covariates in the mean model as well as in the variance and the covariance components. Following covariates were considered: gestational age, sex, chorionicity, placental type, insertion of the umbilical cord, birth order, the origin of the pregnancy (spontaneous, in-vitro-fertilization, induction of ovulation), maternal age, parity, and neonatal death. During gestation, heritability dropped from 37% at 25 weeks to 14% at 42 weeks. After adjusting for covariates the heritability increased: from 58% at 25 weeks to 26% at 42 weeks. The origin of the pregnancy did not contribute to the model. Chorionicity explained part of prenatal growth but had almost no influence on the covariance structure. All other covariates influenced the genetic and environmental components of the covariance structure in different ways. For complex traits such as growth, it is important to explain a large part of the variance and covariance, as it will help to find genes in linkage and association studies. Thus, explaining (birth) weight by modeling the genetic and environmental factors gives a better inside in factors influencing growth during gestation.

Expression profile study of atherosclerosis: microarray analysis in human coronaries with atheromas. *M. Biscuola[1], S. Cagnin[2], C. Patuzzo[1], E. Trabetti[1], M. Iafrancesco[3], A. Forni[3], G. Faggian[3], A. Mazzucco[3], G. Lanfranchi[2], P.F. Pignatti[1]* 1) Department of Mother & Child & Biol-Genetics, University of Verona, Verona, Verona, Italy; 2) CRIBI Biotechnology Centre and Department of Biology, University of Padova, Italy; 3) Division of Cardiac Surgery, University of Verona Medical School, Italy.

Atherosclerosis is a progressive disease characterized by the accumulation of lipids and fibrous elements in the large arteries and it constitutes the single most important cause of cardiovascular disease. Genetic susceptibility, mechanical shear stress, diet and smoke have been involved in the pathogenesis of atherosclerosis but the causal mechanisms are not yet understood. Changes in the expression levels of several genes have been described in animal models. Segments of Left Anterior Descending (LAD) coronary artery were obtained from a total of 13 patients undergoing graft surgery. Coronarography was used to classify individuals into occluded or not. 3 patients with acute myocardial infarction with atherosclerosis were cases. 10 patients presenting with dilatative cardiomyopathy and no atheromas were controls. We performed a preliminary study using the RNA extracted from all the samples. RNA obtained from 6 of the 10 controls was used to create a control pool and this pool was compared with 2 of the 3 cases. Using an oligo DNA microarray carrying 22.000 human genes we looked at differential gene expression profiles. Data were analyzed with a modified t-test using the SAM program (Significance Analysis of Microarrays). We found 6 classes of genes differentially expressed in both cases. They represent genes involved in the pathway for the eicosanoid synthesis, complement and coagulation cascades, response to wounding, innate immunological response, actin binding and cytoskeleton activities, blood and lymph tissue proliferation. After an extension of the number of samples analyzed by microarray, we will perform qRT-PCR in order to confirm the suggested results.

Autosomal dominant early onset Alzheimer disease with cerebral amyloid angiopathy caused by *APP* duplication: clinical and neuropathological study of five French families. *L. Guyant-Maréchal[1,2], L. Cabrejo[1], A. Laquerrière[1], M. Vercelletto[3], F. De La Fournière[4], C. Thomas-Antérion[5], C. Verny[6], F. Letournel[7], F. Pasquier[8], A. Vital[9], F. Checler[10], T. Frebourg[1,2], C. Campion[2], D. Hannequin[1,2]* 1) Departments of Neurology, Genetics, and Neuropathology, University Hospital, Rouen, France; 2) Inserm U614, Faculty of Medicine, Rouen, France; 3) Department of Neurology, University Hospital, Nantes, France; 4) Department of Geriatrics, Pau Hospital France; 5) Department of Neurology, University Hospital, Saint-Etienne, France; 6) Department of Neurology, University Hospital, Angers, France; 7) Laboratory of Cellular Biology, University Hospital, Angers, France; 8) Department of Neurology, University Hospital, Lille, France; 9) Department of Pathology, University Hospital, Bordeaux, France; 10) Institute of Molecular and Cellular Pharmacology, UMR6097 CNRS/UNSA, Valbonne, France.

We recently reported (Rovelet-Lecrux et al. Nature Genetics 2006, 38: 24-26), in 5 French families with autosomal dominant early-onset Alzheimer disease (ADEOAD) and cerebral amyloid angiopathy (CAA), the duplication of the *APP* locus located on chromosome 21, the duplication ranging from 0.58 to 6.37 Mb. The present study describes the phenotype of this new entity. The phenotype was not dependent on the size of the duplication and, remarkably, there was no clinical feature of Down's syndrome (DS). Dementia was observed in all cases (age of onset (AOO): 42-59 years), intracerebral haemorrhage (ICH) was reported in 6 (26 %; AOO: 53-64 years) and seizures occurred in 12 (57%)among 21 affected patients. Neuropathological examination in 5 cases demonstrated AD and severe CAA lesions which were reminiscent from those reported in brains of DS patients. A striking feature consisted in intraneuronal Ax-40 accumulation located in the granular cell layer of the dentate gyrus and in the pyramidal cell layer of the Ammon's horn. It could be speculated that both an overproduction of the A peptide and a possible shift toward a high A40/A42 ratio account for the pathological hallmarks of the disease.

Rare cases from a large sample of Rett syndrome patients, including a MECP2 mutated male and a female with a novel CDKL5 mutation. *F. Cogliati[1], M. Masciadri[1], I. Moroni[2], M. Marchi[1], P. Vigliano[3], R. Gaggero[4], MT. Bonati[1], L. Larizza[5], S. Russo[1]* 1) Ist.Auxologico Italiano,Milano, Italy; 2) Ist.Neurologico C.Besta,Milano,Italy; 3) NPI ASL2,Torino,Italy; 4) Ist.G.Gaslini,Genova,Italy; 5) Polo S.Paolo,Milano University,Italy.

Classical RTT syndrome is a neurodevelopmental disorder predominantly affecting young females which causes retardation with loss in motor and vocal skills and additional problems such as microcephaly,ataxia,growth retardation and scoliosis. 95% percent of classical RTT and 40-50% of atypical cases have been attributed to mutations in the gene coding for methyl CpG binding protein,MECP2.Occurrence of males affected by severe encephalopathy and Rett-like phenotype with mutation in MECP2 is very rare.Recently, mutations in cyclin-dependent kinase-like 5,CDKL5,have been described in atypical variants of RTT including early onset of epileptic seizures and infantile spasms.We addressed the molecular characterization of MECP2 mutations in both the alfa and beta isoforms on over200 females and 30 males,identifying mutations in the isoform MECP2e2 in 42 classical and in 19 atypical RTT girls. Mutational screening of MECP2e1 evidenced the A2V substitution in a girl with Rett classical phenotype not found in her parents and in over100 controls. As regards the males cohort we could identify a maternally inherited C-terminal deletion,1163del44,in a boy affected with severe encephalopaty and early onset of epilepsy. X inactivation test disclosed maternal skewed inactivation. This MECP2 mutated male patient is a tool to investigate MECP2 interactors and underlines the occurrence of maternal transmission of non expressed mutations and their impact on genetic counseling.We also investigated for CDKL5 mutations a cohort of 15 atypical RTT patients selected for the early onset of seizures or infantile spasms and identified a novel IVS4+2 T-C splice mutation.The girl presented with early onset focal seizures and infantile spasms evolved in daily mioclonic seizures.Based on our results we recommend to investigate both MECP2 and CDKL5 genes when there is a suspicious of Rett syndrome and to test males with specific clinical traits.

The G397A (E133K) change in the AGGF1 (VG5Q) gene is a single nucleotide polymorphism in the normal population and not a predisposing mutation for Klippel-Trenaunay syndrome. *S. Gutiérrez[1], L. Magano[1], A. Delicado[1], M.A. Mori[1], M.L. de Torres[1], I. Vallcorba[1], L. Fernández[1], M. Palomares[1], E. Fernández[2], G. Rodriguez Tarduchy[2], J. Molano[1], I. López Pajares[1], P. Lapunzina[1]* 1) Medical and Molecular Genetics, Hospital Universitario La Paz, Madrid, Madrid, Spain; 2) Sequencing Unit, IIB, Madrid, Spain.

Introduction: Klippel-Trenaunay syndrome (KTS) is an unusual, asymmetric overgrowth syndrome (OGS) characterized by vascular malformations and partial overgrowth of the affected region of the body, usually the lower limb. It was recently suggested that the E113K change in the VG5Q (*AGGF1*) gene causes susceptibility to KTS (Nature 2004;427:640-5). In that paper, the authors suggested that this change was responsible of such susceptibility after finding 5 out of 130 KTS patients with heterozygosis for the E113K. **Methods**: We genotyped 768 control DNA samples and 387 DNA samples of patients and/or parents of patients with overgrowth syndromes (OGS): 63 patients and 53 parents with Beckwith Wiedemann syndrome, 97 patients and 130 parents with Sotos, 18 with Simpson-Golabi Behmel, 10 patients and 13 parents with Macrocephaly-Cutis Marmorata telangienctatica and 3 patients with KTS. Genotyping was performed using TaqMan Genotyping Assays (C_25615428_10, Applied Biosystems). **Results**: On 768 normal, age-matched control samples we found that 23 individuals were heterozygous for the E133K change (3 %). In patients with OGS and their relatives, only 3 were heterozygous, 1 Simpson-Golabi-Behmel syndrome and 2 parents of patients with OGS (1 with Sotos syndrome and 1 with Beckwith Wiedemann syndrome). **Conclusions**: the E133K (G397A) change of the *VG5Q* (*AGGF1*) gene seems to be a polymorphism rather than a true predisposing mutation since, similarly to the frequency observed in KTS patients,around 3 % of controls were heterozygous for such a change. The E133K change should not be related to susceptibility to KTS or any other OGS and should be assumed as a normal, infrequent SNP instead.

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Identification of a Telencephalon preferentially expressed new MicroRNA, exploring its roles in Neurodevelopmental Processes. *P. Carotenuto, L. Garzia, A.M. Bello, V. Maffia, G. Vitale, M. Zollo* Biotecnologie Avanzate SCARL, CEINGE, Naples, Italy.

We have recently isolated 48 new microRNA precursors preferentially expressed in murine telencephalon at embryonic days E14.5 (Zollo et al. 2005). Within this collection a novel microRNA (6.A7), was characterized and found involved in differentiation and apoptosis processes of human neuroblastoma SH-SY5Y cells line, thus regulating the expression of target genes including IAP genes (BIRC1, BIRC4, BIRC5), and Delta/Notch EGF receptor (DNER) pathway related genes. We will present its functional correlation to neurodevelopmental processes such as neural differentiation and apoptosis, being potential a new controlling gene involved in human neurodevelopmental disorders.

Four Different ABCA4 Mutations in a Large Family, With Several Loops of Consanguinity, Affected With Autosomal Recessive Cone-Rod Dystrophy : Further Evidence for a High Frequency of ABCA4 Heterozygote Carriers. *D. Ducroq[1], S. Shalev[2], A. Habib[2], A. Blumenfeld[3], A. Munnich[1], J. Kaplan[1], J.M. Rozet[1]* 1) INSERM U781, Hopital des Enfants Malades, Paris, France; 2) Genetics, Institute of Ha'Emek Medical center, Afula, Israel; 3) Genetic Testing Laboratory, Department of Ophthalmology, Hadassah University Hospital, Israel.

This study aimed to identify the molecular defect responsible for autosomal recessive cone-rod dystrophy segregating in a large multiplex and consanguineous family of Christian-Arab ancestry. This pedigree was made of seven nuclear families gathering 12 affected members and seven healthy relatives. All family members underwent general and ophthalmologic examinations. Among affected patients, 9/12 displayed typical signs of cone-rod dystrophy, 1/10 was affected with a typical Stargardt disease. In 2/10 affected patients the exact diagnosis could not be definitely carried with regard to their young age. Linkage analyses were performed in this pedigree using polymorphic markers flanking each of the three hitherto known arCRD loci: CORD3, CORD8 and CORD9, respectively. Subsequently, all 50 exons of the ABCA4 gene at the CORD3 locus were screened for mutations by direct sequencing. Linkage analyses failed to identify homozygosity in the seven nuclear families of this large pedigree at any of three arCORD loci. However, homozygosity was found at the CORD3 locus for three nuclear families and the segregation of four distinct haplotypes at this locus in the whole pedigree suggested the alteration of the ABCA4 gene. This hypothesis was confirmed by the identification of four distinct mutations leading to cone-rod dystrophy in all patients but one. This last patient, suspected to have the same disease as her relatives, displayed in fact typical sypmptoms of Stargardt disease without extension to the peripheral retina. In conclusion, the results of this study emphasize the trap of the search for homozygosity in patients belonging to highly inbred families when the heterozygote carrier frequency is much higher than the mean frequency of heterozygote individuals in the general population.

Lysinuric protein intolerance is present world-wide and is not a rare disorder. *S. Fecarotta, M.P. Sperandeo, P. Annunziata, P. Piccolo, A. Pepe, G. Andria, G. Sebastio* Department of Pediatrics, Federico II University, Naples, Italy.

Lysinuric protein intolerance (LPI; [MIM 222700]) is caused by mutations of the SLC7A7 gene, encoding the light chain of the cationic amino acid transporter 4F2hc/y+LAT-1. LPI was described in Finland where its frequency was estimated at 1:60,000. LPI was considered a rare disease in other countries, apart from Italy and Japan, where small clusters where identified. LPI patients are usually asymptomatic when breast-fed whereas, after weaning, they present vomiting, diarrhea, growth retardation, hepatosplenomegaly, osteoporosis, episodes of coma, lung and renal involvement. A total of 35 mutations of the SLC7A7 gene were identified by three research groups (Italian, Spanish-Finnish, and Japanese) in 115 LPI patients from all continents. Except for the IVS6-2AT mutation, found in all the Finnish patients, only 5 mutant alleles (1670insATCA, 1471delTTCT, W242X, R410X and IVS4+1GA) were found in more than a single LPI family. Private mutations account for most of the mutant alleles of non-Finnish origin. This mutational heterogeneity reaches the highest level in Italy where 10 different mutant alleles were identified in 17 LPI independent families from Southern Italy. This suggests that LPI is present worldwide and that the relatively higher frequency in Finland is due to a founder effect. The diagnosis of LPI is difficult and it is likely that the disease has been under-recognized in countries other than Finland. Clinical data from LPI patients of non-Finnish origin (18 different countries) underline the wide clinical variability in LPI and the lack of specificity of the clinical presentation. In addition, diagnostic laboratory tools rarely provide an unequivocal LPI diagnosis. As a consequence, most of patients receive misdiagnoses such as celiac disease, hemophagocytic histiocytosis, myopathy, infectious diseases and various metabolic disoders. We propose a diagnostic work-up as a rapid diagnosis of LPI is necessary to avoid hyperammonemic crisis by appropriate therapy. In addition, a well designed follow-up is requested to monitor life-threatening complications.

Usher Syndrome Type 1a: From Myth to Reality. *S. Gerber[1], D. Bonneau[2], A. Munnich[1], J.L. Dufier[3], J.M. Rozet[1], J. Kaplan[1]* 1) INSERM U781, Hopital Necker-Enfants Malades, Paris, France; 2) Service de Genetique Medicale, CHU d'Angers, France; 3) Service d'Opthalmologie, Hopital Necker-Enfants Malades, Paris, France.

The purpose of the present study was to check the reality of the "French variety" of Usher Type 1A, thirteen years after its report, in light of unexpected data that occured since then. The USH1A locus was described in 1992 in eight families originating from the small area of the Poitou-Charentes region around the town of Bressuire in France. Subsequently, an enlarged panel USH1 families was considered including the eight original USH1A families and two additonnal ones hailing from Bressuire. Surprisingly, one of these two additional families appeared unlinked to the USH1A locus on 14q32.1. Besides, an additional affected individual in one of the original USH1A families turned to be haplodifferent to his affected sibs at this locus. Therefore we decided to screen for mutations the major USH1 gene, the myosin VIIA in one affected individual of each family using direct sequencing. Among the 10 families originating from Bressuire, 7/10 harboured mutations in the myosin VIIA gene, 1/10 was compatible with the USH1D and USH1E loci, 1/10 excluded all seven hitherto identified USH1 loci. No DNA was available for further linkage studies in the last family. In conclusion, the recent study of additonal families affected with Usher syndrome type 1 originating from Poitou-charentes clearly showed that i)the USH1A locus does not exist; ii) the myosin VIIA gene is the major USH1 gene and finally iii) that, in our series, one family excluded all USH1 loci suggesting that at least one additional gene should be identified.

Traumatic Brain Injury and APOE-e4 allele: cause or risk of Alzheimers Disease? *R. Cittadella[1], V. Andreoli[1], G. Bono[2], M. Mauri[2], E. Sinforiani[3], F. Boller[4], A. La Russa[1], I. Manna[1], E. Altomare[1], G. Nappi[3], A. Quattrone[5]* 1) Inst Neurological Sci, CNR, Cosenza, CS, Italy; 2) Department of Clinical Medicine, VA, Italy; 3) IRCSS C. Mondino,PV, Italy; 4) INSERM U549,Paris, France; 5) University of Magna Graecia,CZ,Italy.

The hypothesis, that traumatic brain injury (TBI) may influence the development of Alzheimers Disease (AD) in human subjects encouraged several research lines. The link between head injury and dementia/AD is controversial. Recent epidemiological studies have shown that head injury is a risk factor for the development of dementia/AD. The e4 allele of Apolipoprotein E and TBI are both risk factors for the development of AD. Several studies have found that individuals who sustained moderate to severe head injury are more likely to develop dementia and AD. These risk factors appear to act synergistically, in that individuals who are ApoE4+; are even more likely to develop dementia if they sustain TBI at some time in their life. For example, ApoE4-; individuals were 10 times more likely to develop AD after TBI than those who were ApoE4-, whereas ApoE4 in the absence of injury was associated with only twice the risk. We present the results of a cross-sectional and longitudinal study of the relationship between these factors in northern and southern Italy. The study included 337 consecutive patients with probable AD and 63 subjects with Mild Cognitive Impairment (MCI). Information concerning head injuries was collected by interview of multiple informants. Twenty-one patients with dementia and 9 with MCI were found to have a history of TBI with loss of consciousness. AD and MCI patients with TBI, compared with a control group matched by age, sex, education and degree of mental impairment, showed more marked depressive and behavioral disturbances (GDS and NPI $p<0.05$). Among the patients with TBI and cognitive impairment, a high frequency of e4 allele was detected. The higher frequency of TBI and e4 genotype among AD and MCI patients confirms the synergistic interaction of enviromental and genetic factors in the development of dementia. This study has been supported by grant Mini-San 2000 IRCSS-C. Mondino-Pavia.

Genetic characterization of a family with asymptomatic high serum gamma-glutamyltransferase (GGT) concentration. *A. De Grandi[1], B. Taibi[1], F. Marroni[1], I. Pichler[1], S. Pedrotti[1], C. Beu Volpato[1], P.P. Pramstaller[1, 2]* 1) Department of Genetic Medicine, EURAC Research, Bolzano, Italy; 2) Department of Neurology, General Regional Hospital, Bolzano, Italy.

GGT is one of the major enzymes involved in the metabolism of glutathione. In humans, hepatic GGT activity is elevated in some liver diseases and also in liver damage. Patients with cholestasis usually have increased serum GGT concentration, which may be due to certain enzyme-inducing drugs or alcohol abuse. This GGT involvement, in the presence of transition metals like iron, is demonstrated by the production of free radicals which cause oxidative stress. GGT is also independently associated with cardiovascular mortality. Its activity in serum is the sum of the activities of at least four heterogeneous isoenzymes. This study involves an Italian family, already described by Bibas et al. (1994), in which 7 members in 3 generations showed unexplained high (100-fold) elevation of serum GGT. There was no history of drug use, alcohol abuse, or any form of viral hepatitis, and all family members studied were healthy and had normal results on other liver function tests. Inheritance appeared to follow an autosomal dominant pattern. A whole genome wide scan analysis was performed and lead to a peak signal in the 22q11.1-q11.2 region. In this region all 7 individuals shared a common haplotype not present in other family members in a region known to have at least 4 genes in the GGT family. Analysis of the chr22 region identified large repeat sequences involving part of the GGT transcript region. The presence of low copy repeats (LCR22s) hindered the search for mutations in the GGT genes. Rearrangements in this region of chr22 were already reported in some rare genetic diseases like VCFS/DGS and cat-eye syndromes. Real time PCR experiments show the presence of a deletion in the GGT2 locus in the 7 individuals with the condition. Further studies to better understand the genomic organization and transcription regulation in this region on chr22 are in progress.

Simple F-ratio test reveals Gene-Gene Interactions in Case-Control Studies. *G. Chen, A. Yuan, J. Zhou, A. Adeyemo, C. Rotimi* Natl Human Genome Ctr, Howard Univ, Washington, DC.

To analysis the gene-gene interactions in human disease case-control studies, we propose a simple F-ratio non-parameter method to measure these interactions. In this three steps method, first we examine dependence of genes in cases and controls, respectively. Second, F-ratio test is used to evaluate the ratio of dependence in cases than controls. Final, False Discovery rate (FDR) to evaluate the multiple F-ratio tests. Comparing with logistical regression method, we show F-ratio test is an efficient approach to measuring gene-gene interactions.

Molecular diagnosis of hypertrophic cardiomyopathy with a high-throughput 12-gene DNA resequencing chip: the first 38 cases. *S. Fokstuen[1], R. Lyle[2], A. Munoz[2], C. Gehrig[2], R. Lerch[3], M. Beghetti[4], A. Perrot[5], K.J. Osterziel[5], F. Mach[3], J. Sztajzel[3], S.E. Antonarakis[1,2], U. Sigwart[3], J.L. Blouin[1]* 1) Medical Genetics, Univ. Hospitals of Geneva, Switzerland; 2) Genetic Medicine and Development, Univ. of Geneva School of Medicine, Switzerland; 3) Cardiology, Univ. Hospitals of Geneva, Switzerland; 4) Pediatric Cardiology, Univ. Hospitals of Geneva, Switzerland; 5) Charité-Universitaetsmedizin Berlin, Germany.

Hypertrophic cardiomyopathy (HCM) is a primary autosomal dominant disorder of the myocardium with a prevalence of 1/500 showing a wide range of clinical features. Over 300 different mutations in 16 genes have been identified so far. The large allelic and genetic heterogeneity of HCM requires high-throughput, rapid and cheap mutation detection technologies in order to efficiently integrate molecular knowledge into clinical practice. We have developed a custom DNA resequencing array for molecular diagnosis of HCM. Our chip is able to sequence nearly 30 kb in a single experiment and contains both strands of all coding exons (160), splice-site junctions and known promoter regions of 12 genes mutated in HCM. We analysed a first series of 38 unrelated patients with HCM (17 familial, 21 sporadic). The mean base call rate of successfully assigned nucleotide was 96%. Overall we identified 20 different functional variants in 22 patients (58 %). Three functional variants were known mutations (1 nonsense, 2 missenses) and four were known functional SNPs. All the others were novel functional changes in highly conserved regions (3 splice, 2 promoter regions, 8 amino-acid replacements). In order to assess the potential pathological role of the 8 new missense variants, we screened 192 chromosomes of a control population of matched ethnic origin. Five variants were absent in the controls, 2 were found in 4 chromosomes and 1 was found in 10. In conclusion, our results demonstrate the need to screen all coding sequences, splice sites and promotor regions of the entire panel of HCM genes for appropriate clinical genetic testing. Our high-throughput HCM array allows to screen efficiently 12 of the so far 16 known HCM genes in a reliable, fast and cost-effective way.

NTRK2 is a susceptibility gene for obsessive-compulsive disorder, while BDNF involvement is limited to patients with positive family history. *M.P. Alonso[1], M. Gratacos[2,3], J.R. Gonzalez[2,3], J.M. Menchon[1], R. de Cid[2,3], C. Segalas[1], M. Bayes[2,3], J. Labad[1], J. Vallejo[1], X. Estivill[2,3,4]* 1) OCD Clinic, Department of Psychiatry, Hospital Universitari de Bellvitge, Hospitalet de Llobregat, Barcelona, Spain; 2) Genes and Disease Program, Barcelona, Spain; 3) National Genotyping Center (CeGen), Center for Genomic Regulation, Barcelona, Spain; 4) Department of Health and Life Sciences, Pompeu Fabra University, Barcelona, Catalonia, Spain.

Growth factors promote cell survival and neuronal differentiation and are known to be involved in synaptic efficiency and neuronal plasticity. Brain-derived neurotrophic factor (BDNF) pertains to the neurotrophin family and has been involved in several psychiatric disorders. To test the involvement of BDNF and its receptor (NTRK2) in obsessive-compulsive disorder (OCD), we performed an association study of 54 TagSNPs covering both genes in a sample consisting on 134 patients (OCD Clinic of Bellvitge University Hospital) and 342 control samples. TagSNPs were selected from the HapMap project (Phase I data freeze, dbSNP b124) and considering the genotypes corresponding to the 60 individuals from the CEPH-30-trios of European ancestry. Eight TagSNPs were selected that cover BDNF and 46 for coverage of NTRK2. We performed both single case-control association analysis and haplotype analysis. No significant differences in genotype and allele distributions of BDNF, including the functional Val66Met variant, were detected between OCD and the control group. A significant association of an intronic SNP in NTRK2 receptor gene and the presence of OCD was identified ($p = 0.001$). When considering OCD family background, both a single SNP and a composite haplotype was identified for BDNF ($p = 0.0003$ and $p = 0.01$, respectively). Furthermore, an intronic SNP from the NTRK2 gene and OCD with positive family history of OCD emerged ($p = 0.0002$). Our findings suggest that NTRK2 is a susceptibility gene for OCD, while BDNF involvement in OCD vulnerability appears to be limited to the subgroup of patients with positive family history of OCD.

Inhibition of topoisomerase I prevents common fragile site expression. *M.F. Arlt, R.L. Ragland, T.W. Glover*
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Common fragile sites (CFS) are regions of the genome that exhibit chromosome gaps and breaks after cells are grown under conditions of replication stress. CFSs are frequently deleted or rearranged in cancers, with the most frequently-expressed CFSs (FRA3B, FRA16D) mapping within tumor suppressor genes (FHIT, WWOX). Partial replication inhibition by aphidicolin (APH), an inhibitor of DNA polymerases, is highly effective in inducing chromosome gaps and breaks at these sites. APH has been shown to uncouple the replicative helicase complex from the polymerases in vitro, causing continued unwinding of template DNA without replication, leading to long stretches of single stranded DNA. We hypothesize that the APH-induced uncoupling of the helicase and polymerase functions is an integral part of CFS expression. Under this hypothesis, if the uncoupling of polymerase and helicase activities were attenuated, CFS instability should be reduced, even in the presence of APH. To test this hypothesis, we treated normal lymphoblasts and primary lymphocytes with APH in combination with low doses of the topoisomerase I inhibitor, camptothecin (CPT). Topoisomerase I reduces the torsional stress ahead of the replication fork and is required for continued DNA unwinding by helicase. We found that, in the presence of APH, varying doses of CPT result in up to a five-fold reduction of total gaps and breaks as well as a ten-fold reduction of specific CFS breaks, almost down to levels seen in untreated cells. This reduction in CFS expression is not due to checkpoint induction, as CPT treatment results in a reduction, rather than an increase, of APH-induced phosphorylation of CHK1, a checkpoint protein important in CFS stability. We are currently investigating if the transcription function of topoisomerase I is involved in CFS expression. These results indicate that, unlike most mammalian proteins examined to date, topoisomerase I is required for the induction of CFS instability, rather than for its suppression. Furthermore, they support models for CFSs based on regions of single-stranded, unreplicated DNA.

The effects of SNP genotyping errors on power of the Cochran-Armitage linear trend test for case/control association studies. *K. Ahn[1,2], D. Gordon[3], S.J. Finch[2]* 1) Health Evaluation Sciences, Penn State College of Medicine, Hershey, PA; 2) Applied Mathematics and Statistics, Stony Brook University, Stony Brook, NY; 3) Genetics, Rutgers University, Piscataway, NJ.

The questions addressed in this work are: What single nucleotide polymorphism (SNP) genotyping errors are most costly, in terms of minimum sample size necessary (MSSN) to maintain constant asymptotic power and significance level, when performing case-control studies of genetic association applying the Cochran-Armitage trend test? Which trend test or 2X3 Chi-squared test is more powerful under standard mode of inheritances (MOIs) with genotyping errors? Our strategy is to expand the non-centrality parameter of the asymptotic distribution of the trend test to approximate MSSN using a Taylor series linear in the genotyping error rates. We apply our strategy to example scenarios that assume recessive, dominant, additive, or over-dominant disease MOIs. The most costly errors are recording the more common homozygote as the less common homozygote and the more common homozygote as the heterozygote for each of the trend tests considered, with MSSN that become indefinitely large as the minor SNP allele frequency approaches zero. Misclassifying the heterozygote as the less common homozygote can be indefinitely costly when using the recessive trend test on data from a recessive MOI. The 2X3 Chi-squared test has power close to but less than the optimal trend test and is never dominated over all MOIs studied by any specific trend test.

FOS - the Fabry Outcome Survey - a newly expanded global outcomes database for patients with Fabry disease.

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Fabry disease is a rare debilitating X-linked lysosomal storage disorder caused by deficiency of the enzyme -galactosidase A. Introduction of enzyme replacement therapy with agalsidase alfa led to the establishment of two multinational outcomes databases: FOS (the Fabry Outcome Survey) based in Europe, and FIRE (the Fabry Information Research Exchange) in the rest of the world. These were recently merged and the expanded database now contains comprehensive information on 1230 patients (604 females, 626 males) from 107 clinical centres in 18 countries. The present analysis of the expanded FOS database was undertaken to assess the compatibility of the two original independent databases and to explore any differences that may exist in patient populations from different regions. The age of patients was 36.017.1 and 36.915.2 years (meanSD) in FOS and FIRE, respectively. In FOS, 48.1% of patients were males vs 56.7% in FIRE. End-stage renal failure was present in 13.4% (95% CI, 10.2-17.1%) of males and 0.7% (0.1-2.0%) of females in FOS and in 14.3% (10.0-19.6%) of males and 1.2% (0.1-4.2%) of females in FIRE. Left ventricular hypertrophy was reported in 41.1% (95% CI, 36.2-46.2%) of males and 25.1% (21.0-29.6%) of females in FOS and in 25.1% (19.6-31.3%) of males and 15.9% (10.7-22.3%) of females in FIRE. In FOS, stroke had occurred in 9.2% (6.6-12.4%) of males and 5.8% (3.8-8.4%) of females; in FIRE, stroke had occurred in 7.6% (4.5-11.9%) of males and 2.9% (1.0-6.7%) of females. Transient ischemic attacks were reported in 8.4% (5.9-11.6%) of males and 7.1% (4.9-10.0%) of females in FOS and in 7.2% (4.2-11.4%) of males and 3.5% (1.3-7.5%) of females in FIRE. We conclude that the two databases are sufficiently similar to be combined for the purposes of further epidemiological investigation of Fabry disease and the effects of agalsidase alfa.

Genome-Wide Study of Isolated Cleft Palate Only (CPO) Using the 50K Affymetrix SNP Chips. *M. Ghassibe[1], B. Bayet[2], N. Revencu[1, 3], Ch. Verellen-Dumoulin[3], Y. Gillerot[3], R. Vanwijck[2], M. Vikkula[1]* 1) Laboratory of Human Molecular Genetics, Christian de Duve Institute of Cellular Pathology, Université catholique de Louvain, Brussels, Belgium; 2) Centre Labiopalatin, Division of Plastic Surgery, Cliniques universitaires St Luc, Brussels, Belgium; 3) Center for Human Genetics, Cliniques universitaires St Luc and Université catholique de Louvain, Brussels, Belgium.

Cleft lip and/or cleft palate is the most frequent craniofacial malformation in humans (~ 1/700). Genetic factors involved in cleft lip with or without cleft palate (CL/P) are thought to be different from those having a role in cleft palate only (CPO). There is a significant challenge in identifying the important genetic and environmental causes of complex traits such as isolated clefting, particularly when the numbers of genes and environmental triggers are expected to be large. We carried out a genome-wide study of 65 patients affected with CPO, 34 of which had isolated nonsyndromic cleft palate only, 18 the Pierre Robin sequence, and 12 a syndromic cleft palate. 50K Affymetrix SNP Chip analysis was performed for every patient. In order to detect copy number changes in the human genome, several analysis softwares were used: CNAT (Copy Number Analysis Tool), CNAG (Copy Number Analyzer for GeneChip) and dChip (DNA-Chip Analyzer). The results were compared to human polymorphism databases in order to eliminate all non causative deletions and duplications. Several copy number variations were identified in our cohort, including a 2p12 amplification (the CTNNA2 gene), 3p26.1 and 10q21.1 deletions. This study shows that deletions and/or duplications may be involved in the occurrence of cleft palate. Yet, several copy number changes located in coding regions may be of no pathological significance, since we can find them in non affected individuals as well. The role of these changes needs to be studied more carefully in order to see if they modify the occurrence of cleft palate. (http://www.icp.ucl.ac.be) (vikkula@bchm.ucl.ac.be).

Detection and mapping of DNA copy number alterations in human chromosome 21 using tiling BAC arrays. *F. Béna[1], C. Gehrig[1], G. Lopez[1], S. Gagos[3], J.M. Delabarre[4], A. Schinzel[5], S. Dahoun[1], J. Lespinasse[6], L. Taine[7], M. Doco-Fenzy[8], L. Colleaux[9], E.A.Y. Graison[10], M. Costantine[4], P.M. Sinet[10], S.E. Antonarakis[1], R. Lyle[2]* 1) University of Medical School, Geneva, Switzerland; 2) Institute of Public Health, Oslo, Norway; 3) Foundation for Biomedical Research of the Academy Athens, Greece; 4) EA 3508 University Paris Denis Diderot, France; 5) Institute of Medical Genetics, Schwerzenbach, Switzerland; 6) Cytogenetic Laboratory, General Hospital, Chambery, France; 7) Department of Genetics, CHU Pellegrin , Bordeaux, France; 8) Department of Genetics, IFR53 Reims, France; 9) INSERM U781 Necker-Enfants Malades, Paris, France; 10) CNRS UMR7637,Paris, France.

A major goal of understanding the molecular pathology of Down Syndrome (DS) is genotype-phenotype correlations, that is identification of Hsa21 genes or other functional elements that contribute to specific aspects of the phenotype. Rare cases of partial trisomy 21 associated with DS features could identify genomic regions associated with specific phenotypes. Studies of other rearrangments involving Hsa21 may also provide information on the contribution of Hsa21 genes to DS. In order to establish high resolution mapping of pathogenic partial aneuploidies and unbalanced translocations involving Hsa21, we constructed a BAC microarray covering 21q. The array consists of 411 Hsa21 BACs (from RPCI and CTD libraries) with a mean overlap of 85 kb giving an approximate 2-fold tiling path. The array also includes 100 BACs for normalisation as well as additional controls. We analysed 45 phenotypically well-characterised pathogenic chromosomal aberrations; including 8 complete trisomies and 32 partial aneuploidies of Hsa21. In each case, the size of the segmental aneuploidy has been estimated and in 20 cases confirmed by real-time quantitative PCR. The breakpoints have been mapped to within 200kb on average. We also tested 5 cases with a normal karyotype on the basis of clinical findings indicative of a DS phenotype. Our study contributes to the understanding of genotype-phenotype correlations in DS pathophysiology.

Association of the 4 integrin subunit gene with autism. *C. Correia[1, 2], M. Martins[1, 2], A.M. Coutinho[1], C. Marques[3], T. Miguel[4], A. Ataíde[4], J. Almeida[3], L. Borges[3], L. Gallagher[5, 6], J. Conroy[5], M. Gill[5, 6], G. Oliveira[3], A.M. Vicente[1, 2]*
1) Inst Gulbenkian Ciencia, Lisbon, Portugal; 2) Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisbon, Portugal; 3) Centro de Desenvolvimento da Criança, Hospital Pediátrico de Coimbra, Coimbra, Portugal; 4) Direcção Regional de Educação da Região Centro, Coimbra, Portugal; 5) Department of Genetics, Smurfit Institute, Trinity College, Dublin, Ireland; 6) Department of Psychiatry, Trinity College, Dublin, Ireland.

Autism is a complex behavioral disorder likely determined by interaction of multiple genes, still largely unidentified. The results from several genome scans have provided convincing evidence for the presence of an autism susceptibility locus on chromosome 2q31-q33. One of the functional candidate genes that maps in this region is the *ITGA4* gene, which encodes the Integrin alpha-4 precursor. In this study, we tested eight single nucleotide polymorphisms (SNP) in the *ITGA4* gene for association with autism in a sample of 164 autistic nuclear families. We found significant transmission disequilibrium of alleles at the rs155100 marker (*P=0.019*) and of specific haplotypes containing this SNP. It is reported that 4-integrins play an important role in the recruitment of circulating activated T-cells, monocytes and macrophages to the central nervous system and in the modulation of the behaviour of microglia. In a previous study, we have shown that an autoreactive response to a specific brain protein occurs significantly more frequently in autistic children than in age-matched controls, which may represent the immune systems neuroprotective response to a previous brain injury occurred during neurodevelopment. The finding that *ITGA4* rs1449263 marker is associated with this specific autoreactivity prompt us to suggest that the association found between autism and the *ITGA4* gene is due to a possible involvement of 4 integrin in this process of neuroinflammation. Our study provides new insights in the role of integrins in the etiology of autism, to which little attention has been paid so far.

RET-DEPENDENT AND RET-INDEPENDENT HIRSCHSPRUNG DISEASES. *L. de Pontual, A. Pelet, D. Trochet, S. ANTONORAKIS, R. TOURRAINE,, A. MUNNICH, F. JAUBERT, M. GOOSSENS, J. FEINGOLD, S. LYONNET, J. AMIEL* Dept Genetics, Hosp Necker-enfants Malades, Paris, France.

Hirschsprung disease (HSCR) stands as a model in the study of diseases with a complex mode of inheritance. A multiplicative oligogenic model with 3 loci has been proposed with RET proto-oncogene being the key player. Indeed, almost all HSCR patients harbor either a heterozygous mutation of the coding sequence or, more frequently, a hypomorphic allele located in a conserved non gene sequence in intron 1. In roughly 30% of the cases however, HSCR is associated to other malformations; Ondines Curse (CCHS), Mowat-Wilson (MWS), Waardenburg type 4 (WS4), Bardet-Bield Syndrome (BBS) and Down syndrome . The penetrance of the HSCR phenotype is incomplete in all cases. To test whether RET could be regarded as a modifier gene for the enteric phenotype in these 5 syndromes, we genotyped the RET locus in CCHS (N=143), MWS (N=70), WS4 (N=28), BBS (N=38) and Down syndrome (N=60). Splitting patients into 2 groups (with or without HSCR) for each syndrome showed a statistically significant over representation of the RET hypomorphic allele in CCHS, BBS and Down syndrome patients whereas HSCR is RET independent in MWS ans WS4 syndromes. These data illustrate the concept of a developmental gene being either a major disease-causing gene or a modifier gene. Finally, we also suggest the possibility of RET dependent and RET independent HSCR cases.

Novel genomic rearrangements of *ATM* gene identified in Ataxia-Telangiectasia Italian patients. *S. Cavalieri[1], A. Funaro[1], P. Porcedda[2], V. Turinetto[2], N. Migone[1], R.A. Gatti[3], A. Brusco[1]* 1) Department of Genetics Biology and Biochemistry, University of Turin, and Medical Genetics Unit, S. Giovanni Battista Hospital, Turin, Italy; 2) Department of Clinical and Biological Sciences, University of Turin, Regione Gonzole 10, Orbassano 10043, Italy; 3) Department of Pathology and Laboratory Medicine, The David Geffen School of Medicine at UCLA, Los Angeles, California, USA.

Mutations in the *ATM* gene lead to loss-of-function alleles. Here we report a multi-step mutation screening strategy on the *ATM* gene in 19 unrelated Italian A-T patients which allowed us to identify 37 of the 38 expected defective alleles. Most mutations (21/37) were novel. Standardized SNP and STR haplotyping followed by DHPLC screening of genomic DNA, allowed us to detect all but 5 mutations (~86%). The remaining mutations were initially investigated by RT-PCR analysis of *ATM* transcript and Southern Blotting of genomic DNA. Three large deletions were found: one 8.5-kb and two 18-kb identical deletions, spanning exons 32-36 and 21-29, respectively; both deletions involved regions rich in repetitive elements. The recent development of a Multiple Ligation of Probe Amplification (MLPA) kit covering 33 of the 65 *ATM* exons led us to screen for deletions and duplications in the remaining 3 patients where only one mutation had been found. We discovered a small deletion containing exon 31 and a duplication of exons 4-20. In conclusion this screening strategy, using SNP/STR haplotyping to rapidly identify known founder mutations, followed by analysis of point mutations and large gene rearrangements by DHPLC, MLPA, RT-PCR and Southern Blotting, allowed us to reach 98% sensitivity. Moreover the frequency of genomic rearrangements found in our patients (5/38, 13%) may suggest a full exon screening of *ATM* exons by the MLPA kit, increasing its sensitivity and reliability.

Mutation analysis of pantothenate kinase 2 (PANK2) gene in patients with pantothenate kinase-associated neurodegeneration (PKAN). *E. Battaloglu[1], B. Bilir[1], C. Yalcinkaya[2], Z. Yapici[3]* 1) Dept Molecular Biol & Gen, Bogazici Univ, Istanbul, Turkey; 2) Istanbul University, Istanbul Medical Faculty, Department of Neurology, Istanbul, Turkey; 3) Istanbul University, Cerrahpasa Medical Faculty, Department of Neurology, Istanbul, Turkey.

Pantothenate kinase-associated neurodegeneration (PKAN), formerly known as Hallervorden-Spatz syndrome (HSS), is a rare autosomal recessive neurodegenerative disorder associated with iron accumulation in the brain. Clinical features include dystonia, rigidity, pigmentary retinopathy and mental deterioration. Age of onset is usually in early adolescence (classic form); however, cases with onset in adulthood (atypical form) have also been reported. Prevalence of PKAN is estimated to be 1-3 in 1,000,000 individuals. It is associated with the mutations in the pantothenate kinase 2 gene (PANK2) on chromosome 20p13-p12.3. PANK2 gene is composed of seven exons, covering approximately 3.5Mb of genomic DNA and encodes a 50.5-kDa pantothenate kinase that is a regulatory enzyme in coenzyme A (CoA) biosynthesis. In the present study, the genetic basis of PKAN was investigated in five unrelated Turkish families with PKAN, including a total of eight patients (five males and three females). Mutation analysis using SSCP and subsequent DNA sequencing techniques revealed that a novel homozygous 664CT transition (Q222X) was present in the third exon of the PANK2 gene in all affected individuals of family 1. The members of this family had classic disease and their parents were heterozygous for the mutant allele. No disease causing mutations could be identified in the other four families, two of which were manifesting atypical phenotypes. Furthermore, a homozygous polymorphism (IVS4-9_8insTTCCCC) was present in the intronic region of the gene in family 1 and in heterozygous condition in the two atypical cases.

***In vitro* and *in vivo* effects of farnesyltransferase inhibitors for Hutchinson-Gilford progeria syndrome.** *B.C. Capell[1], M.R. Erdos[1], M. Eriksson[2], R. Varga[1], M. Olive[1], F. Kolodgie[3], H. Avallone[3], H. San[1], X. Qu[1], L.B. Gordon[4], R. Virmani[3], E.G. Nabel[1,5], W.A. Gahl[1], F.S. Collins[1]* 1) NHGRI, NIH, Bethesda, MD; 2) Karolinska Institutet, Huddinge, Sweden; 3) CVPath, Inc., Gaithersburg, MD; 4) Brown University, Providence, RI; 5) NHLBI, NIH, Bethesda, MD.

Hutchinson-Gilford progeria syndrome (HGPS) is a rare genetic disorder of premature aging and accelerated cardiovascular disease. HGPS is almost always caused by a *de novo* point mutation in the lamin A (*LMNA*) gene that activates a cryptic splice donor site, producing a mutant protein, termed progerin. Wild-type prelamin A is anchored to the nuclear envelope by a modification of the C-terminal CAAX motif by a farnesyl isoprenoid lipid. Cleavage of the terminal 15 amino acids and the farnesyl group releases mature lamin A from this tether. This cleavage site is deleted in progerin. We hypothesized that retention of the farnesyl group causes progerin to remain anchored in the nuclear membrane, disrupting the nuclear scaffold and causing nuclear blebbing characteristic of HGPS. If so, blocking farnesylation should decrease progerin toxicity. Blocking farnesylation of progerin in transiently transfected cells with farnesyltransferase inhibitors (FTIs) has been shown to restore normal nuclear architecture. Treatment of early and late passage human HGPS fibroblasts with FTIs also reduces nuclear blebbing. Therefore, we administered the FTI, tipifarnib (R115777, Zarnestra), to a transgenic mouse model of HGPS that was created by recombineering a 164 kb human BAC containing the *LMNA* gene to incorporate the 1824C>T (G608G) mutation. Mice from a stable transgenic line express the mutant *LMNA* RNA and protein (progerin) products, and display a progressive loss of vascular smooth muscle cells, strikingly similar to the cardiovascular disease of HGPS. Early results in 5 month-old animals treated with R115777 suggest little or no dropout of vascular smooth muscle cells in the media of large arteries, providing encouragement that this drug therapy may work. Based on these observations, we believe that there is sufficient evidence to initiate a clinical trial with an FTI for children with progeria later this year.

The level of HER-2/neu gene amplification may correlate with some relevant histopathological features of early stage breast cancer. *D. Bettio[1], G. Gullo[2], G. Masci[2], A. Venci[1], L. Di Tommaso[3], A. Santoro[2]* 1) Cytogenetic Laboratory, Humanitas Clinical Inst, Milan, Italy; 2) Oncology Dept.,Humanitas Clinical Inst, Milan, Italy; 3) Anatomic Pathology Laboratory,Humanitas Clinical Inst, Milan, Italy.

BACKGROUND HER-2/neu gene amplification and/or protein overexpression predict poor outcome in invasive breast cancer and have become crucial determinants in therapeutic decisions. FISH by means of the PathVysion kit was used to investigate HER-2 gene amplification in order to identify different categories of patients with different levels of amplification and correlate this value with histopathological features rather than IHC evaluation of the protein overexpression and looking for a prognostic role of these parameters. METHODS Our study was performed on 28 early stage breast cancer female patients treated with curative surgery randomly taken from the archives of Pathology. Patient characteristics were: median age: 55 (range: 36-73); invasive ductal carcinoma: 24 (86%), invasive lobular: 3 (11%); mixed (ductal and lobular) type: 1 (3%). RESULTS Seventeen patients showed HER-2 amplification with HER-2/CEP17 ratio ranging from 2.1-8.5, whereas 11 FISH negative patients represented the control group. Patients positive by FISH were divided into two groups according to the HER-2/CEP17 ratio: low-level HER-2 amplification (HER-2/CEP17 ratio 2.1-3.9) and high-level HER2 amplification (ratio 4-8). Relevant histopathologic features of tumors in the three groups, no amplification/low-amplification/high-amplification, respectively, were as follows: high histologic grade (G3): 36/33/63% (p 0.07 Mantel-Haentzel test); vascular invasion present: 45/44/75%, high proliferative MIB-1 index (MIB-1 protein 20%): 27/44/63%. No significant difference was observed for ER and/or progesterone-receptor status. On follow-up ranging from 6 to 40 months the worst clinical outcome was among the group with amplification and chromosome 17 polisomy. CONCLUSIONS This is the first observation of a possible correlation between the level of HER-2 amplification and some relevant histopathological features of breast cancer.

Defining the importance of mitochondrial gene defects in Maternally Inherited Diabetes and Deafness and in neuromuscular presentations suggesting respiratory chain dysfunction: Screening of the entire mitochondrial genome in one hundred patients with Surveyor nuclease strategy. *S. Bannwarth[1,6], V. Procaccio[2,6], C. Rouzier[1], B. Vialettes[3], D. Raccah[3], B. Chabrol[3], C. Desnuelle[4], J. Pouget[3], J.F. Pellissier[3], D. Wallace[2], J.C. Lambert[1], V. Paquis-Flucklinger[1,5]* 1) Department of Medical Genetics, CHU Nice,Nice,France; 2) Center for Molecular and Mitochondrial Medicine and Genetics,University of California,Irvine,USA; 3) CHU Marseille,France; 4) CHU Nice,France; 5) UMR CNRS/UNSA6543,Medicine School,University of Nice-Sophia Antipolis,France; 6) These authors contributed equally to the study.

Mutations of mitochondrial genome(mtDNA)are responsible for respiratory chain defects in numerous patients. Recently,we have developed a new strategy for the rapid identification of heteroplsmic mtDNA mutations. This method,which is based on the use of a new mismatch-specific DNA endonuclease,named "SurveyorTM Nuclease",enables the systematic screening of the entire mitochondrial genome rapidly and can detect different mutants present at as low as 3% heteroplasmy. We have used this new strategy for screening the entire mtDNA in a large population including patients with diabetes,hearing deficit and history of diabetes in maternal relatives on the one hand,and patients with neuromuscular features suggesting respiratory chain dysfunction,on the other hand. Here,we show that,in clinical practice,the search for mtDNA mutations is not highly relevant in maternally inherited diabetes with deafness when 3243 mutation has been ruled out. On the contrary,we identified mtDNA deleterious mutations in 18% of patients (9/50) presenting with neuromuscular symptoms. Among the 9 identified mutations,3 were novel. This result validates the interest of Surveyor TM method in such patients when both 3243 mutation and mtDNA deletions have been ruled out. In one family, we identified the 8344 mutation in association with a second 4295 mutation in the tRNAIle gene.This result suggests that a combination of different mutations can be responsible for variable phenotypes. Last,we show that polymorphisms are frequently found in a heteroplasmy state.

Dosage analysis of the proteolipid protein 1 (PLP1) gene in Pelizaeus-Merzbacher disease. *B. Bilir[1], Z. Yapici[2], C. Yalcinkaya[3], E. Battaloglu[1]* 1) Bogazici University, Department of Molecular Biology and Genetics, Istanbul, Turkey; 2) Istanbul University, Istanbul Medical Faculty, Department of Neurology, Istanbul, Turkey; 3) Istanbul University, Cerrahpasa Medical Faculty, Department of Neurology, Istanbul, Turkey.

Pelizaeus-Merzbacher disease (PMD) is a rare hypomyelinating disorder of the central nervous system with X-linked recessive inheritance, mostly affecting males. The clinical severity and age of onset vary widely among the PMD patients, but common characteristics include nystagmus, ataxia, stridor, spasticity, and mental retardation. The prevalence in the US is estimated to be ~ 1/500,000. About 80 per cent of the patients, clinically diagnosed as PMD, have been shown to carry duplications, point mutations or deletions in the proteolipid protein 1 (PLP1) gene, which is located on chromosome Xq21.3-Xq22. Rest of the PMD cases (about 20 per cent) do not have mutations in the PLP1 gene, suggesting involvement of additional loci or mutations in noncoding regions of the PLP1 gene.

In the framework of this study, a total of 23 patients with PMD phenotype (18 males and five females) from 19 unrelated families were investigated in order to unravel the genetic basis of PMD in Turkey. Since PLP1 gene duplication is the most common (60-70 per cent) type of mutation observed in PMD, testing for the duplication is reasonably the first step for genetic analysis. Various techniques were used in this study in order to detect duplications and deletions of the PLP1 gene, including interphase FISH analysis, quantitative fluorescent multiplex PCR, and restriction fragment length polymorphism (RFLP) analysis. Interphase FISH analysis revealed that PLP1 duplication was present in seven male cases. No deletions were identified in the patients. These results were confirmed by quantitative fluorescent multiplex PCR analysis. Since restriction analysis for the exonic IV/AhaII and intronic DXS 17/TaqI polymorphisms revealed no heterozygosity in males, this method was noninformative for testing of the PLP1 duplication in this cohort of patients.

Intracontinental Distribution of Haplotype Variation: Implications for Human Demographic History. *M.C. Campbell[1], S.A. Tishkoff[2], C. D. Bustamante[3], J.H. Lee[4], A. Carracedo[5], E.J. Parra[6], R. DeSalle[7], R.L. Holloway[1]* 1) Department of Anthropology, Columbia University, New York, NY; 2) Department of Biology,University of Maryland, College Park, MD; 3) Biological Statistics and Computational Biology, Cornell University, Ithaca, NY; 4) Sergievsky Center, College of Physicians and Surgeons, Columbia University, New York, NY; 5) Institute of Legal Medicine, University of Santiago de Compostela, Galicia, Spain; 6) Department of Anthropology, University of Toronto, Mississauga, Canada; 7) Division of Invertebrate Zoology, American Museum of Natural History, New York, NY.

In human evolutionary studies, a continued challenge has been to characterize ancestral genetic structure among ethnically diverse human populations within continental regions, particularly in Africa. To approach this challenge, we examined the distribution of haplotype variation at Sortilin1 (SORT1) on chromosome 1 within major geographic regions (~1400 individuals from 59 human populations). SORT1 is a receptor protein that binds neuropeptides and has been associated with neurological disorders. We selected 18 HapMap SNPs located in the non-coding and coding regions of SORT1 for genotyping by mass spectrometry. SNP haplotypes were reconstructed using PHASE and PL-EM algorithms, and population divergence was measured using Wrights fixation index (F_{ST}) and Raymond and Roussets exact test of sample differentiation in ARLEQUIN. Our results show that the among-population F_{ST} value is significantly higher in sub-Saharan Africa than in Europe, the Middle East, Central/South Asia and East Asia. In addition, exact tests also indicate that sub-Saharan African populations are more differentiated (exact P-value = 0.00368 0.00035) than populations within Europe, the Middle East, Central/South Asia and East Asia. These results suggest that diverse African populations were more subdivided with lower levels of gene flow during human history. This study provides further information regarding intracontinental haplotype variability using a large sample of healthy individuals from diverse human groups, including 11 distinct African populations which are mostly underrepresented in genetic studies.

DMP1 mutations in autosomal recessive hypophosphatemia implicate a bone matrix protein in the regulation of phosphate homeostasis. *A. Benet-Pages[1], M. Bastepe[2], B. Lorenz-Depiereux[1], M. Amyere[3], J. Wagenstaller[1], U. Müller-Barth[4], K. Badenhoop[5], R.S. Rittmaster[6], A.H. Shlossberg[6], J.L. Olivares[7], C. Loris[8], F.J. Ramos[7], F. Glorieux[9], M. Vikkula[3], H. Jüppner[2], T.M. Strom[1]* 1) Institute of Human Genetics, GSF National Research Center and Technical University, Munich, Germany; 2) Endocrine and Pediatric Nephrology Unit, Massachusetts General Hospital and Harvard Medical School, Boston, USA; 3) Human Molecular Genetics, Universite catholique de Louvain, Brussels, Belgium; 4) Medical Genetics, Hanau, Germany; 5) Division of Endocrinology, University Hospital, Frankfurt/M, Germany; 6) QEII Health Sciences Centre, Halifax, Canada; 7) Department of Pediatrics, University Hospital of Zaragoza, Spain; 8) University Children's Hospital Miguel Servet, Zaragoza, Spain; 9) Shriners Hospital for Children, Montreal, Canada.

Monogenic phosphate-wasting disorders leading to rickets typically follow an X-linked (XLH) or autosomal dominant mode of inheritance (ADHR). We have now identified a novel autosomal recessive form of hypophosphatemia (ARHP) in three unrelated families. The affected individuals showed clinical and biochemical parameters that were similar to those observed in XLH and ADHR. We performed a genome-wide linkage analysis using SNP array genotyping. Assuming that the disease alleles could be identical by descent in each family because of their rare occurence, we analysed the data by homozygosity mapping and identified a 4.6 Mb candidate region on chrosomsome 4q21 between the SNPs rs340204 and rs722937 (max. LOD score of 3.1, 2.38 and 4.15, respectively, in families 1, 2, and 3). Sequence analysis of candidate genes identified homozygous loss of function mutations in DMP1 (dentin matrix protein 1), which codes for a non-collagenous bone matrix protein expressed in osteoblasts/osteocytes along with FGF23 (fibroblast growth factor 23) and PHEX (phosphate regulating endopeptidase). FGF23 plasma levels were elevated in two of three individuals indicating that DMP1 is involved in regulating FGF23 expression. Our results add DMP1 to the growing list of genes involved in phosphate regulation.

Family based association study of the 9p22 chromosomal region in Italian subjects with allergic asthma. *A. Begnini, E. Trabetti, G. Malerba, C. Bombieri, L. Xumerle, M. Biscuola, R. Galavotti, P.F. Pignatti* Department of Mother and Child, University of Verona, Verona, Italy.

A genome scan for allergic asthma in 123 Italian families revealed linkage of skin prick test positivity to common aeroallergens (SPT) or elevated total serum IgE (IgE) with chromosome 9p22 markers (Malerba G. et al., Am J Hum Genet. 2002, Vol. 71 n. 4 Abs 1662). An association study was now performed by Transmission Disequilibrium Test (TDT) on a subset of the 19 families with non-negative linkage for SPT or IgE, using 11 SNPs located in the linked region spanning ADAMTSL1 and SH3GL2 genes, showed significant associations with some of the studied SNPs (rs1888067: p = 0.005 for SPT; rs2811807: p = 0.009 for clinical asthma). Also, multilocus analysis revealed preferential trasmission of particular haplotypes with some phenotypes (e.g. rs1928580-rs960232-rs1888067 T-C-T: p = 0.0000087 for SPT and rs2811807-rs1928580-rs960232 C-T-C: p = 0.000056 for atopy). In order to confirm the association, we are extending the analysis of the 4 polymorphisms showing significant association individually or as haplotypes on a sample of 87 asthmatic families (391 subjects) from the same population. Preliminary results on 232 subjects show no significant association with any of the phenotypes (asthma, IgE, SPT, atopy), suggesting that the previous evidence of association might be due to different locations in the 9p22 region.

Somatic instability of CTGn expansion in Myotonic Dystrophy type 1 (DM1) patients and genotype-phenotype correlation. *E. Bonifazi[1], A. Botta[1], R. Iraci[1], L. Vallo[1], V. Romeo[2], M. Gennarelli[3], C. Angelini[2], G. Novelli[1]* 1) Dept. of Biopathology, Tor Vergata University, Rome, Italy; 2) Dept. of Neurosciences, University of Padua, Italy; 3) IRCCS S. Giovanni di Dio;,Brescia, Italy.

Myotonic Dystrophy type 1 (DM1; OMIM #160900), is an autosomal-dominant genetic disorder with multisystemic clinical features associated with a CTG expansion in 3'UTR of the DMPK gene (OMIM # 605377) on chromosome 19q13.3. The number of CTG repeats at DM1 locus is extremely variable (5-35 CTG) but stable in healthy individuals. When the repeats number is between 36 to 49, they became potentially unstable (premutation) and with a repeats length above 50, the pathology arises. The DM1 mutation shows somatic instability and the CTGn expansion size correlates with the severity of the pathology and the age of onset. In this study we analyzed the intra-individual dynamics of CTGn expansion and its possible relationship with the DM1 disease progression in a ten years-period (initial time=T0; final time =T10). For this purpose, 19 unrelated DM1 patients have been phenotypically and genetically re-evaluated, ten years after their first clinical and molecular diagnosis of DM1. Data colleted at time T10 have been compared to the phenotypic classification and to the CTGn expansion established on from peripheral blood at time T0, using a Long-PCR based protocol. Our data show that CTGn expansion containing up to 300 repeats (n=8 patients) remains relatively stable in time, whereas there is a variable increase of CTGn expansion in patients (n=11) with a repeats number 300. Percent variation of CTGn expansion size ranged from 7,2% to 55,1% and the calculated mean value of this variation is of 25,7%. Genotype-phenotype correlation showed that clinical parameters which are indicative of DM1 disease progression, correlate with the CTGn repeat length only for expansions up to 300 units. No obvious genotype-phenotype correlation has been observed in DM1 patients with CTGn expansion 300, possibly because of an higher degree of somatic instability. Our experience has confirmed that direct molecular analysis for the DM1 mutation is of considerable value in pre- and postnatal diagnosis and in phenotype prediction.

Association analysis of RAGE and BTNL2 genes with sarcoidosis in the Italian population. *C. Bombieri[1], I. Campo[2], M. Zorzetto[2], I. Ferrarotti[2], P.F. Pignatti[1], M. Luisetti[2]* 1) Section Biology & Genetics, DMIBG, Univ Verona; 2) Biochemistry and Genetics lab, Resp Dis Clinic, IRCCS, Univ of Pavia; Italy.

Sarcoidosis is a polygenic multisystemic immune disorder with predominant manifestations in the lung, characterised by infiltration of CD4+ activated T-cells leading to the formation of specific non-caseating granulomas. We performed a case-control association analysis with RAGE and BTNL2 genes, which could be implicated in the inflammatory process surrounding granuloma, and are located in a region (6p21) in linkage with sarcoidosis. RAGE -374T/A (rs1800624) and BTNL2 16071G/A (rs2076530) polymorphisms were analyzed in 2 series of DNA samples from sarcoidosis patients (99 from biopsies obtained for confirmation, and 101 from blood of a second replication set) and in 262 geographically matched controls (staff members and blood donors). RAGE -374 TT/AT genotype frequency was significantly higher in sarcoidosis biopsies than in controls (90% vs 76%, p=0.004). This findings was confirmed in the second group of patients (94% vs 76%, p=0.000105). Genotype and allele frequencies of BTNL2 16071G/A polymorphism showed no statistical difference between patients (A 60%) and controls (A 67%). BTNL2 16071G/A was associated with sarcoidosis in German and African-American populations (Valentonyte et al., Nat Genet 2005, 37:357-64; Rybicki et al., Am J Hum Genet 2005, 77:491-9). Data obtained in this study from the Italian population do not confirm the possible involvement of BTNL2 16071G/A in sarcoidosis susceptibility, as suggested by previous findings on other populations. In conclusion, this work provides evidence for a possible role of the RAGE gene as a risk factor for sarcoidosis.

Megamitochondria formation in a murine model of Mut class methylmalonic acidemia. *R.J. Chandler[1], M.S. Tsai[1], J. Sloan[1], P.M. Zerfas[2], V. Hoffmann[2], C.P. Venditti[1]* 1) Genetic Disease Research Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, United States of America; 2) Division of Veterinary Resources, Office of Research Services, National Institutes of Health, Bethesda, Maryland, United States of America.

Mut class methylmalonic acidemia (mut-MMA) is an autosomal recessive inborn error of metabolism caused by a defect in the adenosylcobalamin dependent enzyme, methylmalonyl-CoA mutase. Affected patients suffer from life-threatening intermittent metabolic decompensation, metabolic strokes, and renal failure; the etiology of these complications remains unknown. Current treatments include dietary restriction of precursors, vitamin B12 supplementation in responsive patients as well as liver and/or kidney transplantation in the most severely affected patients. Paradoxically, liver transplantation does not appear to substantially reduce the plasma levels of methylmalonic acid in comparison to non-transplanted patients with intact renal function, but does confer greatly increased metabolic stability. Murine models of mut-MMA were created to recapitulate the phenotype observed in human mut-MMA and mitochondrial morphology was studied. The use of a mixed genetic background afforded increased survival for some animals that were homozygous for the knock-out allele. Megamitochondria and increased lipid droplet formation in the hepatocytes in the mut-MMA mice were documented by ultrastructural studies utilizing electron microscopy. The proximal tubules of the affected animals were also found to have abnormal mitochondria, which were enlarged with distorted cristae and contained inclusions and unusual lamellar structures. Mitochondria in the skeletal muscle were normal. These findings indicate a tissue specific response inherent to mut-MMA and for the first time, provide evidence to link mitochondrial function as the etiology of organ system dysfunction seen in this disorder. Furthermore, these observations explain the protective effects of liver transplantation in methylmalonic acidemia and should guide the development and testing of new therapies for affected patients.

A de novo case of Alpha-Thalassemia Mental Retardation Syndrome detected by genome-wide analysis using BAC arrays. *W. Gibson[1], C. Harvard[1,2], Y. Qiao[1,2], M. Somerville[3], S. Lewis[1], E. Rajcan-Separovic[2]* 1) Dept Medical Genetics, UBC, Vancouver, Canada; 2) Dept Pathology, UBC; 3) Molecular Diagnostic Laboratory, Edmonton, AB.

We report an 8 year-old girl who presented with developmental delays and mild dysmorphic features. She spoke her first words at 2 years, and walked independently at 3 years. She had myringotomy tubes for conductive hearing loss. She exhibited delays in motor, social, academic and personal/self-care skills. She also had low truncal muscle tone. Assessment with the Stanford-Binet Intelligence Scales found her intellectual abilities ranged from below the 0.1st centile (Working Memory) to the 10th centile (Quantitative Reasoning). Growth was normal. Facial features included a broad forehead with up-slanting palpebral fissures, a prominent nasal root and bridge, and flattened maxilla. She had a high, arched palate and a tongue frenulum. Hb electrophoresis at 13 months showed H bodies with normal Hb A2. CT scan showed reduced volume of the periventricular white matter posteriorly. MRI showed a thin corpus callosum. Whole-genome BAC array analysis revealed a sub-microscopic loss of 16p material involving clones RP11-344L6 at 0.1Mb, RP1-121I14 at 0.2Mb and RP11-334D3 at 1Mb. FISH confirmed the deletion of RP1-121I14 was de novo, and revealed a slight difference in signal intensity for RP11-334D3, suggesting that a break occurred within this, the most proximal clone. Her deletion may thus be as large as ~1Mb. Deletions of the distal end of 16p are associated with Alpha-Thalassemia Mental Retardation Syndrome, (ATR-16; MIM No. 141750), characterized by mental retardation with mild, nonspecific dysmorphic features. Hb electrophoresis can provide a critical clue to diagnosis by showing mild hypochromic anemia and occasional Hb H inclusion bodies. The disorder is caused by a contiguous deletion on chromosome 16p that removes both alpha-globin genes, in addition to others such as SOX8, which are believed to be involved in brain development. Testing for SOX8 is pending in our patient. To facilitate future diagnoses, we review the main clinical features established so far and compare them with our patient.

Expanded newborn screening using tandem mass spectroscopy in Mississippi in 2005. *C.A. Friedrich[1], T. Carey[2], P. Hoggatt[2], B. Polk[2], D. Freer[3], H-G.O. Bock[1]* 1) Dept Prev Med, Medical Gen, Univ Mississippi Medical Ctr, Jackson, MS; 2) Mississippi State Dept of Health, Jackson; 3) Pediatrix, Pittsburgh, PA.

In June 2003 Mississippi became the first state to screen all newborns with an expanded test panel that included tandem mass spectroscopy (MS/MS) and selected DNA tests. Tests were performed under contract by Pediatrix (Pittsburgh, PA). In 2005, the most recent year for which complete results are available, 42,877 samples from 41,199 newborns were tested. 3.95% required repeat samples because the first sample was unacceptable, inconclusive, or positive. 95.65% showed no abnormalities. 1.73% were unacceptable, 1.25% were inconclusive, 0.93% were obtained < 24 hours after birth, and 0.44% were positive. 1140 (2.66%) were unacceptable including those drawn at < 24 hours. Of these 87% were partially acceptable. The most common technical reason for an unacceptable sample was insufficient quantity (60%). Of the presumptive positive samples 75 (0.17%) had a hemoglobinopathy, 33 (0.08%) had congenital hypothyroidism (one confirmed), 4 (0.01%) had congenital adrenal hypoplasia (one confirmed), 10 (0.02%) had galactosemia (4 confirmed by genotyping), 11 (0.03%) had biotinidase deficiency (5 confirmed), 7 (0.02%) had cystic fibrosis (6 confirmed clinically, 7 confirmed by genotyping), 2 (0.005%) had phenylketonuria), 4 (0.01%) had elevated methionine (one confirmed homocystinuria), 2 (0.005%) had elevated tyrosine, 5 (0.012%) had elevated propionylcarnitine (1 confirmed propionic acidemia), 3 (0.007%) had elevated butyrylcarnitine), 2 (0.005%) had MCAD (both confirmed by genotyping), and 1 each (0.002% each) had elevations of isovalerylcarnitine, 3-OH-isovalerylcarnitine, MADD deficiency, or tetradecenoylcarnitine. Additionally, many heterozygotes for sickle cell trait or cystic fibrosis were also detected but the program was not designed to provide long-term follow-up for those babies. Hurricane Katrina had a minor and transient impact on the receipt of samples for testing. The expansion of the traditional newborn screening program posed no insurmountable obstacles to the states public health and medical communities.

The Role of RUNX1 Gene Expression Variation in Down Syndrome-Related Leukemia. *K.J. Duffy, M. Olivier*
Physiology, Medical College of Wisconsin, Milwaukee, WI.

Trisomy 21, the cause of Down syndrome, affects 1 in every 800 to 1000 live births today. The phenotypes associated with this chromosomal abnormality range in severity and include an increased risk for several types of leukemia. Because children with trisomy 21 have a 20 times greater chance of developing acute myeloid leukemia and a 500 times greater chance of developing acute megakaryoblastic leukemia (AMKL), it is reasonable to postulate that the over-expression of genes on chromosome 21 may play a critical role in this process. RUNX1, a gene located on chromosome 21, plays a crucial role in the development of other acute myeloid leukemia (AML) subtypes. Here, we examined the linkage disequilibrium structure of the RUNX1 gene region and analyzed whether individual common haplotypes lead to over-expression of RUNX1, an effect that would be further potentiated in trisomy 21. Using the HapMap CEPH genotype data, we identified 95 blocks of high linkage disequilibrium (LD) across a 2Mb region of interest. We determined the common haplotypes (frequency >5%) in each of the eight largest LD blocks, the average size being 30kb. We subsequently identified CEPH individuals homozygous for common haplotypes within each block. Using lymphoblastoid cell lines (Coriell Cell Repository) for these homozygous individuals, we measured the relative mRNA expression levels of RUNX1 by real-time PCR. One of the eight blocks of LD showed a significant difference in RUNX1 mRNA expression between haplotypes. The block is 35kb in size and located in the middle of the RUNX1 gene. Six tag SNPs define seven common haplotypes in this block. Of these, cell lines homozygous for haplotype 2 (frequency 30%) showed significantly increased RUNX1 mRNA expression versus all other haplotypes tested. Expression was elevated approximately 1.7 fold ($p<.0002$). This haplotype-specific difference in RUNX1 gene expression in chromosomally normal cell lines suggests that sequence variants within RUNX1 combined with an over-expression of RUNX1 in trisomy 21 may play a functional role in the development of trisomy 21-related AMKL.

Haplotype analysis of the angiotensinogen (AGT) gene in 62 worldwide populations. *R.R. Ferrucci[1], A. Pace[2], S.W. Watkins[1], T. Nakajima[3], L.B. Jorde[1]* 1) Dept Human Genetics, Univ Utah, Salt Lake City, UT; 2) University of Utah School of Medicine, Salt Lake City, UT; 3) Department of Molecular Pathogenesis, Medical Research Institute and Laboratory of Genome Research, School of Biomedical Science, Tokyo Medical and Dental University.

We have constructed four-SNP haplotypes for the AGT gene in 62 worldwide populations using the Foundation Dausset-Centre dEtude du Polymorphisme Humain (CEPH) Human Genome Diversity Project (HGDP) panel and additional population samples from our laboratory (N = 1572 individuals). Our previous studies demonstrated high frequencies of ancestral AGT alleles in tropical African populations. Ancestral AGT alleles are associated with high levels of sodium retention, which, while predisposing adults to hypertension, may have been selectively advantageous in the sodium-poor tropical environment. We, therefore, would expect other tropical populations to have high frequencies of these alleles. Our results in South American and Oceanian populations are consistent with the salt retention hypothesis. Further analysis was performed on the South Asian Indian population sample, composed of Brahmins, two lower caste populations, and two tribal populations. Though some of these populations show consistency with the salt retention hypothesis, others do not. This likely reflects the effect of genetic drift. In addition, low heterozygosities and fixation or near-fixation for ancestral AGT alleles are given as evidence for founder effects in the peopling of South America and Oceania. Finally, a mutational model was developed for AGT evolution.

Niemann-Pick type C disease: molecular genetic study in a cohort of eleven patients with highly variable clinical manifestations. *L. Dvorakova, M. Hrebicek, M. Bouckova, H. Vlaskova, L. Stolnaja, M. Elleder* Inst Inherit Metabol Disorders, Charles University, 1st Sch Medicine, Prague, Czech Republic.

Niemann-Pick type C disease (NPC, OMIM #257220) is a severe autosomal recessive neuro-visceral disorder characterized by progressive neurological deterioration and hepatosplenomegaly. The most prominent biochemical feature is complex lipid storage of free cholesterol and glycolipids due to dysregulation of the cellular lipid trafficking. Molecular defects in two late endosomal/lysosomal proteins (NPC1 and NPC2) are involved in manifestation of NPC.

During the last 35 years about 40 NPC patients from Czech and Slovak populations (15 mil.) were diagnosed by histochemical and biochemical methods. Only one of these patients was proved to belong to NPC2 complementation group. To characterize the molecular basis of the disease, we established the methods for mutation analysis in *NPC1* and *NPC2* genes. Up to now we analyzed 11 patients coming from Czech Republic, Slovakia, Brazil and Germany. The patients had highly variable clinical manifestations - from infantile neurological form to adult solely visceral form. Although both genes (*NPC1* and *NPC2*) were examined in the patients, the molecular defects were detected only in *NPC1* gene. We have identified 17 different mutations on 21 NPC1 alleles, 7 of them being novel. Besides missense and nonsense mutations we found a 1bp deletion, a 2bp insertion and a small insertion/deletion leading to a frameshift. Out of 12 missense mutations two were located in sterol sensing domain and five resided in cysteine rich domain. Correlation with clinical phenotype suggests classification of several mutations. Mutation A927V was found in homozygosity in the patient with late onset of neurological symptoms. The adult solely visceral form was associated with S666N and N961S. On the contrary, R958X was present in homozygosity in the patient with infantile form of NPC. By this way mutation analysis may contribute to studies of the protein structure-function relationship.

The work was supported by IGA MZ CR NR/8351-3, VZ MSM CR 0021620806 and VZ MZ CR 64165.

Visual Interpretation of Epistasis Models using the Open-Source Multifactor Dimensionality Reduction (MDR) Software Package. *N. Barney, W. Holden, B.C. White, J.H. Moore* Computational Genetics Laboratory, Dartmouth Medical School, Lebanon, NH.

Epistasis is a central feature of the genetic architecture of complex traits. However, detecting epistasis in population-based studies is challenging due to the dimensionality associated with modeling combinations of genotypes from multiple loci. We have previously developed a multifactor dimensionality reduction (MDR) approach for detecting epistasis. MDR uses constructive induction for changing the representation space of the data to make it easier to detect interactions using methods such as logistic regression. Although MDR has good power for detecting interactions, the multilocus models it generates can sometimes be difficult to interpret. We have previously evaluated an approach for the statistical interpretation of epistasis models that makes use of graphical models and information theory (Moore et al., *J Theor Biol* 2006). We showed that estimation of interaction information makes it possible to decompose an MDR model into redundant, independent, and synergistic effects. We have implemented these entropy-based measures into the freely available and open-source MDR software package. We provide two different graphical models to facilitate interpretation of epistasis models. First, we organize the entropy results as a gene network that is color-coded according to the nature of the interaction (redundant vs. synergistic). The advantage of this is that both main effects and interactions are displayed. Second, we provide the same entropy results in the form of interaction dendrograms derived from a hierarchical cluster analysis that builds a distance matrix based on the strength of the interactions. The advantage of this is that variables with stronger interactions are grouped together. The dendrograms are also color-coded to allow quick visual interpretation of the nature of the interactions in addition to their strength. These new entropy-based tools and graphs make it possible for the first time to combine statistical and visual interpretation with the MDR algorithm for mining epistatic relationships. (Supported by NIH R01s AI59694 and LM009012, PI-Moore).

Validation of the Infinium Whole Genome Genotyping assay for segmental aneuploidy profiling. *S. Colella[1], C. Yau[2], J. Taylor[1], G. Mirza[1], H. Butler[1], P. Clouston[3], A.S. Bassett[4], A. Seller[3], C. Holmes[2], I. Ragoussis[1]* 1) Wellcome Trust Centre for Human Genetics, University of Oxford, UK; 2) Department of Statistics, University of Oxford, UK; 3) Oxford Medical Genetics Laboratories, Churchill Hospital, Oxford, UK; 4) Centre for Addiction & Mental Health, University of Toronto, Canada.

Inherited copy number changes in the genome are linked to human disease and somatic chromosomal alterations are a key event in carcinogenesis. Several microarray platforms have been used to detect copy number variation, including BAC and oligo arrays. Among them, SNP arrays offer the opportunity to analyse segmental aneuploidy events while providing additional information from the genotyping data. The recently developed two Illumina Infinium whole genome genotyping assays on universal bead arrays (Infinium I and II) are promising SNP based platforms for copy number analysis in samples of clinical interest. We validated the performance of both Human-1 and Humanhap300 Genotyping arrays in detecting copy number changes using a set of well characterised patient samples. Twelve samples were tested, including: six 6p deletions, the parents of one 6p deletion patient, two 6p duplications, one *DMD* deletion and one Charcot-Marie-Tooth duplication. We used BeadStudio Genotyping module software tools for loss-of-heterozygosity(LOH)/copy number(CN) detection and also developed a new analysis strategy based on Hidden-Markov Models (HMM). With our method we were able to use all SNP genotyping calls, in contrast to the BeadStudio scores that use only the heterozygous calls (reducing the data to ~1/3). The higher data content, together with the HMM approach, allowed us to obtain a better mapping of the specific breakpoints. The combination of both datasets (total SNPs ~400,000) brought the mapping resolution to a few kilobases (down to 4kb), depending on the SNPs density on the arrays in the region of interest. The results obtained were in agreement with FISH and molecular mapping data available. We conclude that both Infinium assays are suitable for high-throughput analysis of copy number alterations and mapping of deletion/duplication breakpoints.

Hermansky-Pudlak Syndrome in Non-Puerto Rican Hispanic Children. *G.A. Golas, R. Hess, A. Helip-Wooley, M. Huizing, W.A. Gahl* MGB, NHGRI, NIH, Bethesda, MD.

Hermansky-Pudlak Syndrome (HPS) is a recessive disorder of organelle biogenesis characterized by oculocutaneous albinism, with abnormal melanosomes, and prolonged bleeding, with absent platelet dense bodies. Some patients have pulmonary fibrosis or granulomatous colitis. There are 8 known genetic subtypes, with different prognoses and management. Only HPS-1 and HPS-4 patients are at risk for pulmonary fibrosis. HPS-1 is common in northwest Puerto Rico (PR). We describe 3 non-Puerto Rican Hispanic children. Patient #125-5 is an 8 mo-old male of Mexican, German and Native American ancestry. He had nystagmus as an infant, with brown hair and irides, light skin, and decreased retinal pigment. Bruising and epistaxis led to the discovery of absent platelet dense bodies. There is no colitis. Sequencing of HPS5 revealed compound heterozygosity for c.1423dupC (exon 12) and c.835C>A (ex7); p.Q279K. Patient #150-4 is a 2 y-old male from Uruguay with blond hair, eyebrows and lashes and gray irides. Nystagmus and iris transillumination are present. Bloody stools began at 18 mo, along with nosebleeds and prolonged gingival bleeding after trauma. Dense bodies are absent. Molecular analysis revealed a heterozygous c.45delG mutation in exon 3 of HPS4. No second mutation was identified. Patient #163 is a 16-year old male of Honduran and Salvadoran ancestry. He has pale skin and his blond hair has darkened considerably. His irides are brown, and nystagmus is present. Mild bruising occurred during childhood; his platelets have no dense bodies. Episodes of abdominal pain, bloody diarrhea, and anal fissures prompted treatment for Crohns disease at age 13. Weight loss and persistent symptoms led to a colectomy with end ileostomy at age 16. A perforated bowel was found and he received 18u of platelets. There are no pulmonary symptoms. Molecular subtyping is pending. These cases provide examples of the varied clinical manifestations and molecular bases of HPS among non-PR Hispanic children. Such patients should not be assumed to have the HPS-1 subtype typical of northwest PR patients. Accurate molecular subtyping has significant prognostic and therapeutic implications.

Association tests in combined samples of nuclear families and unrelated subjects, allowing for uncertain haplotypes and missing genotypes. *F. Dudbridge* MRC Biostatistics Unit, Cambridge, United Kingdom.

In recent years many methods have been proposed to allow for haplotype uncertainty and missing genotypes in samples of case-parent trios and unrelated individuals. Rather less attention has been given to larger nuclear families and combined samples of families and unrelated subjects. Here I propose a general model for association, based on the multinomial logistic regression, which accommodates nuclear families of any size together with unrelated subjects in a single analysis. Missing genotype data is accommodated by summing over a distribution that is estimated by maximum likelihood. In special cases the model is equivalent to well-known methods for the transmission/disequilibrium test, haplotype relative risk, and logistic regression for unrelated subjects. Quantitative traits and genetic and environmental covariates are accommodated. A novel conditioning strategy is developed that allows valid tests of association in the presence of linkage for nuclear families of any size. The methods are implemented in a computer program, COCAPHASE, that is available from the author.

Haplotype analysis of Hailey-Hailey disease in Tunisian families. *M. BCHETNIA[1], S. DEGHAIS[2], R. BENMOUSLY[2], C. CHARFEDDINE[1], S. KASSAR[3], M. MOKNI[4], S. BOUBAKER[3], A. DHAHRI BEN OSMAN[4], I. MOKHTAR[2], S. ABDELHAK[1]* 1) Human genetics, Pasteur Institute of Tunis, Tunis Belvédère, Tunisia; 2) Department of Dermatology, Habib Thameur Hospital - Tunis; 3) Department of Pathology, Institut Pasteur Tunis; 4) Department of Dermatology, La Rabta Hospital - Tunis.

Hailey-Hailey disease (HHD), also known as familial benign chronic pemphigus, is a rare autosomal dominant genodermatosis characterized by persistent blisters, crusted erosions of the skin and warty papules. It typically presents in the third or fourth decade of life and is due to mutations in the ATP2C1 gene coding for a calcium ATPase localized to the Golgi in keratinocytes. In this study, we performed haplotype analysis of three unrelated consanguineous Tunisian families including 16 patients and a sporadic case using three microsatellite markers flanking the ATP2C1 gene which are D3S3606, D3S1587 and D3S3514. Results showed that there was four haplotypes cosegregating with the HHD in the tested Tunisian families. This haplotype heterogeneity provides evidence of mutation heterogeneity.

Analyzing the effects of mitochondrial tRNA gene of leu/lys and ATPase6, mutations as a possible cause of Friedreichs Ataxia in Iranian FA patients. *S. EtemadAhari[1,2], S. Kasraie[1,2], M. Houshmand[2], M. Moin[3], M. Bahar[1]*
1) Azad University of Science and Research, Tehran, Iran; 2) Molucular Medical Genetic Department, National Institute for Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran; 3) Immunology, Asthma & Allergy Research Institute, Tehran, Iran.

Friedreich's Ataxia (FA) is a severe inherited spinocerebellar ataxia that primarily affects the nervous system and heart leading to death. The gene defective in FA, FRDA, encodes a mitochondrial protein known as frataxin. A triplet repeat expansion within intron 1 of the FRDA gene results in a marked decrease in frataxin expression. Over the last years it has become clear that this results in mitochondrial iron accumulation that generates oxidative stress and results in mitochondrial iron accumulation that generates oxidative stress and results in damage to critical biological molecules. Due to the important role of the mitochondria we are analyzing mitochondrial tRNA gene of leu/lys and ATPase6 (because they have a lot of hot spots)to find point mutations,in about 20 Iranian patients. Some point mutations have been identified by PCR method and automated DNA sequence and we are going to confirm more point mutation by same methods to evaluate any possible specified point mutation related to the mitochondrial tRNA that can show the pathogenesis of the mitochondria in FA.

Mutation analysis in a cohort of 115 cases of Oral-facial-digital type 1 syndrome: an international collaborative effort. *B. Franco, C. Prattichizzo, V. Novelli, G. Giorgio, The International OFD collaborative research group* TIGEM, Fondazione Telethon, Naples, Italy.

Oral-facial-digital type 1 (OFD1; MIM 311200) syndrome belongs to the heterogeneous group of developmental disorders known as Oral-facial-digital syndromes (OFDs) of which at least nine different forms have been described. OFD type 1 is transmitted as an X-linked dominant condition with embryonic male lethality and, similar to all other forms of OFDs, is characterized by malformations of the face, oral cavity and digits, and by cystic kidney which is specific to type 1. The identification of the gene responsible for OFD1 has provided an important tool for genetic counseling and a molecular test is now available to patients and clinicians for OFD1 diagnosis and prenatal testing. To better define the clinical spectrum of Oral-facial-digital type 1 syndrome we collected though an international collaborative effort a cohort of 115 patients which includes typical OFD1 and cases with more general signs of oral-facial-digital syndromes. Mutation analysis for the OFD1 gene was performed in this collection on the 23 coding exons and the alternative spliced exons by DHPLC followed by direct sequencing. The analysis has been so far completed for 90 out of 115. Mutations were identified in 72 patients (80%). A total of 58 different mutations have been identified in exons 1 though 16, with most of the mutations occurring in exons 3, 13, and 9, respectively 15.5%, 13.8% and 10.3%. The mutations identified include frameshifts (58%), missense (13%), non sense (13%) and splicing mutations (13%). Interestingly, no mutations have been identified, so far, in the coding region spanning exons 17-23. According to our results, the incidence of polycystic kidney is underestimated in this pathology. In fact, 20 out of the 72 patients in which a mutation in the OFD1 gene has been identified and for which clinical information are available have cystic kidneys, thus indicating an incidence of polycystic kidney in this condition of at least 27.8%. Finally, an X inactivation study showed non-random X-inactivation in a third of the samples.

A Study of Mixed Mullerian Tumor with Thirteen Oncogene Probes. *S. Faruqi[1], C. Harsch[1], H. Spector[2], J. Noumoff[1]* 1) OB/GYN, Div Gynecologic Oncol, Crozer-Chester Medical Ctr, Upland, PA; 2) Department of Histology Crozer-Chester Medical Ctr, Upland, PA.

Mixed Mullerian tumors often present with multiple elements, principally including carcinoma and sarcoma. Little is known genetically about the induction of the oncogenic process or the differentiation/evolution of the tumor elements. We present here a study of a mixed Mullerian tumor (MMT) with a panel of 13 oncogenic DNA probes, selected on their potential for abnormality in general gynecologic cancers (and in MMTs). Sections of this tumor were hybridized with DNA probes for fluorescence in-situ hybridization (FISH). Probes used were single probes her-2/neu, p53, RB-1 (13q14), ZNF217 (20q13.2), c-myc, n-myc, Inversion 16 (inv16), and Cyclin D1, Multiple oncogene probes used were PML/RARA, LAvysion, Igh/myc, 15q22/6q21, 19q13/17p13, and FIP/CHIC/PDGFRA. Probes that showed significant deviation from the expected response of a healthy cell included that of ZNF217, a zinc finger protein gene recently implicated in several tumors with a relation to the female steroid hormones, and her-2/neu, also found to be amplified in breast and ovarian cancers. The multi-target probes of 15q22/6q21 and FIP/CHIC/PDGFRA showed significant amplification as well. Several other probes showed low-level amplification, defined as having an average probe count per cell of more than 2.3, but fewer than 3. The single probe for tumor suppressor gene p53 showed a low level deletion. Additionally, the dual probe 19q13/17p13 (p53) suggested deletion of at least one copy of p53 in all cells, and a low level deletion in 19q13. In general, these results have indicated areas that may be involved in the various stages of evolution and differentiation that are seen in a mixed Mullerian tumor.

Human *TRIM5* Polymorphisms and Haplotypes are Associated with Susceptibility to Human Immunodeficiency Virus Infection. *P. An[1], G.W. Nelson[1], G.D. Kirk[2], R. Detels[3], S. Buchbinder[4], S. Donfield[5], J.J. Goedert[6], J. Sodroski[7], S.J. O'Brien[8], C.A. Winkler[1]* 1) Laboratory of Genomic Diversity, SAIC-Frederick, Inc., Frederick, MD; 2) Johns Hopkins University, Baltimore, MD; 3) UCLA School of Public Health, Los Angeles, CA; 4) University of California, San Francisco, CA; 5) Rho, Inc., Chapel Hill, NC; 6) National Cancer Institute, Bethesda, MD; 7) Harvard Medical School, Boston, MA; 8) Laboratory of Genomic Diversity, National Cancer Institute, Frederick, MD.

Introduction TRIM5 protein in mammals is a major barrier to cross-species transmission of retroviruses. TRIM5 in rhesus monkey blocks infection of human immunodeficiency virus (HIV-1). Human TRIM5 is less potent at suppressing infection of HIV-1 *in vitro*. It is unclear whether human TRIM5 protects human from HIV-1 infection *in vivo*. We examined the effect of SNPs and haplotypes of human *TRIM5* on restricting HIV-1 infection in HIV-1 natural cohorts.

Methods Allelic frequencies were compared between high-risk exposed HIV-1-uninfected individuals (HREU) versus HIV-1 seroconverters (SC), and HIV-1 seronegatives (SN) versus seroconverters (SC),by the chi-square test. The Mantel-Haenszel test was used to assess the trend among SC, SN, and HREU groups with increasing resistance to HIV-1. The overall significance of the association was evaluated by the permutation test.

Results Four of 12 *TRIM5* SNPs tested were associated with modified risk of HIV-1 infection in African Americans. Two non-coding SNP variant alleles were elevated in HIV-1-infected groups and the variant alleles for H43Y and R136Q were elevated in HIV-1-uninfected groups. The ancestral haplotype specifying wild-type alleles was protective against HIV-1 infection. Permutation results indicated an overall significant association of variants with HIV-1 infection.

Conclusion These results suggest that genetic variation of human *TRIM5* influences susceptibility to HIV-1 infection. (Funded by NCI Contract N01-CO-12400).

Common coding variants in CASP8 and TGFB1 increase breast cancer risk. *A. Cox[1], A. Dunning[2], P. Pharoah[2], M. Garcia-Closas[3], D. Easton[4], The Breast Cancer Association Consortium* 1) Institute for Cancer Studies, University of Sheffield, Sheffield, United Kingdom; 2) Department of Oncology, University of Cambridge, Cambridge, United Kingdom; 3) Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, MD; 4) Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom.

Association studies are a useful tool to search for common low-penetrance breast cancer susceptibility alleles, but can often be under-powered. The Breast Cancer Association Consortium (BCAC) has been established to conduct combined analyses to confirm or refute putative genetic associations with breast cancer. In the present study we genotyped 9 single nucleotide polymorphisms (SNPs) for which there was prior evidence (published or from within the consortium) of an association with breast cancer: CASP8 D302H (rs1045485), IGFBP3 -202 c>g (rs2854744), SOD2 V16A (rs1799725), TGFB1 L10P (rs1982073), ATM S49C (rs1800054), ADH1B 3UTR a>g (rs1042026), CDKN1A S31R (rs1801270), ICAM5 V301I (rs1056538) and NUMA1 A794G (rs3750913). Data from 9-15 groups, comprising 11,323-17,229 cases and 13,733-21,643 controls, were included. Genotype-specific odds ratios (ORs) and 95% confidence intervals (95% CIs) were estimated by logistic regression, adjusting for study. A case-only analysis was carried out to look for age-specific effects.

We found strong evidence of an association with breast cancer risk for TGFB1 L10P (OR (95% CI) 1.07 (1.01, 1.13) and 1.16 (1.07, 1.25) for heterozygote and rare homozygote genotypes respectively, P-trend = 0.000094) and CASP8 D302H (0.89 (0.84, 0.94) and 0.77 (0.65, 0.92), P-trend = 0.000002). Both of these associations remained significant after excluding the original study (P= 0.0029 and P= 0.000018 respectively). There was no evidence of effect modification by age. There was weak evidence of a recessive effect of IGFBP3 -202 c>g (0.93 (0.87, 0.99), but no consistent evidence of association with the other six SNPs. TGFB1 L10P and CASP8 D302H are the first common alleles with convincing evidence of an association with breast cancer risk.

X-inactivation patterns in Aicardi syndrome. *T.N. Eble[1], P. Fang[1], W. Jin[1], V.R. Sutton[1], R.A. Lewis[1], I.B. Van den Veyver[1,2]* 1) Molecular and Human Genetics; 2) Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX.

Aicardi syndrome (AIC) is a severe sporadic neurodevelopmental disorder, characterized by a classic triad of agenesis of the corpus callosum, chorioretinal lacunae, and infantile spasms. Because nearly all affected individuals are female and the few known males with AIC have a 47,XXY karyotype, it is thought that the condition is caused by mutations in a gene subjected to X inactivation. Previous limited X-inactivation (XI) studies in AIC have had conflicting results. Therefore we studied XI in AIC, determined the parental origin of the inactive allele when skewed and investigated correlation between the severity of the phenotypes and the ratio of XI skewing, which has not been documented previously. The human androgen receptor assay was performed on DNA extracted from peripheral blood leukocytes (PBL) of 18 affected girls and their parents. Samples for this initial study came from girls with AIC who had all features of the classic triad. Phenotypic features of the study participants were confirmed with neurological and ophthalmologic examinations, parental interviews and review of medical records and were recorded in a database. With a cut-off of >70%:30%, the assay determined that 6 of 18 samples (30%) showed skewed patterns of XI. A tendency for skewing (66%:34%) was seen in 1 sample, and 1 sample was uninformative. Of the 4 samples with non-random XI where both parents could be studied, 3 preferentially inactivated the paternally inherited X chromosome. This is consistent with the hypothesis that AIC is caused by de novo X-linked mutations, which primarily arise on the paternal X chromosome. As subjects were selected by their more typical presentation, it was not surprising that we found no correlation between XI patterns and overall phenotypic features. However our data suggest a correlation with non-neurological features. DNA from additional individuals, including those with less severe phenotypes and from two brain samples, will be studied to confirm this and determine the extent of the XI-phenotype correlation. In conclusion, these initial findings support the hypothesis that the mutated gene for AIC is X-linked.

Increased power to detect disease genes in genetic linkage and whole genome wide association studies by employing a functional human gene network. *L. Franke[1], B.P.C. Koeleman[1], F. Dudbridge[2,3], M. Egmont-Petersen[4], C. Wijmenga[1]* 1) DBG-Department of Medical Genetics, University Medical Centre Utrecht, Utrecht, The Netherlands; 2) MRC Rosalind Franklin Centre for Genomics Research, Cambridge, United Kingdom; 3) MRC Biostatistics Unit, Cambridge, United Kingdom; 4) Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

Identifying the disease genes in complex diseases is proving difficult, since hundreds of genes may reside in the susceptibility loci identified by linkage analysis. To help discriminate between the causative gene(s) and the non-disease genes contained in multiple loci, we have reconstructed a functional human gene network, (Franke *et al*, 2006, AJHG), available at www.genenetwork.nl, and used it to prioritize the individual genes in each locus by assuming that disease genes will usually be functionally closer together than the other non-disease genes. We have now applied this method to genome-wide SNP association studies, which permit the analysis of hundreds of thousands of SNPs. Usually, researchers only follow-up the top set of most significantly associated SNPs (Dudbridge *et al*, 2004, AJHG). By assuming that the real causative SNPs map within genes that are closely related in the gene network, we should be able to distinguish between these SNPs and the non-disease SNPs in this set. To assess the power of this method, we simulated genotypes for 300,000 SNPs in 750 cases and 750 controls for 96 different diseases, for which at least three disease genes were known for each disease. The SNPs that resided within the disease genes were assigned an odds ratio of 1.25. Initially, in each disease, we assessed how many of the true causative SNPs were contained in the top set for a fixed number of the most significantly associated SNPs. We then analyzed a SNP set of equal size that was generated by applying our method: the probability that the true causative genes lay within this adjusted set improved substantially, thus indicating that a functional gene network can help to identify causative genes in complex diseases.

Molecular analysis of the CYP1B1 gene from FTA cards in primary congenital glaucoma in European patients.
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Primary congenital glaucoma (PCG) is a rare ocular disorder characterized by marked elevation of intraocular pressure (IOP) at birth or in early childhood, leading to ocular enlargement (buphtalmos) and corneal oedema, and then to irreversible damages on the optic nerves if it is not treated urgently. The frequency of this defect is 1 in 12,000 to 18,000 and represents 1% to 5% of all glaucomas. An autosomal recessive pattern of inheritance is common. A major gene, CYP1B1, a member of the cytochrome P450 gene family, mapping on chr.2p22.2, has been identified. This gene contains 3 exons and codes for the 544 amino acids cytochrome P4501B1 protein. In this study, we analysed the CYP1B1 gene from saliva on FTA cards of 15 cases with PCG. The male: female ratio was 8:7. Consanguinity was observed in 3 cases. The analysis, i.e. DNA extraction and PCR, was performed in our laboratory which is automated and fully computerised. By DNA sequencing analysis of the CYP1B1 gene, we found mutations in 8 patients and 3 of them were novel mutations. These data show that our process from saliva on FTA cards in an automated laboratory is reliable and rapid. This is particularly interesting for patients because of a non-invasive and painless sampling. In this study, more than 50% of the patients have at least one mutation in the CYP1B1 gene and we can conclude that PCG is not the consequence of mutations in this gene for about one of 2 patients.

Quality of life in families: children with birth defects. *B. Ballesteros[1], M. Novoa[1], I. Zarante[2], F. Suárez[2]* 1) Facultad de Psicología, Pontificia Universidad Javeriana, Bogota D.C., Colombia; 2) Instituto de Genética Humana, Pontificia Universidad Javeriana, Bogotá.

AIM: To investigate the psychological impact on family members, with a child less than 2 years old, affected with major birth defects. METHODS: A 62-item questionnaire divided in four blocks was designed to elicit from parents, and any relative living with the affected patient, the potential change in quality of life after the birth of a child with major birth defects. The first block considered the functional status of each individual in the family, the second block, evaluated the perception of the relatives, about the symptoms and the gravity of birth defects in the affected child. The third block evaluated the psychological impact in the family after the birth of the affected child. The last block measured the social behavior of the family members. 36 families (72 members) completed psychological interviews and questionnaires. No of the families had a prenatal diagnostic. The 36 affected children was less than 2 years old. The birth defects included neural tube defects, abdominal wall defects, limb defects and facial clefts. RESULTS: Stress, incomprehension, complaints about the health care system, medical appointments and prenatal procedures, lack of information about the children disease, and anxiety were often noted. But the overall score of the scales did not show any decrease in the quality of life of the family members. In fact a better cohesion between the family members was noted in almost every case. DISCUSSION: all of the familys members show behaviors associated to the unexpected birth defect but without a demonstrable decrease in quality of life. We believe that the potential change in quality of life, between the family members, are expected to appear during the future development of the affected, which is expected to be not easy, due to the complications and difficult for the required treatments and recuperation.

The Role of Protein Tyrosine Phosphatase 1 Beta (PTP1B) Sequence Variants in Obesity and the Metabolic Syndrome. *J. Eckert, T. Wang, J. Kim, A. Kissebah, M. Olivier* Physiology, Medical College of Wisconsin, Milwaukee, WI.

PTP1B negatively regulates insulin and leptin signaling. Individual single nucleotide polymorphisms (SNPs) in the gene for PTP1B have been shown to be associated with type 2 diabetes, insulin resistance, Body Mass Index (BMI) and alterations in plasma lipid levels. Recently, PTP1B haplotypes have been associated with hypertension, type 2 diabetes, and insulin sensitivity. In our study, we analyzed the linkage disequilibrium (LD) and haplotypes structure of the PTP1B genomic region on chromosome 20, and explored the association to obesity and metabolic syndrome related phenotypes. We genotyped 22 SNP in the gene region on 499 unrelated females of Northern European decent from the Midwestern U.S. A high LD region containing 16 intronic and synonymous SNPs and covering the entire gene region defined 8 haplotypes with a frequency greater then 1% and accounted for 92% of the total diversity. A common haplotype (28.6%) showed association with waist and hip circumference, BMI, fasting plasma insulin and leptin, and insulin glucose ratio (p=0.0006-0.0079) in a weighted regression model. In an effort to elucidate the potential function of this risk haplotype, we investigated its effect on mRNA expression levels by examining lymphoblast cell lines homozygous for the risk and other common haplotypes. The CEPH and Coriell Variation Panel were genotyped and homozygous females were identified. mRNA levels were quantified relative to the expression of stably expressed genes using TaqMan real-time PCR. The initial study examined six independent cell lines from female individuals, and we observed a 1.5 fold over-expression of PTP1B mRNA in cell lines homozygous for the risk haplotype compared to the most common haplotype (p=0.0001). A second confirmatory analysis in an additional set of twelve female cell lines confirmed this over-expression (p=2.2x10-6). Our analysis provides the first functional evidence for a cellular effect mediated by the associated PTP-1B risk haplotype, and the observed over-expression of the negative regulator may explain the association with insulin and leptin resistance in our cohort.

Sporadic Congenital Lymphedema Can Be Caused By A *De Novo VEGFR3* Mutation. *A. Ghalamkarpour[1], S. Morlot[2], J.B. Mulliken[3], L.M. Boon[1,4], M. Vikkula[1]* 1) Laboratory of Human Molecular Genetics, Christian de Duve Institute of Cellular Pathology, Université catholique de Louvain, Brussels, Belgium; 2) Praxis für Humangenetik, Aerztliche Partnerschaft WagnerStibbe, Hannover, Germany; 3) Vascular Anomalies Center, Division of Plastic Surgery, Children's Hospital, Harvard Medical School, Boston, USA; 4) Centre for Vascular Anomalies, Division of Plastic Surgery, Cliniques Universitaires Saint-Luc, Brussels, Belgium.

Mutations in the *vascular endothelial growth factor receptor 3* gene, *VEGFR3/FLT4*, have been identified in a subset of families with hereditary lymphedema type I or Milroy disease (MIM 153100). In this study, we report a *VEGFR3* mutation in a patient with sporadic congenital lymphedema. The phenotype included bilateral swelling of the lower limbs, prominently below the knees, accompanied by upturned toenails and mild hydrocele. The mutation was heterozygous and not present in the non-affected parents and thus constitutes a dominant *de novo VEGFR3* mutation. All earlier reports have described *inherited* mutations in familial congenital lymphedema. Our data indicate that *VEGFR3* can be the pathophysiological cause of *sporadic* congenital lymphedema and *VEGFR3* screening in such patients may lead to a specific diagnosis. This has implications for follow-up care, as such individuals have a nearly fifty percent risk for occurrence of lymphedema in their children, compared to the low incidence in general population. In addition, as *VEGFR3* mutations can cause generalized lymphatic dysfunction, we conclude that sporadic hydrops fetalis may be a result of a *de novo VEGFR3* mutation. (http://www.icp.ucl.ac.be/vikkula) (vikkula@bchm.ucl.ac.be).

Evidence for Association of the KIAA0319-Like (KIAA0319L) gene on Chromosome 1p to Reading Disabilities.

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Developmental dyslexia or specific reading disabilities (RD) is a learning disability characterized by difficulties with fluent word recognition and by poor spelling and decoding abilities. These difficulties result from deficits in the phonological components of language, and can occur despite at least average intelligence and effective classroom instruction. Many studies have found linkage and/or association of RD to several chromosomal regions. A locus on chromosome 1p34-36 (DYX8) has been previously linked to RD in three independent samples. Within this region we have identified a gene KIAA0319-Like (KIAA0319L) as a potential candidate based on its position, as well as its homology to KIAA0319, a gene that has been named as a RD susceptibility candidate on chromosome 6p (DYX2) in a number of independent association studies. In this study, association of KIAA0319L was assessed using 5 tagging SNPs in a sample of 263 nuclear families ascertained through a proband with reading difficulties. Evidence of association was found for rs7523017 ($c2 = 6.737$, $p = 0.009$; corrected $p = 0.035$) to RD defined as a categorical trait. We also observed evidence for association to quantitative measures of spelling and word reading efficiency (a composite of word identification and decoding). We conclude that KIAA0319L is likely the RD susceptibility gene on chromosome 1p and contributes to multiple reading components. These results also strengthen the case for KIAA0319 as the susceptibility gene on 6p. Studies are currently underway to find functional DNA changes in KIAA0319L that contribute to RD.

Metabolic disturbances in mice heterozygous for mevalonate kinase deficiency. *K.M. Gibson[1], A.S. Pappu[2], R.D. Steiner[2], G.F. Hoffmann[3], E.J. Hager[1]* 1) Pediatrics, Pathology, Human Genetics, Children's Hospital, Pittsburgh, PA; 2) Pediatrics and Medicine, Oregon Health & Science University, Portland, OR; 3) Pediatrics, University of Heidelberg, Germany.

Mevalonate kinase (MK; OMIM 251170/260920) catalyzes the first committed step in cholesterol and isoprene synthesis. Human MK mutations associate with diverse disorders, from hyperIgD/periodic fever syndrome to severe mevalonic aciduria. We ablated the murine MK gene employing a gene-trap disrupted ES cell line. Chimeric mice were generated by ES cell injection into blastocysts from strain C57BL/6, followed by implantation of injected embryos into pseudopregnant females for development to term. Male chimeras showing extensive ES cell-derived agouti coat color were bred with C57BL/6 females for germline transmission and identification of heterozygous MK +/- mice. Sequential MK +/- matings yielded no MK -/- animals; small litter sizes were dominated by MK +/- animals. Genotyping of 12 embryos (gestational day (E)14) revealed only MK +/- mice. Successful targeting was verified by RT-PCR and liver gene-dosage [MK/control enzyme: MK +/+; (n=4), 5.04 (1.66, SD); MK +/- (n=18), 2.54 (1.02, SD); p=0.001]. MK+/- mice appeared phenotypically normal; nonentheless, we characterized these animals for evidence of biochemical pathology. Quantitation of mevalonate in tissue extracts (heart (H), kidney (K), brain (B) and liver (L); n=8 MK +/+, n=6 MK +/-; mean (SEM), pmol/g tissue; all animals 6 weeks old) were: (H) MK +/+, 1134 (111); MK +/-, 2360 (65) ($p<0.0001$, nonparametric t test): (K) MK +/+, 2782 (300); MK +/-, 4727 (343) ($p<0.002$): (B) MK +/+, 482 (55); MK +/- 574 (46) (p=ns): (L) MK +/+, 5921 (894); MK +/-, 6784 (966) (p=ns). Metabolic disturbances in MK +/- mice suggest that additional pathway stress (e.g., statin, isoprene intervention) may induce a more prominent phenotype, and yield a model in which to explore the association of altered MK activity with disease pathology. Murine models in which MK is depleted will be useful to examine the biological role of MK related to tumorigenesis, hormone regulation, inflammatory processes, and development.

Mechanism of Isodicentric (Xq) Chromosome Formation in Turner Syndrome Patients. *N. Cohen[1], N.B. Kardon[1], W. Edelmann[2], L. Edelmann[1]* 1) Human Genetics, Mount Sinai School of Medicine, New York, NY; 2) Albert Einstein College of Medicine Bronx, NY.

Turner syndrome (TS) results from monosomy of the X chromosome and is mediated by haplo-insufficiency of genes that normally escape X-inactivation. Although half of all TS cases have a 45,X karyotype, the remaining half have other X-chromosome variants, the most frequent of which is the isodicentric (Xq) chromosome (idic(Xq)). Idic(Xq) is a spontaneously occurring chromosomal aberration that is present in 18% of TS cases, and it is the most common constitutional isochromosome in humans. The incidence of idic(Xq) is estimated at 1 in 13,000 females making idic(Xq) formation a significant cause of TS in live born females. Nine cases of idic(Xq) were analyzed to define the breakpoint regions and determine the mechanism of formation. In addition, seven translocations involving Xp11.2 were ascertained and the breakpoint regions were examined. Fluorescence In Situ Hybridization (FISH) also was performed using BAC clones from the physical map of the X chromosome that span 11 Mb from Xp11.21 - Xp11.23. Our data from the idic(Xq) and translocation cell lines indicate that most of the breakpoints fall within a 6 Mb region from 52 to 58 Mb on the physical map of Xp11.2 with clustering into at least four distinct breakpoint regions. Two of the regions contain repetitive gene clusters, including the SSX and GAGED genes, and one region harbors the inverted duplication that includes the ZXDA and ZXDB genes. Although there is not one specific region of susceptibility for idic(Xq) rearrangements, the Xp11.2 region contains a number of repetitive gene clusters which may contribute to the overall instability of this region. We are currently using high resolution oligonucleotide array CGH analysis to elucidate the exact sequences at each of the breakpoints. These studies will aid in our understanding of the role of genomic architecture on Xp11.2 in the generation of chromosomal rearrangement disorders.

Medical Sequencing at the Extremes of Human Body Mass. *N. Ahituv[1,2], N. Kavaslar[3], J. Cohen[4], R. Dent[3], R. McPherson[3], L.A. Pennacchio[1,2]* 1) Genomics Division, Lawrence Berkeley National Laboratory, Berkeley, CA; 2) US DOE Joint Genome Institute, Walnut Creek, CA; 3) University of Ottawa Heart Institute, Ottawa, Canada; 4) University of Texas South Western, Dallas, TX.

Human body weight is a quantitative trait with significant and complex heritability. To explore genetic contributors to this phenotype, we undertook a large-scale medical sequencing approach. We resequenced the coding exons and splice junctions of 58 BMI-related genes in 379 obese (average BMI 49.0 kg/m2) and 378 lean (average BMI 19.5 kg/m2) Caucasian individuals. In total, we generated over 90 Mb of sequence and detected 1074 genetic variants, the majority of which were rare (n=822, minor allele frequency <1%) of which 271 result in amino acid substitutions. While familial segregation of several characterized monogenic obesity genes failed to show complete correlation with BMI, analysis of their rare non-synonymous variants showed a significant frequency skew between the obese and lean panels (23 limited to obese versus 10 in lean). We thus used this paradigm as a filter for the other 51 genes and found 6 novel genes that may be implicated with human BMI (DGAT1, DGAT2, NMUR1, PRKAG3, and SIM1 with obesity and NTS with leanness), all showing significantly large skews between the two panels. In addition, using familial segregation analysis on a portion of the rare non-synonymous variants within the remaining novel genes, enabled us to detect other promising BMI-influencing variants. Association analysis of the 252 common variants (>1% minor allele frequency) that we discovered, failed to show any significant correlations with BMI, including previously suggested ones. Combined, these results point out the importance of complete variation identification in human genetic investigations to complement existing strategies focused on common variant mechanisms of disease susceptibility.

Early Markers of Disease Severity in Female Patients in the Fabry Outcome Survey. *P.B. Deegan[1], D.A. Hughes[2], F.A. Baehner[3], M-A. Barba-Romero[4], M. Beck[3] on behalf of the European FOS Investigators* 1) Department of Medicine, Addenbrooke's Hospital, Cambridge, United Kingdom; 2) Royal Free Hospital, London, UK; 3) Childrens Hospital, Mainz, Germany; 4) Albacete University Hospital, Albacete, Spain.

BACKGROUND: Fabry disease is an X-linked lysosomal storage disorder with heterogeneous expressivity in heterozygotes. It causes, inter alia, hypertrophic cardiomyopathy, stroke and renal impairment. Hypohidrosis commonly leads to heat intolerance. Once established, major organ involvement is often irreversible. Enzyme Replacement Therapy (ERT), though expensive, is available in many countries and may prevent progression of organ damage. Criteria for initiation of ERT in heterozygotes are still debated. AIMS: A method to predict future disease severity would assist in making decisions about early treatment. METHODS: An anamnestic questionnaire for completion by female patients was made available to clinicians participating in the Fabry Outcome Survey (FOS). Patients were asked whether they recalled experiencing less sweating, less physical activity, more days off school through ill-health and more days in hospital in childhood than their peers. Results were compared with the patients current disease severity as assessed by a modification of the Mainz Severity Score Index, corrected for age. 81 patients over 30 years of age were analysed separately. Differences in age-corrected severity scores were compared by unpaired T-test. RESULTS: 106 completed questionnaires were received. The 81 patients over 30 yrs of age were representative of the female cohort in FOS. In the group of 106 patients of all ages, all four parameters were associated with severity of disease ($p<0.05$). In 81 patients >30yrs, two parameters were associated with disease severity: less sweating and more time in hospital (p0.001). CONCLUSION: Female patients with severe features of Fabry disease recall less sweating and more admissions to hospital in childhood than their peers. These features may prove useful in predicting future disease severity in girls with Fabry disease and may inform decisions about treatment.

Cerebrotendinous Xanthomatosis: early presentation and response to treatment with Chenodeoxycholic acid.

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Cerebrotendinous xanthomatosis (CTX) is an autosomal recessive disorder due to deficiency of the mitochondrial sterol 27-hydroxylase. It is characterized by deficiency of bile acids esp. chenodeoxycholic acid (CDCA) and accumulation of cholestanol. We report a 6 year old boy who was referred at 5 years of age after surgical removal of 2 xanthomas from his left anterior thigh. He had bilateral cataracts that were removed. He had failure to thrive and long standing diarrhea. Plasma cholestanol was elevated and he had typical abnormal urinary bile acid and plasma sterol profiles. Brain MRI and BAERS were normal but VEPs were reduced. Liver function tests and vitamins D, E and K levels were normal. Psychometrics were in the average range with decreased visual and fine motor skills. CTX was confirmed by finding homozygosity for a known pathogenic mutation, c.1183C>T; p.Arg395Cys. Both parents are heterozygous with no history of consanguinity. He was started on CDCA, 15mgs/kg/day in 3 divided doses. There was cessation of diarrhea and increase in growth parameters from the 5th to the 25th centiles. A foot xanthoma resolved on therapy. Plasma cholestanol declined and other plasma sterols normalized. CDCA appears to be an effective treatment for this condition.

CDCA mg/Kg	Chol mg/L	8-DHC	Cholestano	Cholesteno	Desmo	7-DHC	Latho
0	1563	21	17.5	22.2	1.3	10.4	9
13	1861	0	9.7	0.4	1.3	0.2	1.1
12	1862	0	1.5	0	1.9	0.1	0.9
13	1962	0	6.1	0.3	0.5	0.2	0.6

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Detection of genomic deletions of the *TP53* gene in the Li-Fraumeni syndrome. *G. Bougeard[1,2], S. Vasseur[1], C. Martin[1], L. Brugières[3], A. Chompret[4], B. Bressac-De Paillerets[5], P. Jonveaux[6], H. Sobol[7], L. Gladieff[8], P. Gesta[9], S. Baert-Desurmont[1,2], T. Frebourg[1,2]* 1) Department of Genetics, Rouen University Hospital, F-76000 France; 2) Inserm U614, Faculty of Medicine, Rouen, F-76000 France; 3) Department of Pediatric Oncology; Gustave Roussy Institute, Villejuif, F-94800 France; 4) Department of Oncogenetics - Gustave Roussy Institute, Villejuif, F-94800 France; 5) Department of Genetics - Gustave Roussy Institute, Villejuif, F-94800 France; 6) Department of Medical Genetics - Nancy University Hospital, F-54000 France; 7) Marseille Cancer Institute, Molecular Oncology Department, UMR599 Inserm and Paoli-Calmettes Institute, Marseille, France; 8) Department of Oncogenetics - Claudius Regaud Institute, Toulouse, F-31000 France; 9) Department of Oncology - Niort Hospital, F-79000 France.

Absence of detectable germline mutation of the *TP53* gene in a fraction of families with Li-Fraumeni syndrome (LFS) led us to screen for *TP53* genomic rearrangements, using QMPSF (Quantitative Multiplex PCR of Short Fluorescent fragments), families without detectable point mutation. We recently reported the first case of a complete heterozygous germline deletion of *TP53*, removing all the exons, in a large French family. The screening for *TP53* rearrangements has now been integrated into the routine diagnosis of LFS, using a QMPSF assay which explores the 11 exons and the promoter of the *TP53* gene. Analysis, using both sequencing and QMPSF, of 259 families suspected to present a LFS according to the Chompret criteria allowed us to identify a *TP53* germline alteration in 52 families (20%). We identified 3 large genomic deletions, indicating that *TP53* rearrangements represent about 6% of the *TP53* alterations. In 2 distinct families, we identified 2 deletions removing exon 1 and the promoter but respecting the 10 coding exons. These results constitute a definitive argument demonstrating that the LFS results from a haploinsufficiency at the *TP53* locus and suggest that screening for *TP53* rearrangements should be integrated in the LFS molecular diagnosis.

Exploring Managed Care Decision-Maker Perspectives on the Utility and Economics of Genetic Cancer Risk Assessment: Focus groups and an educational intervention. *J. Culver[1], K. Blazer[1], H. Bichkoff[2], J. Weitzel[1]* 1) Clinical Cancer Genetics, City of Hope, Duarte, CA; 2) California Cancer Care, Inc, Greenbrae, CA.

Genetic cancer risk assessment (GCRA) is considered standard-of-care for hereditary cancer syndromes, but many patients report barriers to access, in part due to insurer denial of coverage for cancer risk consultation and genetic testing. Given that a growing proportion of care is delivered via managed care organizations (MCOs), efforts to increase access to appropriately-recommended GCRA services will need to include the dissemination of evidence-based information about the clinical utility and economics of GCRA to decision makers within MCOs. As part of an educational intervention we conducted a formal focus group to identify key themes and then conducted 4 roundtable sessions with managed care decision makers (n=20 participants), such as medical directors of independent practice associations. Each roundtable session included a continuing medical education (CME) lecture about the process, economics, and utility of GCRA, followed by an abbreviated focus group component to elicit current practices and information needs. Participants GCRA knowledge and practices were surveyed. While 32% of participants reported being very aware of the process and utility of genetic testing for cancer risk, only 11% rated their organization similarly. Thirty-seven percent stated their organizations have a written policy regarding genetic testing for cancer risk, and 47% were aware of cancer genetics resources in their service area. Content analysis of the focus groups indicated a need for more evidence-based information about the utility of GCRA, concise summaries of syndromes, and cost-effectiveness data. The focus group process also yielded valuable information regarding knowledge, opinions, perceived need for and current organizational policies regarding GCRA authorization. The educational intervention was tailored using feedback from participants and will be disseminated to top-tier decision and policy makers about the process, efficacy and economics of GCRA.

Proteomic analysis of the purkinje cell degeneration mouse yields evidence for a bioenergetics basis for the neurodegeneration. *L. Chakrabarti, S. Ryu, B. Gallis, S. Schaffer, D. Goodlett, A. La Spada* Laboratory Medicine, University of Washington, Seattle, WA.

The Purkinje cell degeneration (*pcd*) mouse is a unique recessive model of neurodegeneration, as *pcd* mice undergo a dramatic postnatal degeneration of Purkinje cell neurons and retinal photoreceptors, yielding a phenotype of ataxia and blindness. In 2002, the causal gene for *pcd* was found to be *Nna1*. The Nna1 protein contains an evolutionarily conserved zinc carboxypeptidase domain, suggesting that loss of this protease function somehow results in rapid neurodegeneration and neuron cell death. Using a proteomics approach, we compared the protein expression profiles of retinas from sets of presymptomatic *pcd* mice and their wild-type littermates (n = 4 / group). After trypsin digestion, peptide counts were obtained by LC-MS/MS analysis on an LTQ-FT. The resulting tandem mass spectrometry data (obtained in triplicate) was searched using SEQUEST, and identified proteins filtered using the Protein Prophet probability score. Protein relative abundance was measured using protein sequence coverage, peptide/spectrum count, and peptide intensity area/height, using the spectral count method. Finally, ratios were calculated for relative abundance between mutant *pcd* and wild type samples, yielding a list of the most significantly changed, highly abundant proteins ($p < 0.05$ and abundance >15). Most of the proteins showing altered expression in pcd retina are involved in energy metabolism, with 2/3 localizing to mitochondrial metabolic pathways. Especially noteworthy was a preponderance of enzymes from the glycolytic pathway, whose expression alterations in retina and cerebellum have been confirmed by Western blot analysis. Our findings thus suggest that loss of Nna1 function resulting in a bioenergetics defect underlies *pcd* pathogenesis.

The P2 Promoter of Hepatocyte Nuclear Factor-4 alpha Gene is Associated with Type 2 Diabetes, the AADM Study. *Y. Chen[1], G. Chen[1], J. Zhou[1], A. Doumatey[1], H. Huang[1], A. Adeyemo[1], G. Dunston[1], F. Collins[2], C. Rotimi[1]* 1) National Human Genome Ctr, Howard Univ, Washington, DC; 2) National Human Genome Research Institute, Bethesda, MD.

Hepatocyte Nuclear Factor-4 alpha (HNF4a), located at chromosome 20q12-q13.1, is a transcription factor important to the development and function of the beta-cell. A number of studies based on several different populations including the Finns, Ashkenazi Jews, Pima Indians and Japanese, have demonstrated association of genetic variations of HNF4a with type 2 diabetes. African American is one of the ethnic groups with the highest prevalence of type 2 diabetes, which is twice as high as that of Caucasians. Our previous genome wide scan showed evidence of linkage of type 2 diabetes to 20q11.2-q13.3 region in West Africans, the founder population of African Americans. In this study, a total of 756 individuals from four tribes of two West African countries, Nigeria and Ghana, were genotyped. We found that the G allele of SNP rs6031558, located at the P2 promoter of HNF4a gene, was associated with 1.43-fold increased risk of type 2 diabetes. Considering that the frequency of G allele in this population is 49.5%, it appeared that HNF4a played an important role in the development of type 2 diabetes in West Africans.

Determining relative values of interphase Panel FISH and conventional chromosome studies in hematological malignancies. *R.A. Conte, L.A. Cannizzaro, D.T. Walsh, M. Zohouri, K.H. Ramesh* Department of Pathology, Montefiore Medical Center, Bronx, NY.

Characterization of hematological malignancies (HM) with greater certainty is warranted for improved patient care. Flow cytometry and bone marrow (BM) histology/hematology are routinely used to type the HM, cytogenetic testing usually follows. Our goal was to determine if interphase Panel FISH (PF) analyses, (diagnosis-specific battery of DNA probes), improved this capability. Using BM or unstimulated peripheral blood samples of 119 patients we determined relative values of chromosome analyses (CA) and PF results. There were 29 patients with myelodysplastic syndrome (MDS), 23 with acute myeloid leukemia (AML), 27 with acute lymphocytic leukemia (ALL), 15 with chronic lymphocytic leukemia (CLL), 8 with non-Hodgkin lymphoma (NHL), 13 with multiple myeloma (MM) and 4 with myeloproliferative disease (MPD). In 8/119 patients (6.7%), CA and PF results were abnormal; in 5/119 patients (4.2%), PF results were normal and CA results were abnormal; in 58/119 patients (48.7%), PF and CA results were normal, and in 48/119 patients (40.3%), the PF results were abnormal and the CA results were normal, had failed or inconclusive. Abnormal PF results (when CA were normal, had failed or inconclusive) were consistent with the suspected clinical diagnoses in 6/29 (20.7%) patients with MDS; 7/23 (30.4%) with AML; 16/27 (59.3%) with ALL; 10/15 (66.7%) with CLL; 2/8 (25.0%) with NHL; 6/13 (46.2%) with MM and 1/4 (25%) with MPD. Results revealed that in 40.3% of the patients with normal, failed or inconclusive CA, abnormalities were detected by PF analyses alone. PF analysis was most promising in detecting abnormalities in ALL (59.3%), CLL (66.7%) and MM (46.2%) where the detection of abnormalities by CA was normal, had failed or was inconclusive. If an abnormality has been established by PF, then the determined specific probes can be used to continually monitor the HM. This study showed that PF, when used alone or in conjunction with CA, is a vital and essential diagnostic tool that provides improved diagnostic capabilities to physicians in planning treatment modalities and monitoring prognoses.

Heritability of physical performance measures in older Amish. *P. Gallins[1], J.L. McCauley[1], L. Jiang[1], P.C. Gaskell[1], A.E. Crunk[2], M. Creason[1], L. Caywood[1], D. Fuzzell[2], C. Knebusch[2], M.C. Morey[1], E.R. Hauser[1], C.E. Jackson[3], J.R. Gilbert[1], M.A. Pericak-Vance[1], J.L. Haines[2], W.K. Scott[1]* 1) Duke University, Durham, NC; 2) Vanderbilt University, Nashville, TN; 3) Scott & White, Temple, TX.

Cognitive and physical decline are common, but not universal, features of aging. Variation in the degree of physical function has been observed, and several measures of physical function (such as grip strength and total score in the EPESE physical performance battery) are heritable in twin samples. We have measured several aspects of physical performance in our ongoing studies of successful aging (SA), or healthy longevity, in older Amish adults. The Amish communities of northeastern Indiana and central Ohio were founded in the mid-1800s by a small number of individuals and have remained socially and genetically isolated. Thus, they may have reduced numbers of genetic polymorphisms governing these traits and smaller variation in the traits due to environment, making identification of complex trait loci more feasible in this population. We have measured hand grip strength, lower extremity function (balance, gait speed, and standing from a chair), and cognition (3MS) in 246 Amish adults over age 65 clustered in 13 kindreds of closely related members. Heritability of these quantitative measures of function was determined using the polygenic model function of the SOLAR package. All measures were normally distributed or transformed to a normally distributed measure. Models contained sex, body mass index, age, and cognitive status (3MS) as covariates. Several performance measures were significantly heritable: log grip strength (h^2=0.43, p=0.03), total lower extremity function [sum of balance, gait speed, and chair stands from EPESE physical performance battery] (h^2=0.40, p=0.02), balance (h^2=0.45, p=0.006), and gait (h^2=0.36, p=0.04). These results suggest that several measures of physical performance are heritable in older Amish adults, confirming the prior twin studies. These traits might be useful intermediate traits for mapping genes involved in successful aging.

Multipoint PPL analysis of cleft lip with/out cleft palate (CL/P) provides compelling evidence in favor of linkage.

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Non-syndromic CL/P, the most commonly occurring congenital craniofacial anomaly, presents a special challenge for testing and identifying candidate genes and regions due to its complex etiology, epidemiology, and ethnicity-specific prevalence. This necessitates analytical approaches, such as the posterior probability of linkage (PPL), capable of modeling expected heterogeneity and geographic variation in CL/P. The PPL integrates over the trait model parameters, explicitly allows for intra- and inter-sample heterogeneity, and accumulates evidence for/against linkage across heterogeneous data sets/subsets via sequential updating. Application of the PPL to a 2-point 10cM genome scan (Govil et al, ASHG, 2005) of ethnically diverse families indicated regions for multipoint analysis on chromosomes 1, 2, 6, 9 and 12. Since computing the PPL involves evaluation of a multi-dimensional likelihood integral with no analytical functional form, it is approximated using grid enumeration, which imposes a considerable computational burden. In the case of multipoint analyses, the complexity of a single likelihood calculation adds to the complexity of the method. For CL/P data from China, India, Philippines, Turkey and Pittsburgh, computation of the 3-point imputed PPL for select regions took 6 months on 36 processors. However, the statistic, computed for the pooled data and updated over 5 populations of origin (POP) and/or 4 cleft phenotypic groups (PG), provides striking results. First, based on POP, the PPL indicates a 90% probability of a gene linked to a 14cM region on 6q. Notably, association results were also observed on 6q (Marazita et al, this meeting). Second, updating over both PG and POP provides an 82% PPL for 2p, as compared to 10% for the pooled data. While effort is ongoing to improve the computational speed for the PPL, these results are exciting and clearly indicate very specific regions for further analysis. NIH grants R01-DE09886, R01-DE012472, R37-DE08559, R01-DE016148, P50-DE016215, M01-RR00084; CIDR NIH contract N01-HG-65403; Carver Charitable Trust UIRF 05-2211.

Molecular characterization of a pericentric inversion of chromosome 3 in a 3-generation family with short stature. *U. Dutta, F. Matthes, K. Peisker, I. Hansmann, D. Schlote* Institut für Humangenetik und Medizinische Biologie, Halle/Saale, Germany.

Structural chromosomal aberrations are often associated with a specific phenotype and they are a significant cause of human disorders.Characterization of the breakpoints may lead to the identification of the responsible gene.Here we report a case of short stature in a girl with a karyotype of 46,XX,inv(3)(p24.2q26.1). Cytogenetic analysis had revealed a familial inversion 3, being heterozygous in the proband, mother and her grandmother. In order to characterize the breakpoints FISH (Fluorescence- *in situ* -hybridization) experiments were performed. Initially YAC (Yeast Artificial Chromosome) clones were selected by *in silico* analysis using the respective human genome database. From the four p-specific YACs selected , YAC clone CEPHy904H0787 (1090 kb) gave a split signal on the metaphase chromosomes of the proband assigning the breakpoint to 3p24.2.The split signal indicates that the target sequence carries the inversion breakpoint. YAC insert end sequencing was also done which lead to the identification of BACs (Bacterial Artificial Chromosome) for further FISH analysis. Out of the 15 YACs selected on the q arm, YAC CEPHy904G07889 (1610 kb) showed a split signal assigning the breakpoint to chromosomal band 3q26.1. Further analysis using 10 BACs in this region narrowed down the breakpoint to 100 kb represented in BAC RP11-12N13 thus giving a split signal. Using subcloned fragments of this BAC as well as Long range PCR products as probes the breakpoint is now located with in a region of 13 kb. This should help us in identifying putative candidate genes in the region of interest correlating the inversion with the phenotype of the proband.

The MICA gene distinguishes the extended HLA Type 1 Diabetes (T1D) associated risk haplotypes: findings from an association study and a meta-analysis. *B.Z. Alizadeh[1,2], P. Eerligh[2], A. van der Slik[2], A. Shastry[3], A. Zhernakova[1], J.G. Bruining[4], C.B. Sanjeevi[3], C. Wijmenga[1], B.O. Roep[2], B.P.C. Koeleman[1,2]* 1) Complex Genetic Section, Dept. Medical Genetics, UMC Utrecht, The Netherlands; 2) Dept. Immunohematology & Blood Transfusion, LUMC, Leiden, the; 3) Dept. of Molecular Medicine Karolinska Hospital, Stockholm, Sweden; 4) Dept. of Pediatrics, EMC, Rotterdam, the Netherlands.

The association of HLA complex cannot explain the total linkage of HLA region on chromosome 6 to autoimmnue disorders, leading to the hypothesis that there are other causal genes in the HLA region for immune related diseases. The MICA gene on chromosome 6 is involved in immune signal transduction, and was associated to autoimmune diseases, including T1D. Its variations are informative genetic indicators of recent human migration. Thus, a co-analysis of MICA with the HLA DQ DR haplotype may reveal secondary genes involved in T1D. In our cases (n=350) and controls (n=540), we found that the MICA*A5 and MICA*A6 variants were significantly associated with an increased and decreased risk for T1D, respectively. In a meta-analysis of 14 published studies, we confirmed the significant association of MICA*A5 to T1D only in Caucasians, but the protective effect of MICA*A6 in T1D was found worldwide. We found a significant LD between MICA and HLA complex. Thus, we performed a conditional analysis on T1D-associated high risk HLA DQ-DR haplotypes. Overall, conditional analysis did not confirm a significant HLA-independent association of MICA with T1D. MICA*A6 showed the highest risk for T1D when it was coinherited with T1D-risk HLA DQ2-DR3 haplotypes. In carriers of the T1D-risk HLA DR4-DQ8 haplotype, MICA*A9 showed a significant association to increased risk for T1D. Despite the initial findings of our data and the meta-analysis that confirmed the association of MICA with T1D, we found no HLA-independent relationship between MICA and T1D. Our results showed the highest T1D-associated risk lies with the co-inheritance of MICA*A6-HLA DQ2-DR3 haplotype, indicating this is the most likely haplotype to harbor the additional T1D-associated genetic factors in the HLA region.

Variants in *TCF7L2* are Associated with Predictors of Type 2 Diabetes Mellitus in Pima Indians. *T. Guo, Y. Muller, M. Traurig, J. Mack, L. Ma, R. Hanson, S. Kobes, C. Bogardus, L. Baier* Diabetes Molecular Genetics Section, NIDDK, NIH, Phoenix, AZ.

The transcription factor 7-like 2 gene (TCF7L2 or TCF4) has been reported to be associated with type 2 diabetes mellitus (T2DM) in Iceland,Danish and US populations.In that report,the strongest association was observed with marker DG10S478,positioned within intron 3 of TCF7L2.To investigate whether *TCF7L2* also has a role in T2DM susceptibility in the Pima Indians,a population with the worlds highest prevalence of T2DM,the marker DG10S478 and 38 SNPs that span *TCF7L2* were genotyped in 1311 Pima Indians.DG10S478 was essentially monomorphic for the protective allele in Pima Indians (1290 of the 1311 subjects were homozygous for allele 0). Only one SNP (rs1225404) within intron 10 was associated with T2DM (p= 0.01).However,several other SNPs were associated with pre-diabetic traits among non-diabetic Pima Indians who had been metabolically phenotyped (N=356).Five SNPs,all in high linkage disequilibrium (LD:D>0.9),spanning the 5-flanking region and exon 1 were associated with BMI (p=0.02-0.0002).Three additional SNPs, in high LD among themselves, within intron 3 were also associated with BMI (p= 0.01-0.04).Six SNPs spanning intron 3-4,the region containing marker DG10S478,were associated with measures of insulin secretion (acute insulin response as determined following an intravenous bolus glucose injection,p=0.02-0.002, and 30 min. plasma insulin concentration after an oral glucose tolerance test,p=0.03-0.0075) among 188 normal glucose tolerant Pima Indians. Several SNPs spanning exon 5 through the 3UTR were modestly associated with measures of insulin action, with the strongest association coming from rs10787475 (p= 0.00001 for 60 minute glucose response to an OGTT, and p=0.03 for glucose uptake rate in response to physiologic insulin concentrations during a clamp).Expression profiling of *TCF7L2* identified the major transcripts in all tissues that were tested, including pancreas, islet, adipose, liver and skeletal muscle. These results indicate that *TCF7L2* may have multiple roles in T2DM susceptibility, by affecting BMI, insulin secretion and insulin resistance.

Prenatal screening by array comparative genomic hybridization: A proposal for clinical practice guidelines. *M. Cho, The CIRGE Genetics Policy Working Group* Pediatrics/Genetics, Stanford Center for Biomedical Ethics, Palo Alto, CA.

Array-based comparative genomic hybridization (aGCH) and other techniques for genome-wide analyses have begun to be used for genetic screening, including the prenatal screening context. These techniques may also be used soon on single cells for preimplantation genetic diagnosis (PGD). These technologies, relying primarily on measures of copy number variation compared to a normal reference standard, raise a number of clinical, social and ethical issues that should be addressed before widespread use. Uncertainty about the clinical meaning of the data generated by aCGH, the potential for widespread use in conjunction with amniocentesis or chorionic villus sampling, the vast number of conditions and loci that could be included in aCGH screening, the complexity of genetic counseling necessary for such screening, and access are just some of the issues that should be considered in the clinical application of aCGH. In the summer of 2006, the Center for Integration of Research on Genetics and Ethics (CIRGE) at Stanford University invited a group of nationally known clinicians (including clinical geneticists, genetic counselors, and obstetrician/gynecologists), genetic researchers and ethicists to attend a workshop to address these issues. The group also includes members representing professional societies such as ACMG and ACOG. The purpose of this workshop is to develop a framework for clinical practice guidelines, with input from a variety of disciplinary perspectives. The goal of this group effort is to create a proposal for guidelines for the translation of aCGH from the research to the clinical setting that will be useful for clinicians and researchers developing similar technologies at universities and biotechnology companies. The proposed guidelines will address the currently proposed applications (including prenatal screening) and those proposed for the near future (including PGD) and will serve as a resource for development of practice guidelines by professional societies.

Identification of a novel locus on chromosome 7 in familial cases of Premature Ovarian Failure. *S. Caburet[1], P. Laissue[1], S. Christin-Maitre[2], P. Bouchard[2], Z. BenNeriah[3], S. Shalev[4], S. Copelli[5], D. Bacq[6], S. Heath[6], M. Lathrop[6], R.A. Veitia[1], M. Fellous[1]* 1) Team 21, Cochin Institute, Paris, France; 2) Service of Endocrinology, St-Antoine Hospital, Paris, France; 3) Hadassah Hospital, Jerusalem, Israel; 4) Haemek Medical Center, Afula, Israel; 5) Caece University, Buenos Aires, Argentina; 6) National Center for Genotyping, Evry, France.

Premature ovarian failure (POF) is a disease affecting 1 to 3 % of women before the age of 40 years and 0.1 % of women below the age of 30 years. Patients present a primary or a secondary amenorrhea and an hypergonadotroph hypogonadism. Genetic alterations or mutations have been involved in POF. The most frequent anomalies are chromosome X alterations, such as monosomy associated with Turner syndrome, deletions or X:autosome translocations. Unfortunately, in more 90 % of POF patients, the aetiology of the disease is still unknown. For this reason, we set up an international network in order to perform a positional cloning approach in familial cases. We have collected a large panel of familial cases (9 families with 30 POF cases). A genome-wide genotyping was performed using 457 microsatellite markers for all the individuals (affected or not). Evidence for significant linkage was detected on the long arm of chromosome 7 with a peak lod-score of 3.85 in 7q21-22, defining a 62 Mb-long region between markers D7S502 and D7S530. This large region was refined using 35 microsatellites between D7S502-530. The region of interest is presently 10 Mb long and contains more than 130 genes. As some of the studied families present consanguinity, we also analyzed the results of the genome scan by homozygozity mapping. The identified region on 7q coincides with a minimal region of homozygosity by descent shared between the affected members of the consanguinous families. We are currently refining the region of interest by adding new individuals and new families in our study. In addition, two functionnal candidate genes present in the region will be sequenced soon. Finding new genes involved in POF will contribute to a better understanding of normal folliculogenesis and ovarian physiopathology.

Anti-inflammation treatment by Indomethacin extended the life span of the Krabbe disease variant saposin A deficient mice. *S. Barnes, Y. Sun, G. Grabowski* The Division and Program in Human Genetics, Children's Hospital Research Foundation, and University of Cincinnati College of Medicine, Department of Pediatrics, Cincinnati, OH.

Prosaposin is the precursor of four ~80 amino acid proteins known as saposins (termed A, B, C, or D) that are necessary for the activity of several lysosomal hydrolases in the glycosphingolipid metabolic pathway. Genetic deficiencies of individual saposins lead to lysosomal storage diseases that phenotypically mimic the deficiencies of the cognate enzymes e.g., saposin A is essential for galactosylceramidase (Krabbe enzyme) activity. We seek to characterize and elucidate the differential cellular characteristics and temporal events important to central nervous system deterioration in complete or partial prosaposin deficiencies, including the role of proinflammatory reactions. Here, 21 day-old saposin A (Sap A -/-) deficient mice were treated with low doses (0.8 mg/kg/day) of indomethacin, an inhibitor of the COX II (cyclooxygenase) proinflammatory pathway. Indomethacin treated mice lived longer (>7 days) than their untreated littermates. Immunohistochemical studies showed decreased levels of CD68 positive staining in the brains and spinal cords of indomethacin-treated mice that correlated with increased survival. In contrast, no effects on CD68 were observed in sciatic nerve. Western blot analyses revealed significantly decreased Il-1 and TNF- and glial fibrillary acid protein levels in Sap A -/- mice receiving indomethacin. However, at end stage (12 wks), treated mice showed similar levels of these inflammatory-related molecules as untreated mice. This study demonstrates that inflammation is a component of disease progression. Activation of the cyclooxygenase pathway may contribute, but is not the major inflammatory pathway involved in the pathogenesis of saposin A galactosylceramidosis.

Clinical and molecular study of 348 children with Marfan syndrome and related type I fibrillinopathies out of a series of 1057 probands with a pathogenic FBN1 mutation. *L. Faivre[1,2], C. Stheuner[3], G. Collod-Beroud[4], P. Chevallier[3], A. Child[5], B. Callewaert[6], C. Binquet[2], E. Gautier[2], E. Arbustini[7], K. Mayer[8], A. Kiotsekoglou[5], P. Comeglio[5], B. Loeys[6], J. De Backer[6], P. Coucke[6], U. Francke[9], L. Ades[10], A. De Paepe[6], C. Boileau[3], G. Jondeau[3]* 1) Dept Genetics, Dijon, France; 2) Inserm CIE1, Dijon, France; 3) Consultation pluridisciplinaire Marfan, Hôpital Ambroise Paré, France; 4) IURC, Montpellier, France; 5) St Georges Hospital, London, UK; 6) Medical Genetics, Ghent, Belgium; 7) Molecular Diagnostic, Pavia, Italy; 8) Human Genetics, Martinsried, Germany; 9) Stanford University Medical Center, USA; 10) Royal Alexandra Hospital, Sydney, Australia.

From a large series of 1057 probands with a FBN1 mutation, data from 348 patients aged less than 18 years were analyzed (33%). The population was classified as follows: 17% neonatal MFS, 18% severe MFS, 31% classical MFS and 34% probable MFS (defined by incomplete Ghent criteria in childhood). No differences were noted in the frequency of specific diagnostic manifestations when compared to adults, ie ectopia lentis (EL, 56%), ascending aortic dilatation (AAD, 70%) and major skeletal involvement (22%). In contrast, striae distensiae (18%) and family history (29%) were underrepresented. When excluding patients with neonatal MFS, the mean age at diagnosis was 8.2 years. The first diagnostic feature was EL in 18%, AAD in 28%, both EL and AAD dilatation in 29% and other presentations in 25% of cases. 32% of mutations were located in exons 24-32 vs 13% in adults ($p<0.0001$), 27% in the 5 region vs 30% in adults (p NS), 26% of mutations resulted in premature stop codons vs 36% in adults ($p=0.0006$) and 64% were missense mutations involving a cysteine vs 58% in adults (p NS). Only 55% of the children met the Ghent criteria, and this proportion rose to 81% when the presence of a FBN1 mutation was considered as a major feature. Because of the low sensibility of clinical Ghent criteria during childhood, we recommend mutation screening in children with one major and one minor criteria and aortic follow-up even in children with incomplete Ghent criteria.

Wnt Signaling in Craniofacial Patterning. *S. Brugmann[1], A. Gregorieff[2], P. Leucht[1], H. Clevers[2], J. Helms[1]* 1) Department of Plastic Surgery, Stanford University, Stanford, CA; 2) Hubrecht Lab, Netherlands Institute for Developmental Biology, The Netherlands.

Wnt signaling has been implicated in numerous aspects of craniofacial development, including the generation of neural crest cells and the patency of cranial sutures. Herein, we examined the role of Wnt signaling during development of the facial prominences using the TOPgal reporter mouse line as an indicator of cells that have responded to a canonical Wnt signal. We observed that Wnt responsive cells define distinct boundaries within the frontonasal prominence (FNP). By E9.5 the central region of the FNP is devoid of Wnt responsive cells. This marked absence of Wnt responsiveness in the FNP is maintained to E15.5 and beyond, and delineates the midline infranasal depression (also known as the philtrum) from the more lateral regions of the midface. To confirm a role for Wnt signaling in medial/lateral patterning we used two separate methods to attenuate Wnt signaling. First, we inhibited Wnt signaling by knocking out two intracellular enhancers of Wnt signaling, Lef1 and TCF4 (i.e., Lef1-/-/TCF4-/-). Second, we used in utero gene transfer to inhibit Wnt signaling by infecting murine embryos with an adenovirus expressing the soluble Wnt inhibitor, Dickkopf 1 (Dkk1). Attenuating Wnt signaling using both experimental conditions resulted in the expansion of facial midline. Midline expansion, defined by an increased distance between lateral populations of Wnt responsive cells in TOPgal reporter mice, was often accompanied by a flattened infranasal depression and/or a dysmorphic nasal septum. We examined the in situ hybridization patterns for Wnt target genes and performed histological analyses in embryos with expanded midlines to characterize the molecular and cellular nature of this phenotype. Collectively these data begin to elucidate a role for Wnt signaling, independent of neural crest generation, in the development and patterning of the craniofacial complex.

MAOA promoter polymorphism is associated with brain structure volumes in autism. *L. Davis[1], C. Hazlett[2], M. Mosconi[2], J. Piven[2], T. Wassink[1]* 1) Department of Psychiatry, University of Iowa Carver College of Medicine, Iowa City, IA; 2) Neurodevelopmental Disorders Research Center and Dempartment of Psychiatry, University of North Carolina, Chapel Hill, NC.

Monoamine oxidase A (MAOA) is an enzyme expressed in the brain that metabolizes dopamine, norepinephrine, epinephrine and serotonin (Lenders, 1996). Abnormalities of serotonin neurotransmission have long been implicated in the psychopathology of autism. A polymorphism exists within the promoter region of the MAOA gene that influences MAOA expression levels. This polymorphism has four common alleles that can be grouped into high activity and low activity based on in vitro and in vivo expression studies (Sabol et al., 1998). The low activity alleles are associated with increased neurotransmitter levels in the brain. Individuals with autism often exhibit elevated serotonin levels (Anderson et al., 1987). This suggests a deficit in serotonin metabolism that may be accounted for by low activity MAOA expression. Additional studies indicate that the low activity allele may be associated with lower IQ and more severe autistic symptoms (Yirmya, 2002). In this study we genotyped the MAOA promoter polymorphism in a group of 33 males (age 2-3 yrs) with autism for whom brain MRI data was available. Using ANCOVAs, we tested relationships between MAOA genotype and frontal lobe, temporal lobe, parietal-occipital lobe gray and white matter volumes and cerebellar gray and white matter volumes. Covariates included age at the time of scan and head circumference. We found a consistent association between the low activity allele and larger brain volumes for several regions of the brain, including total cerebral gray ($F_{1,25}=5.31$, $p=0.03$), total cerebral white ($F_{1,25}=4.81$, $p=0.04$), frontal white matter ($F_{1,25}=7.29$, $p=0.01$), and frontal gray matter ($F_{1,25}=4.10$, $p=0.05$). These results correlate well with the replicated finding that children with autism often have macrocephaly. Future studies will determine if there is a more severe clinical phenotype associated with both the low activity genotype and the larger brain volumes in our sample.

Surgical interventions in MPS I patients: a significant burden in all phenotypes. *P. Arn*[1], *E. Wraith*[2] 1) Div Genetics, Nemours Children's Clinic, Jacksonville, FL; 2) Willink Biochemical Genetics Unit, Royal Manchester Children's Hospital, Manchester, UK.

Mucopolysaccharidosis I (MPS I) is caused by a deficiency of -L-iduronidase, the lysosomal enzyme that hydrolyzes the terminal -L-iduronic acid residues of dermatan and heparan sulfate. Patients with MPS I have traditionally been classified as having Hurler, Hurler-Scheie, and Scheie syndromes, however there is substantial clinical overlap between the phenotypes. All patients with MPS I suffer from a number of progressively debilitating symptoms affecting multiple organ systems. BioMarin/Genzyme LLC established the MPS I registry to track the outcomes of patients with MPS I. The registry was queried to assess the surgical needs of patients with MPS I. Of the 425 patients enrolled in the registry at the time of the query, 919 surgical interventions were reported. Repetition of the same surgery (ex multiple PE tube placements) was considered a single procedure. Age at the time of the surgical intervention ranged from 2 months to 65 years. The most common interventions were PE tubes, hernia repair, and adenoidectomy. Median age at these procedures was 1.8 years for PE tubes and for hernia repair and 2.8 years for adenoidectomy. Of 141 patients reporting PE tube placement 29 procedures were done prior to diagnosis of MPS I; of 136 reporting hernia surgery, 58 were performed prior to diagnosis, and of 124 adenoidectomies 26 were done prior to diagnosis. In total, 177 surgical procedures were performed prior to the diagnosis of MPS I. These procedures included 8 VP shunts, 5 spinal cord decompressions, 3 cardiac valve replacements, and a corneal transplant. Surgery prior to diagnosis was reported in all classifications of MPS I, Hurler, Hurler-Schie, and Scheie. The type and ages at intervention for all procedures will be reported. We conclude that the surgical burden of all phenotypes of MPS I is high. A large number of MPS I patients undergo surgical procedures prior to diagnosis, potentially putting them at risk for complications. Education programs aimed at surgeons may assist in earlier diagnosis of these patients.

Ascertainment of multiplex families can decrease the power of association analysis. *M.A.R. Ferreira[1], P. Sham[2], M.J. Daly[1], S. Purcell[1]* 1) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA; 2) Institute of Psychiatry, King's College London, UK.

Many Mendelian diseases have been reported to have both sporadic and familial forms, i.e. present in either one or multiple relatives in a family. Familial cases are thought to arise from the inheritance of a genetic mutation and so are the natural choice for gene mapping of Mendelian traits. The same assumption is often made in the context of family-based designs for complex diseases. Indeed, many association studies use families with multiple affected relatives in the hope that this will increase power to detect a disease locus. However, our recent work and that of others has indicated that the converse is often true - for a range of scenarios that are likely to be encountered for common diseases, preferentially ascertaining multiplex families can actually reduce power, even for the same total sample size. Critically, the performance of different ascertainment strategies depends on residual sources of variation (i.e. other than the locus being studied), a fact that has often been ignored in previous work. Here, we describe a set of web-based tools that quantify the relative merits of different design strategies, tailored to the specifics of the disease under study. These tools implement a liability threshold model that allows researchers to estimate the power of the TDT for different effect sizes, residual heritability, shared environmental variance and family structures. Users can also specify more than one disease model in the population and so assess the impact of etiologic heterogeneity. For example, the power provided by 400 trios selected with disregard of the affection status of other relatives was 0.885 (= 0.05), assuming a disease prevalence of 5%, a locus that explains 1% of the variance in liability to disease (MAF = 5%), heritability of 40% and shared environmental variance of 10%. By contrast, the power provided by 400 trios selected conditional on having 2 additional affected sibs was 0.686. With these tools, researchers can determine whether the extra effort involved in selecting multiplex families will be helpful or counterproductive.

Gender-specific association of the UGT2B17 gene deletion with decreased glucuronidation of NNAL and increased risk for lung cancer. *C.J. Gallagher, J.E. Muscat, A.N. Hicks, A.M. Dyer, G.A. Chase, J.P. Richie Jr., P. Lazarus* Penn State Cancer Institute, Penn State College of Medicine, Hershey, PA.

UGT2B17 is a phase II metabolizing enzyme that mediates the detoxification of the tobacco smoke carcinogen, NNAL, by converting it to its glucuronide (NNAL-Gluc). A deletion allele encompassing the entire UGT2B17 gene was identified and found to reduce the rate of NNAL glucuronidation in human liver microsomes *in vitro*. The goal of the present study was to determine if the UGT2B17 deletion genotype is associated with reduced rates of glucuronidation *in vivo* and with increased risk for lung cancer. We developed a novel multiplex real-time PCR assay to genotype the UGT2B17 gene deletion. In a screening of 82 white smokers, the urinary concentrations of NNAL and NNAL-Gluc were measured. A significant decrease in urinary NNAL-Gluc to NNAL ratio was observed among smokers with the UGT2B17 deletion [(0/0)] genotype when compared to smokers with at least one intact UGT2B17 allele (p-value=0.049). This association was only observed in females (p-value=0.058); no association was observed in males (p-value=0.597). In an analysis of 398 white subjects with newly-diagnosed primary lung cancer and 697 white controls, a significant increase in risk for lung cancer was observed in women with the UGT2B17 (0/0) genotype when compared to women with at least one intact UGT2B17 allele (OR=2.0, 95% CI=1.04-3.75). No association was observed between the (0/0) genotype and lung cancer risk in men (OR=0.7, 95% CI=0.39-1.39). The association between the (0/0) genotype was stronger in women with adenocarcinoma (OR=3.2, 95% CI=1.48-6.90); this association was also not present in men (OR=1.3, 95% CI=0.58-3.09). The association of the UGT2B17 gene deletion was highest for adenocarcinoma, which is consistent with animal model studies demonstrating that NNAL is a selective inducer of lung adenocarcinoma. In summary, the UGT2B17 (0/0) genotype was shown to be associated with decreased glucuronidation of NNAL and increased risk for lung cancer (specifically for adenocarcinoma) in women. This is the first study to show a genetic basis for the observed excess risk of lung cancer in women versus men.

An association study of CD74 and Metallothioneins in multiple sclerosis. *K. Duvefelt[1,3], M. Swanberg[2], C.M. Lindgren[1], O. Lidman[2], T. Olsson[2], H. Harbo[4], J. Saarela[5], A. Oturai[6], J. Hillert[3]* 1) Mutation Analysis Facility, Clinical Research Center, Karolinska University hospital, Stockholm, Sweden; 2) Neuroimmunology Unit, Department of Clinical Neuroscience, Karolinska Institutet,Sweden; 3) Division of Neurology, Department of Clinical Neuroscience, Karolinska Institutet, Sweden; 4) Institute of Immunology, Rikshospitalet University Hospital, Oslo, Norway; 5) Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland; 6) Department of Neurology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark.

We studied two gene loci, CD74 (also known as invariant chain) on chromosome 5q33 and Metallothionein (MT) on chromosome 16q, for a possible importance in determining susceptibility to Multiple Sclerosis (MS), based on their upregulated transcription in the spinal cord in an experimental animal model of inflammation and neurodegeneration. Seven single nucleotide polymorphisms (SNP) in the CD74 gene and 9 SNPs in the MT cluster were analyzed in a Swedish cohort consisting of 890 MS patients and 775 controls. Three SNPs belonging to the same linkage disequilibrium (LD) block in the MT region were found to be associated with the risk of MS (P0.05, OR: 1.24-1.47) as well as one haplotype (P=0.018) consisting of the three associated SNPs and two additional SNPs. One SNP in CD74 showed borderline signs of association, while the other SNPs in the same LD block did not show any association. The associated SNPs in the MT gene region were subsequently studied in a Nordic case control material of 1277 MS samples and 1396 controls. In the Nordic material no association to the risk of MS were found for the studied SNPs.

A Direct Long SAGE Tag-to-Gene Mapping Scheme. *A.E. Dellinger, T. Wang, C.B. Rickman, M.A. Hauser* Center for Human Genetics, Duke University, Durham, NC.

Serial Analysis of Gene Expression (SAGE) is a commonly used, open source technique that evaluates the level of genes expression via total RNA. cDNA is synthesized from RNA and 14bp tags are cut by *NlaIII* and *MmeI*. One difficulty of SAGE is determining the most appropriate method of mapping this tag to a gene. With the advent of the 21bp Long SAGE tag, we can map the tag directly to its gene(s). A straightforward yet biologically thorough method of Long SAGE tag-to-gene mapping has not been defined until now. Our method provides a quick transition from raw data to the goal of SAGE- the analysis of gene expression.

A virtual Long SAGE library was generated by scanning Build 36 of the human genome for tags associated with *NlaIII* sites on both forward and reverse strands. In 56 Long SAGE libraries, 48% of tags mapped to exon regions are antisense. Thus, we always consider both strands. Each tag was mapped to its physical location(s) in the genome. Not all tags map by this method. Over 8% of all human genes contain at least one SNP associated tag (1). SNPs can alter tags or create them by creating *NlaIII* sites. A PERL script found the SNP generated tags in SNPdb (2). Another PERL script found the 5.3% of virtual Long SAGE tags that cross exon boundaries. These tags were reconstituted by concatenating the partial tags with a sufficient number of nucleotides from the next transcribed exon to complete the 17 bp tag. Alternative splicing resulted in multiple tags. Tags from these three techniques were mapped to exons by comparing the genomic locations of the tags and the exons in our exon database, which consolidates the Ensembl (3), UCSC known genes, and EST databases (4). The results of the initial genome scan can be downloaded and the SNP and exon programs run all in minutes. Once the full virtual tag library is created, a Long SAGE library can be quickly mapped to exons. Thus, any library can be ready to search for genes of interest in minutes.

1. Silva, et al. Nucl. Acids Res. 32(20):6104. 2. Sherry, et al. Nucl. Acids Res. 29(1):308. 3. Birney, et al. Nucl. Acids Res. 34:D556. 4. Karolchik, et al. Nucl. Acids Res 31(1):51.

The Language Acquisition Discrepancy (LAD) score: A new phenotyping strategy for genetic analyses in autism (AUT). *M. Cuccaro[1], J.S. Brinkley[1], J. Jaworski[1], R.K. Abramson[2], H.H. Wright[2], J.P. Hussman[3], J.R. Gilbert[1], E.R. Martin[1], M.A. Pericak-Vance[1]* 1) Ctr Human Genetics, Duke Univ Med Ctr, Durham, NC; 2) USC-SOM, Columbia, SC; 3) Hussman Foundation, Ellicott City, MD.

A variable feature in the AUT phenotype is age at language acquisition (age at first words, age at phrase speech). While genetic analyses of AUT have used language acquisition variables in stratified analyses, these variables have not been studied in a joint fashion. We developed the Language Acquisition Discrepancy (LAD) score, based on the difference in age at first words and age at phrase speech as measured by the ADI-R. Clustering algorithms using LAD score, age at first words and ADI-R composite scores (reciprocal social interaction, nonverbal communication, verbal communication, repetitive behaviors) yielded four clusters within our AUT dataset (N = 693 families; M:F = 4.5:1). Defined by a bivariate trait based on age at first words [normal 18 mos delayed] and LAD score [normal 15 mos delayed] the four clusters are: DD = delayed first words/delayed LAD score; DN = delayed first words/normal LAD score; ND = normal first words/delayed LAD score; NN = normal first words/normal LAD score. The clusters differed significantly with respect to ADI-R domains and developmental indicators with the exception of age at walking. The DD cluster was more impaired across variables. Using the pedigree disequilibrium test (PDT) we tested for association in 473 families stratified on the basis of the clusters. We tested 69 SNPS in 14 known autosomal GABA receptor subunit genes. Allelic association (p .05) was detected for SNPs on chr4 in GABRG1 (rs2350439, rs1826923), GABRA4 (rs2280073), and GABRB1 (rs1372496, hcv11353524, rs6289). These results confirm our earlier work suggesting that GABRA4 and GABRB1 interactions may be involved in risk for AUT. Two SNPs on chr15 showed evidence of association (hcv428306, rs1426217). None of the positively associated SNPs fell in the DD cluster suggesting that association is concentrated in the verbal clusters. These results highlight use of LAD in conjunction with age at first words as a potential language based phenotypic metric for genetic analyses of AUT.

Autozygosity mapping of a consanguineous family with an autosomal recessive fifth mandibular incisor phenotype using the 500K SNP Affymetrix microarray. *J.M. Gee[1], S. El-Toum[2], G.A. Mendoza[3], T.J. Pemberton[3], A. Cassia[2], A. Feki[4], A. Megarbane[5], P.I. Patel[1,3]* 1) School of Dentistry, University of Southern California, Los Angeles, CA; 2) Department of Oral Diagnostic Sciences, Lebanese University School of Dentistry, Beirut, Lebanon; 3) Institute for Genetic Medicine, University of Southern California, Los Angeles, CA; 4) Department of Oral Medicine and Oral Surgery, University Hospital of Strasbourg, France; 5) Medical Genetic Unit, Saint-Joseph University, Beirut, Lebanon.

Supernumerary teeth, or the presence of an extra tooth in the oral cavity, is a condition that is present within 0.5-3.8% of the general population. While they can be found in any region of the dental arch, they are usually located in the upper maxillary region between the two central incisors. We have ascertained a highly consanguineous extended family with a very rare autosomal recessive phenotype where four individuals display five incisors in the anterior mandible. We performed autozygosity mapping using the Affymetrix 500K SNP microarray on the four affected individuals, each from a distinct branch of the pedigree separated by seven generations. Analysis of the SNP data using an MS Excel EXLUDEAR-based method for homozygosity determination identified 48 regions of homozygosity within these four affected members that were between 0.2 to 1 MB in length. Microsatellite markers from within these regions were genotyped within 16 family members (affecteds and selected first-degree relatives of affecteds) to confirm or refute putative homozygosity. The five largest regions of homozygosity identified in the SNP scan were interrogated and have been ruled out as candidate regions. The remaining regions are being analyzed by similar microsatellite marker genotyping to identify the candidate region and to guide mutational analysis of candidate genes.

The absence of endothelin-2 partially rescues photoreceptor (PR) death in a model of inherited photoreceptor degeneration (IPD). *A. Bramall[1,2], M.J. Szego[1,2], L. Pacione[1,2], P. D'Orléans-Juste[3], M. Yanagisawa[4], R.R. McInnes[1,2]* 1) Progs in Genet & Dev Biol, Hosp for Sick Children, Toronto; 2) Dept Mol & Med Genet, Univ of Toronto; 3) Dept Pharm, Med School, Univ de Sherbrooke; 4) HHMI & Dept of Mol Genet, Univ of Texas Southwestern Med Center, Dallas.

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) but function remarkably well for years to decades in humans. To identify genes that may influence the risk of death, we performed microarrays and real-time PCR on wild-type (wt) and mutant retinas. Endothelin-2 (*Edn2*) mRNA was 32-fold (p 0.0006) and 14-fold (p 0.009) in the $Rds^{+/-}$ and *Tg(RHO P347S)* mouse models of IPD, respectively. Edn2 is one of three Edn peptides with vasoconstrictive, prosurvival and proapoptotic effects. In $Rds^{+/-}$ retinas, *in situ* hybridization revealed increased *Edn2* mRNA solely in PRs; no *Edn2* mRNA was detectable in wt retinas. By HPLC and RIA, the Edn2 peptide was also increased: minimally 3-fold, but likely more, as Edn2 was undetectable in wt retinas. The mRNA levels of *Edn1*, *Edn3*, and of both endothelin receptors, were unchanged. To determine if Edn2 is pathogenic in IPDs, we generated $Edn2^{-/-}$; $Rds^{-/-}$ and $Edn2^{-/-}$; *Tg(RHO P347S)* mice. At age 40 days, $Edn2^{-/-}$; *Tg(RHO P347S)* retinas showed a 41% rescue of PR degeneration: PR nuclear layer thickness of 94.4 7.6 units vs. 564.7 units in wt mice (p0.003; n=5/genotype). At 40 days, no rescue effect was detectable in the slower degenerating $Edn2^{-/-}$; $Rds^{-/-}$ retinas. We conclude that the protective effect of the $Edn2^{-/-}$ genotype in *Tg(RHO P347S)* retinas is due to either to i) a pathogenic role of Edn2, ii) an *Edn2* tightly linked modifier, or iii) downstream effects of the $Edn2^{-/-}$ genotype, such as the capillary vasodilation we see in $Edn2^{-/-}$ retinas, or Muller cell activation (indicated by increased GFAP staining). Since the *Edn2* mRNA increase (~18 days of age) occurs well after the onset of PR death (~10 days in $Rds^{-/-}$ mice), Edn2 may contribute to the progression of PR death but not its initiation. These findings have therapeutic implications, since Edn receptor blockers might slow PR death in IPDs.

Genetic structure analysis of three Hispanic populations from Costa Rica, Mexico and the Southwest United States, using Y-STRs markers and mtDNA sequence. *R. Campos-Sánchez[1], R. Barrantes[2], S. Silva[1], M. Escamilla[3], A. Ontiveros[4], H. Nicolini[5], R. Mendoza[6], R. Muñoz[7], H. Raventós[1]* 1) CIBCM, Universidad de Costa Rica; 2) Universidad de Costa Rica, Escuela de Biología; 3) Department of Psychiatry, University of Texas Health Science Center at San Antonio; 4) INFOSAME, Monterrey México; 5) Grupo de Estudios Médicos y Familiares Carracci S.C, Ciudad de México; 6) David Geffen UCLA School of Medicine; 7) Family Health Centers of San Diego.

We studied the genetic structure of three Hispanic populations from Mexico, the Southwest USA and Costa Rica, in order to characterize their genetic relationships. The sample of 217 men utilized was drawn from two NIMH (National Institute of Mental Health) funded studies to map schizophrenia susceptibility genes. Our study reports the maternal and paternal relationships between these groups, based on the analysis of 12 Y-STRs and HVI mtDNA sequences. Similarities between these populations have not been previously reported. Our results demonstrate that these populations are genetically related to each other. They are very similar when we compare their internal genetic characteristics as revealed by analyses of diversity. The relationship is stronger through their maternal lineage than the paternal, as a higher number of shared haplotypes and polymorphisms are seen in the mtDNA (compared to Y-STRs). These results provide evidence of previous contact between these populations and shared histories, as explained by common migration movements from Europe, the slavery trade from West Africa, and similar native communities of Amerindians. An analysis of molecular variance (AMOVA) revealed no genetic differentiation for the mtDNA (Fst=0.006) for the three populations but does for the Y-STRs (Rst=0.04). Genetic distance analysis confirms that these populations are very closely related, probably due to migration between close neighbors, as indicated by shared haplotypes. These results suggest that Latin American and Hispanic populations can be studied together for specific purposes as genetic mapping and association studies, which are of most interest in these schizophrenia related samples.

Tubulopathy and pancytopenia with normal pancreatic function: A variant of Pearson syndrome. *A. Atale[1], A. Rotig[2], A. Fischer[3], S. Perez-Martin[1], C. Thauvin-Robinet[4], P. Bonneau-Amati[5], F. Huet[1], L. Faivre[4]* 1) Pediatrics, Hopital d'Enfants, Dijon, France; 2) Immuno-Hématologie Pédiatrique, Hôpital Necker-Enfants Malades, Paris, France; 3) Genetics, Hôpital Necker-Enfants Malades, Paris, France; 4) Génétique, Hôpital dEnfants, Dijon, France; 5) Biologie Moléculaire, CHU Angers, France.

We report on two patients with an atypical presentation of Pearson syndrome, including growth deficiency, pancytopenia and tubulopathy but an absence of malfunction of exocrine pancreas. Patient 1 was a 2 ½- year-old boy with a personal history of thrombopenia and spontaneous regression of a metabolic acidosis in a context of gastroenteritis at 1 year of age. A new episode of gastroenteritis revealed growth retardation, central pancytopenia, mild cytolysis, metabolic acidosis hyperlactacidemia leading to the diagnosis of proximal tubulopathy. A cystinosis and a tyrosinemia were ruled out. Despite normal exocrine pancreatic deficiency, study of mitochondrial DNA revealed a 3.5 kb deletion leading to the diagnosis of Pearson syndrome. Follow-up showed two episodes of acidosis and the finding of hyperinsulism at 3 ½ years of age. Patient 2 had a personal history of pancytopenia necessitating blood transfusions at 11 years of age. She was seen at the age of 2 years for the evaluation of growth retardation. Metabolic acidosis leaded to the diagnosis of proximal and distal tubulopathy but exocrine pancreatic deficiency could not be evidenced. She died at 30 months of age in a context of intractable metabolic acidosis. Postmortem studies revealed a 4.9 kb deletion of the mitochondrial DNA, in favour of Pearson syndrome. A review of the literature revealed that this type of presentation of Pearson syndrome with tubulopathy, pancytopenia without exocrine pancreatic deficiency was rare and often lead to multiple investigations and diagnostic delay. In conclusion, Pearson syndrome should be searched in a child presenting with the association of tubulopathy, pancytopenia and growth retardation even in the absence of exocrine pancreatic deficiency.

Gathering evidence for an adaptive evolution of skin pigmentation in humans. *S. Alonso-Alegre[1], I. Smith-Zubiaga[2], N.P. Smit[4], D. Boyano[3], J.L. Díaz-Pérez[5], I. Garcia[1], N. Izagirre[1], C. de la Rua[1]* 1) Genetics, Physical Anthropology and Animal Physiology. Fac. Science and Technology UPV/EHU, Barrio Sarriena s/n. Leioa, Bizkaia, Spain; 2) Zoology and Animal Cell Biology. Fac. Science and Technology. UPV/EHU, Barrio Sarriena s/n. Leioa, Bizkaia, Spain; 3) Cell Biology and Histology. Fac. Science and Technology. UPV/EHU, Barrio Sarriena s/n. Leioa, Bizkaia, Spain; 4) Leiden University Medical Centre. Department of Clinical Chemistry, Bldg 1, L01-036 Albinusdreef 2, P.O. Box 9600 2300 RC Leiden. the Netherlands; 5) Cruces Hospital. Dermatology Service. Plaza de Cruces s/n, 48903 Barakaldo, Bizkaia, Spain.

While the combination of pale skin and intense sun exposure results in an important health risk for the individual, it is less clear if at the population level this risk has possessed an evolutionary meaning. In this sense, a number of adaptive hypotheses have been put forward to explain the evolution of human skin pigmentation. It is expected that if skin pigmentation is adaptive we might be able to see the signature of positive selection on some of the genes involved. In order to detect this signature we have a) data-mined the available resources and analyzed a battery of 81 candidate loci by means of phylogenetic and population genetic tests; b) resequenced 4kb of the 5' region of the main melanogenic loci TYR, TYRP1 and TYRP2 (DCT) in a total of 116 chromosomes from worldwide populations, including Europeans, European melanoma patients, Senegalese, Pygmies, Chinese and Australians; and c) investigated the gene expression profile (Affymetrix U133Av2 chips) of 7 cultured human melanocytes from donors of light (n = 4) and dark (n = 3) pigmentation, and compared them to the expression profiles of the same cells after repeated irradiation with dayly doses of approximately 50mJ/cm2 of UVA and UVB (once a day for consecutive 6 days). Overall, we find evidence of positive selection, which indicates that skin pigmentation may be adaptive. This adaptation may be related to skin photoprotection against DNA damage.

In the year 2020: Representations of the Promise of Personalized Medicine. *A. Adair[1], T. Caulfield[1, 2, 3]* 1) Health Law Institute, University of Alberta, Edmonton, AB, Canada; 2) Faculty of Law, University of Alberta; 3) Faculty of Medicine and Dentistry, University of Alberta.

In the popular media and scientific publications alike the dream of personalized medicine has resulted in an ongoing narrative of medical revolution that broaches science fiction and biotechnological advances, raising public expectation and venture capital. Building on the recent concern about genohypethe overstatement of technological advance in the biotechnology spherethis paper reviews the pressures behind the creation of a cycle of hype in the context of personalized medicine. Concrete examples of genohype in science journals, the popular press and industry publications from 1989 to 2006 are examined to investigate the role that optimistic messaging strategies play in the representation of genetic and biotechnological developments in Canada and the United States. This paper considers not only a tautology of hype surrounding the representation of personalized medicine, but responses to this hypedepictions that are deliberately understated or complicated. Moreover, the articles reveal a number of strains of representation of personalized medicine: as a panacea, a re-personalization of medical care, a movement away from one-size-fits-all medicine, and a means of empowering the health care patient/consumer. The tenor and scope of these predictions reveal more about the relationship between the scientific community, the media and the public, than about medical and biotechnological advances. Almost twenty years since claims first appeared in public discourse, the personalized medicine narrative has evolved largely in the absence of concrete results or government policy. Its original representation has shifted to a encompass pharmaceuticals targeted to self-identified racial groups; it has been imbricated in the justification for the creation of broad-based biobanks; and it has become an international focus of investment. As ongoing claims about personalized medicine draw government and consumer attention, capital and consent, this paper cautions against the power of genohype in the public consumption of biotechnology.

Prevalence of vertigo in GJB2 deafness. *K.M. Dodson[1], K.A. Arnos[2], S.K. Burton[2], R.S. Marin[2], K.O. Welch[2], V. Norris[2], S. Ackley[2], W.E. Nance[3], A. Pandya[3]* 1) Department of Otolaryngology, Virginia Commonwealth University, Richmond, VA; 2) Department of Biology, Gallaudet University, Washington, DC; 3) Department of Human Genetics, Virginia Commonwealth University, Richmond, VA.

Mutations in GJB2 (connexin-26) are the most common cause of autosomal recessive nonsyndromic hearing impairment. Despite the widespread expression of connexin-26 throughout the vestibular system, vertigo has not previously been reported as a common finding in connexin deafness. Based upon our observations of vertigo accompanying connexin deafness in several large families, we hypothesize that vertigo may actually be a common, yet often overlooked component of GJB2 deafness. To define the prevalence of vertigo in connexin deafness, a vertigo survey was designed and distributed to deaf subjects previously identified with pathogenic mutations in GJB2 and/or GJB6 through our research protocols. The survey quantified duration, frequency, and length of episodes of vertigo, as well as past medical history and a prior history of other vertiginous disorders including Menieres disease, benign paroxysmal positional vertigo, and migraine. 105/214 surveys were completed and returned (49%). The mean age of respondents was 37.6 (range 13-76 years of age). 54/105 (51%) of the responders reported a history of vertigo, which was intermittent in nature in 98%. The majority of patients (57%) reported that vertigo lasted for minutes to hours and had a positional component in 70%. Most (81%) suffered with vertigo for greater than 1 year, with a mean of 9.9 years duration. Most (65%) patients had to lie down in order for vertigo to subside and 50% reported that vertigo interferes with activities of daily living. In conclusion, vertigo appears to be more common in GJB2 deafness than previously reported, and frequently affects activities of daily living in these patients. Further study, including association with objective vestibular testing, is underway.

Why does increasing sample size often dim rather than illuminate? A question of locus heterogeneity. *C.W. Bartlett, V.J. Vieland* Center for Quantitative and Computational Biology, Columbus Children's Research Institute, The Ohio State University, Columbus, OH.

Over the past few years, researchers have increasingly collaborated to create linkage samples with hundreds of families pooled together. With very few exceptions, the best-pooled results are generally similar in magnitude what any individual sample may have produced; furthermore, many if not all detected loci are novel. We set out to recreate these conditions in a simulation paradigm, examining the relative performance of various linkage detection statistics. We simulate a single marker in a sample of N=160 general nuclear families under locus heterogeneity with 0, 1, 2, 4, 6, 8 or 10 background genes. Replicates with observed HLODs > 3 were retained as an initial finding and four unselected samples of 160 families were simulated using the same heterogeneity model in order to simulate follow-up samples; analysis was performed for N=160, 320, 480, 640 & 800 families using the Kong and Cox LOD score (KCL), the HLOD, the MOD (an HLOD maximized over all model parameters), and a statistic that integrates rather than maximizes over the parameter space, the Posterior Probability of Linkage (PPL). Increasing sample size does increase linkage detection with 6 genes or less, though increases become more modest with more genes. When 8 genes are present the average KCL, HLOD and MOD all decrease from N=160 to N=320 (e.g., the KCL decreases from 2.5 to 2.0), then increases again as more samples are added (e.g., the KCL increases from 2.0 at N=320 to 2.4 at N=800); in contrast, the PPL continues to increase as sample size increases (e.g., going from 0.52 to 0.71 with no decreases). In the 10 gene condition, average KCL, HLOD and MOD all decrease from N=160 to N=320 and largely level off (e.g., the KCL drops from 2.5 to 1.8 and stays approximately constant thereafter); again, the PPL continues to increase with increasing sample size (from 0.51 to 0.61 with no decreases). As expected, all statistics will on average decrease under null simulations. We discuss the implications of this work for complex disease gene detection and provide extensions to multipoint localization.

Classical and Duarte galactosemia: Newborn screening, biochemical phenotype, and clinical outcome. *A. Cunningham[1,2], C. Techakittiroj[1,2], R. Sharma[1], C.T. Dvorak[1, 2], P. Hooper[3], D. Baye[3], H.C. Andersson[1,2]* 1) Hayward Genetics Center, Tulane University Health Sciences Center, New Orleans, LA; 2) Department of Pediatrics, Tulane Health Sciences Center, New Orleans, LA; 3) LA State Genetics Program, Office of Public Health, New Orleans, LA.

Newborn screening for galactosemia is routine in 50 US states, but detects many newborns with partial galactose-1-phosphate uridyltransferase (GALT) deficiency (less than 10% normal activity). Lactose restriction for the early months has been proposed for partial GALT deficiency, including Duarte variants, but no clinical outcomes of a prospective cohort of partial GALT deficiency patients has been reported. We report newborn screening data for LA and the clinical outcome of a prospective treatment protocol forpartial GALT deficiency patients. 151,468 infants in Louisiana from Dec 2002 -April 2005 were screened by detecting GALT; 112 presumptive positive cases of galactosemia were reported. After confirmatory testing of 106 cases, 5 cases were normal, 65 cases were galactosemia carriers (G/N) and 37 were affected with galactosemia (3G/G, 7D/D, 27D/G). The incidence of galactosemia in LA is 1: 4,094 live births (including Duarte variants). Duarte variant galactosemia patients (D/G, D/D) were lactose-restricted and challenged with galactose at age 1 year. 14/14 DG patients had normal Gal-1-P (less than 1.0 cM/L) when challenged with lactose-containing diets at age 1y and were placed on an unrestricted diet. At 2y, 6/6 DG and 2/2 D/D patients had normal eye exams, growth, liver function studies and normal Gal-1-P levels. While further follow-up is needed to determine whether Duarte variant patients develop late sequelae of galactosemia, no pathology was demonstrable at 2 years of age with galactose restriction discontinued at 1y.

Testing a genome-wide panel of non-synonymous SNPs for association to multiple common disease phenotypes.
P. Deloukas for the Wellcome Trust Case-Control Consortium Human Genetics, Wellcome Trust Sanger Inst, Cambridge, United Kingdom.

Systematic studies of sequence variation in humans have allowed the compilation of a comprehensive list of circa 10 million common variants, predominantly SNPs. Exonic SNPs that cause amino acid changes or alter STOP codons, non-synonymous (nsSNPs), are by definition very likely to have an impact on phenotype including disease. The Wellcome Trust Case-Control Consortium (WTCCC; http://www.wtccc.org.uk) which is a collaborative network of 24 laboratories across the UK, set up an experiment to test the impact of nsSNPs in four common diseases namely ankylosing spondylitis (AS), breast cancer (BC), multiple sclerosis (MS) and autoimmune thyroid disease (ATD). The study design includes 1000 cases for each disease (national UK Caucasian samples) and 1500 common controls from the 1958 British Birth Cohort (58 BBC). We selected a non redundant set of 15,897 nsSNPs with frequency above 1% in Caucasians and use them together with 1216 tag SNPs from the MHC region to generate a custom chip for use with the Infinium assay (Illumina). We also included 114 ancestry informative markers. Following synthesis, the chip harboured 15,436 loci of which 14,639 (94.8%) passed our quality metrics. We further flagged 385 loci deviating from Hardy-Weinberg Equilibrium ($p < 10^{-3}$). We have completed typing the controls and three of the disease cohorts so far, 5000 samples, and obtained a call rate of 99.4% for markers that passed QC. The 58 BBC controls have also been typed across the Affymetrix 500K arrays; comparison of a set of 980 markers common to both arrays showed concordance above 99%. Assocation analysis is ongoing and results will be discussed at the meeting.

Association Study of Neurotrophic Tyrosine Kinase Receptor 3 Gene in Childhood-Onset Mood Disorder. *Y. Feng[1], N. King[2], A. Vetro[3], E. Kiss[3], K. Kapornai[3], G. Daroczy[3], J.L. Kennedy[2], M. Kovacs[4], C.L. Barr[1,5], The International Consortium for Childhood-Onset Mood Disorders* 1) Dept Cell & Molecular Biol, Toronto Western Hosp, Toronto, ON, Canada; 2) Neurogenetics Section, Centre for Addiction and Mental Health, Toronto, Ontario, Canada; 3) Department for Child and Adolescent Psychiatry, Szeged University, Szeged, Hungary; 4) University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA; 5) The Hospital for Sick Children, Toronto, Ontario, Canada.

Major depressive disorder (MDD) is multifactorial disorder with moderate heritability. Family and twin studies show the highest relative risk for MDD in families of probands with early age at onset and/or recurrent episodes compared with the risk in the general population. Chronic antidepressant treatment up-regulates the neurotrophin signaling pathways which is involved in neural plasticity and survival. Deficits in neural plasticity have been suggested to underlie the development of depression. A genome scan of families multiply affected with recurrent, early onset MDD by Holmans et al. (2004) revealed significant linkage on chromosome 15q25.3-q26.2 (LOD = 3.73). One candidate gene located in this 15q region, NTRK3 (neurotrophic tyrosine kinase receptor 3) was particularly interesting because NTRK3 is a key gene involved in neural plasticity. The results of family based association studies of the NTRK3 gene show three markers have significant association with childhood-onset mood disorder (COMD) in our sample ($p<0.035$). The sample consists of 394 families with 459 affected children recruited from 21 mental health facilities across Hungary. Further investigation of the NTRK3 gene will be important to establish the contribution of this gene in the etiology of COMD.

Allelic association of the interleukin 7 receptor gene (IL7R) with multiple sclerosis (MS). *S.G. Gregory[1], S. Schmidt[1], J. Hart[1], A. Prokop[1], J. van der Walt[1], L.F. Barcellos[2], C. DeLoa[3], J.R. Oksenberg[3], S.L. Hauser[3], J.L. McCauley[4], M.A. Pericak-Vance[1], J.L. Haines[4]* 1) Dept Medicine, Ctr Human Genetics, Durham, NC; 2) School of Public Health, University of California, Berkeley; 3) Department of Neurology, University of California, San Francisco; 4) Center for Human Genetics Research, Vanderbilt University Medical Center.

Multiple sclerosis (MS) is the prototypic human demyelinating disease, with evidence for genetic influences arising from numerous sibling risk, adoption and twin studies. The disease is most common in young adults, with less than 10% of identified cases beginning after the age of 55 and less than 5% before the age of 14. Females are 2-3 times more frequently affected than males and disease course can vary from some patients suffering major disability several decades post-diagnosis, while others reach wheelchair dependency within a few months or years upon onset. We identified 8 genes, from eight published MS RNA expression analyses, that were differentially expressed within at least two of the studies. SNPs within one of these candidate genes, the interleukin 7 receptor gene (IL7R), are significantly associated with MS by PDT analysis within our combined dataset of almost 950 patients and their relatives. One SNP, localizing to alternatively spliced transmembrane domain of the protein, shows strongest association with risk (p=0.00006). Two independent studies have previously implicated IL7R with primary progressive MS, however, our analysis finds that this gene is also associated with the risk of developing the more common relapsing-remitting MS. Our result implicating the involvement of IL7R, therefore, not only represents a functionally relevant gene involved with the risk of MS development but also the replication of two previous studies. Molecular work to investigate the effect that the coding SNP has upon the functioning of the protein is currently underway.

Predicting BRCA1 and BRCA2 Mutations in Women with Carcinoma In Situ of the Breast. *T. Geva[1, 2], J.N. Weitzel[1], A. Dagis[1], J.A. Longmate[1], J.O. Culver[1], M.R. Palomares[1]* 1) City of Hope National Medical Center, Duarte, CA; 2) California State University, Northridge, CA.

BACKGROUND: The utility of existing BRCA mutation probability models for women with carcinoma in situ (CIS) of the breast has not been established. We evaluated existing BRCA mutation prediction models in women with a personal diagnosis of CIS who underwent genetic testing. METHODS: The Couch, Myriad, BRCAPRO, and Tyrer-Cuzick models were tested in 63 CIS patients who were enrolled in an IRB-approved registry and had BRCA analysis in the City of Hope Cancer Screening & Prevention Program Network between April 24, 1997 and March 31, 2005. Since all the models were based on invasive breast cancer history, they were adapted to accommodate the CIS history in three ways: (1) including CIS as invasive breast cancer at the age of CIS diagnosis; (2) including CIS as invasive breast cancer with 10 years added to the age at diagnosis; and (3) excluding CIS and utilizing only the remaining family history. Probability estimates were compared using Wilcoxon rank-sum tests and receiver operating characteristics (ROC) analyses. RESULTS: None of the models evaluated performed well in our sample of women with CIS, regardless of whether or how their CIS diagnoses were included in the probability calculation. Deleterious mutations were found in 6 of the 63 subjects (9.5%). The proportion of CIS patients with a detectable BRCA mutation was similar to that reported with invasive breast cancer, and BRCA carriers with CIS were younger at diagnosis than non-carriers (40.5 versus 46.0, respectively). Not all women with positive test results had a significant family history of breast and/or ovarian cancer. CONCLUSIONS: These findings suggest that BRCA testing is appropriate for women with a personal diagnosis of CIS at a young age and for those with otherwise compelling family cancer history, but that it is necessary to establish a more accurate method of estimating mutation probability for this population.

Frequency of Fragile X in multiplex autism: testing the AGRE families. *W.T. Brown[1], S.L. Nolin[1], C.S. Dobkin[1], G.S. Houck[1], A. Glicksman[1], X.D. Ding[1], S.J. Spence[2], D.H. Geschwind[2]* 1) Dept Human Genetics, NYS Inst Basic Research, Staten Island, NY; 2) Neurology Dept, UCLA School of Medicine, Los Angeles, CA.

Autism has high heritability. The Autism Genetic Resource Exchange (AGRE) is a publicly available resource of well-characterized multiplex families for genetic studies of autism. To better characterize this resource, we conducted fragile X DNA analysis (Brown 93) on one proband in each of 480 AGRE families, with follow-up family studies when indicated. Testing revealed 6 families to be positive for fragile X. These have been flagged in the AGRE database. Review of 326 available medical records showed 114 (35%) had prior negative genetic testing. Thus, the prevalence of fragile X among the approximately 312 previously untested AGRE families was ~ 1.9%. An estimate of the IQ score of the autistic subjects was 8035 with range 34-144, based on the Raven. Thus, the AGRE sample is likely to have a higher IQ distribution than typical for fragile X subjects (mean ~4025). Previous prevalence studies of fragile X in autistic samples range from 0 to 16% with mean ~4% (Feinstein 98). Our 1.9% is similar to a report of 1.6% among 123 unrelated autistic individuals (Bailey 93), but lower than the 13% we found on an earlier multicenter study of 183 individuals (Brown 86). An additional 166 families added to AGRE subsequent to this study, who had received a more rigorous prescreen for genetic conditions, have also been tested and found negative for fragile X. Conclusions: A growing awareness of fragile X syndrome may increase the probability of prior fragile X screening in multiplex autism families and their exclusion from AGRE. The observed frequency of 1.9% is lower than the expected 4%. This appears due to higher mean IQs in AGRE subjects than typical for fragile X syndrome. It confirms an association of fragile X and autism.

Expression pattern of WHSC3 (Wolf-Hirschhorn syndrome candidate gene 3) during neocortex and cerebellum development suggests a role in neuronal migration. *A. Boerdlein, S. Schlickum, A. Winterpacht, S.U. Endele* Institute of Human Genetics,University of Erlangen-Nuremberg, Erlangen, Germany.

Wolf-Hirschhorn syndrome (WHS) is a complex and variable malformation syndrome which is caused by partial deletion of the short arm of one chromosome 4. WHS is thought to be a contiguous gene syndrome with a yet unknown number of genes contributing to the phenotype. We focused on the neurological features of the syndrome (mental retardation, seizures) and further investigated a recently cloned gene (*WHSC3*) from the Wolf-Hirschhorn syndrome critical region (WHSCR1) which is strongly expressed in brain and testis. Human *WHSC3* encodes a protein of 90 amino acids, which probably encodes a preprohormone or neuropeptide precursor. Here, we present a detailed expression study of *Whsc3* during murine neocortex and cerebellum development by RNA-in situ-hybridization of mouse embryos. Expression in the cerebral cortex starts after the preplate stage (> E12). At E14.5 to E17.5 *Whsc3* is expressed in the intermediate zone and in the marginal zone (most probably in the Cajal Retzius cells) of the developing cortex. In newborn mice, we could detect signals in neurons of the cortical plate and in Cajal Retzius cells (marginal zone) whereas in adult brain signals were present in all cortical and subcortical regions of the brain. In the developing cerebellum, expression could be detected in the intermediate zone at stages E.13 to 17.5, in the molecular layer in newborn mice and in the granular and molecular layer in adult mice (most probably in the interneurons). In conclusion, the expression pattern suggests a potential role of this gene in neuronal migration processes, and thus in the pattern formation of human cerebral and cerebellar structures.

Genome-wide association study combining incomplete high resolution SNP data with sparse markers from a linkage scan. *W-M. Chen, G.R. Abecasis* Center for statistical Genetics, Dept. of Biostatistics, Univ. of Michigan, Ann Arbor, MI.

With millions of single nucleotide polymorphisms (SNPs) identified and characterized, genome-wide association studies are underway to identify susceptibility genes for complex traits and diseases. Given limited genotyping resources, we propose an approach that can produce large increases in power for genome wide association scans. We show that, by combining high-resolution SNP genotypes for just a few individuals with sparse marker data from a typical linkage scan, genotypes for many related individuals can be inferred with high accuracy, and power for the genome-wide association analysis can be substantially increased.

First, our algorithm involves calculation of a probability distribution for each missing genotype in pedigree. We implemented this step using the Elston-Stewart algorithm, for larger pedigrees, and the Lander-Green algorithm, for smaller pedigrees. Next, observed genotypes and probability distributions for unobserved genotypes are combined in a rapid association test that characterizes association between genetic variants and quantitative phenotypes in families.

We investigate the properties of the method in different family structures by simulation. We identify optimal family genotyping strategies for many different pedigree structures and show that in many cases it is sufficient to genotype <50% of the individuals in family to infer >90% of genotypes with near certainty. To illustrate our method, we carried out genome-wide association analysis for 27 gene expression phenotypes in 20 CEPH families, in which ~0.8 million SNPs are only genotyped in grandparents and parents, and a subset of 6,564 SNPs are genotyped in all 168 CEPH individuals. In addition to increasing evidence for association of 15 previously identified cis-acting associated alleles, our genotype inference algorithm allowed us to identify 4 novel cis-acting associated alleles that were missed when analysis was restricted to individuals genotyped by the HapMap project. Our genotype inference algorithm and the proposed association test are implemented in computer programs GHOST and Merlin.

dup(15)(q15q25) as a sole anomaly in a case of polycythemia vera: Trisomy 15 in hematological disorders revisited. *A. Adeyinka, S. Wei, J. Sanchez* Dept Medical Genetics, Henry Ford Health System, Detroit, MI.

Trisomy 15 as a sole clonal autosomal anomaly in hematological malignancies reportedly is uncommon. Nonetheless, trisomy 15 as a sole abnormality has been documented in a wide range of hematological disorders, especially myelodysplastic disorders. However, complicating an understanding of the possible role of this anomaly in tumorigenesis and/or tumor progression are its frequent association with loss of the Y chromosome in males, the fact that it is commonly seen in older patients and its presence in individuals without hematological malignancy. We report a cytogenetic and fluorescent in situ hybridization (FISH) analysis in a case of polycythemia vera with dup(15)(q15q25). G-banded metaphase cells obtained from short-term culture of a bone marrow sample from an 87-year-old female patient were 46,XX,dup(15)(q15q25)[5]/46,XX[15]. The dup(15q) was further delineated by FISH studies using whole chromosome 15 painting probe (WCP15), *SNRPN*, *PML*, and D15Z1 probes. FISH analysis confirmed duplication of 15 q-arm distal to *SNRPN* and encompassing the *PML* locus. Furthermore, we identified through a search of our database of cytogenetically characterized hematologic disorders, 22 cases with trisomy 15 as the sole autosomal anomaly. Including the case of dup(15q), all 23 samples with gain of 15q were from older patients, age range 65-92 years with a median age of 81 years, who were mostly males (70%). In nine (1 female and 8 males) (39%) of the 23 individuals, trisomy 15 was associated with loss of a sex chromosome. Among all 23 individuals, a wide range of hematological conditions was associated with trisomy 15/gain of 15q, including hematological malignancies and non-neoplastic marrow. Our data show that trisomy 15 is associated with hematological malignancies as well as non-neoplastic hematological conditions, making it a poor marker of malignancy. Nonetheless, our present finding of dup(15)(q15q25) as a sole abnormality in polycythemia vera and a previously published case of acute promyelocytic leukemia with dup(15)(q15q26) as a sole abnormality suggest that duplication of genes on 15q may be of pathogenic importance in some hematological malignancies.

Families with nonsyndromic cleft lip with or without cleft palate (CL/P) have an increased frequency of lip print whorl patterns, which may be associated with IRF6. *K.W. Chirigos, K. Neiswanger, K.M. Bardi, C.A. Brandon, M.E. Cooper, M.L. Marazita* Ctr Craniof Dent Genet, U Pittsburgh, Pittsburgh, PA.

CL/P is a common birth defect with genetic and environmental components. Van der Woude syndrome (VWS) is an autosomal dominant form of CL/P, in which cleft palate only and/or lower lip pits also affect some family members. VWS is due to mutations in IRF6; different IRF6 variants increase susceptibility to CL/P (see Marazita et al, this meeting). Lip prints are permanent and unique to each individual, with several basic pattern types. Lip whorls consist of one medial whorl on the upper lip and/or two paramedial whorls on the lower lip. As part of the Pittsburgh Oral-Facial Cleft study, we analyzed lip prints from 232 subjects: 167 from cleft families (43 individuals with CL/P, 124 unaffected relatives) and 65 controls with no family history of clefting. Lip prints were taken using the Faurot inkless method, electronically scanned, and contrast-enhanced. Patterns were scored by two independent raters blind to the cleft status of the subjects. 30.5% (51/167) of the subjects from cleft families had whorls, compared to 10.8% (7/65) of the controls ($p = 0.001$). The frequency of whorls did not differ significantly between family members with CL/P (16/43, 37%) and their unaffected relatives (35/124, 28%; $p = 0.34$). 117 cleft and non-cleft family members also were genotyped for three IRF6 SNPs. One of the three SNPs (rs2013162, highly associated with CL/P) showed an association with whorls, both by genotype ($p = 0.03$) and with the common allele ($p = 0.02$). When subjects with CL/P and unaffected relatives were analyzed separately, the trend continued for both genotypes and alleles, with somewhat more significance in the unaffected relatives. If these results are confirmed with additional IRF6 SNPs and larger samples of unrelated individuals, they suggest that individuals with whorl patterns may carry IRF6 variants that increase their risk of having children with CL/P. These results are particularly interesting since the VWS phenotype includes lip pits. Supported by NIH grants R01-DE016148, P50-DE016215, CIDR NIH contract N01-HG-65403.

Replication Stress Induced Tumor-like Deletions of FRA3B in Human/Mouse Cell Hybrids. *S.G. Durkin, R.L. Ragland, T.W. Glover* Dept Human Genetics, Univ Michigan, Ann Arbor, MI.

Common fragile sites (CFS) are regions of the genome that form gaps and breaks on metaphase chromosomes when DNA synthesis is partially inhibited. While normally stable in somatic cells, CFS and associated genes are frequently rearranged in cancer cells and may be indicators of DNA replication stress early in tumorigenesis. We and others have previously reported the induction of large scale chromosome rearrangements, including translocations and terminal deletions with breakpoints at CFS in normal cells in vitro. However, the great majority of rearrangements observed at CFS in cancer cells are not these types of rearrangements, but are intra-locus deletions spanning tens to hundreds of kb. Although it has been assumed that these tumor deletions are due to replication stress-induced CFS destabilization, it has been unclear if replication stress and CFS breaks in vitro can also result in tumor-like deletions. To this end, we have utilized human/mouse cell hybrids harboring a single human chromosome 3 that contains the most frequently broken human CFS, FRA3B. To induce replication stress, these cells were exposed to a low-dose of aphidicolin, which induces CFS breaks, for five days followed by one day of recovery. Seventy-four resulting clonal cell populations were analyzed by PCR spanning FRA3B at approximately 10-30 kb marker intervals to detect deletions. Thirty clones (40%) were detected to have deletions within the CFS critical region, previously defined to lie between intron 3 to intron 7 of FHIT. Ten of the 30 deletion clones were shown to have contiguous deletions within this region ranging from approximately 300 to 800 kb, similar in size and location to those deletions observed in esophageal adenocarcinomas, renal carcinomas and other cancers while other clones exhibited smaller deletions. We are currently cloning the specific breakpoints of these deletions to gain insight on the sequences involved in these rearrangements and how they are repaired, and are analyzing deletion clones for CFS breakage. These findings support a direct mechanistic relationship between replication stress and CFS breaks in vitro, and genome rearrangements in cancer.

Fine mapping of chromosome 19 candidate region shows association in autism. *A.L. Collins[1], D.Q. Ma[1], R. Rabionet[1], I. Konidari[1], E.R. Martin[1], H.H. Wright[2], R.K. Abramson[2], M.L. Cuccaro[1], J.R. Gilbert[1], M.A. Pericak-Vance[1]* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) School of Medicine, University of South Carolina, Columbia, SC.

Autism is a neurodevelopmental disorder of complex genetics characterized by impairment in social interaction and communication as well as repetitive behavior. Evidence from several genome-wide screens suggests the involvement of Chromosome 19 in autism. Results of our linkage study on 210 multiplex families ascertained through Duke and AGRE gave significant evidence for heterogeneity showing that a subset of AGRE families (AGRE1) was significantly different with respect to these data (p=0.001) resulting in a maximum heterogeneity linkage score (HLOD) of 3.56 at marker D19S593 (17.2Mb), Thus, we used the AGRE1 subset (N=99) to fine map the region surrounding this marker (13.7Mb-19.9Mb) by genotyping 424 SNPs with MAF>0.01 across this region, resulting in an average spacing of one SNP every 15kb. We identified two clusters of associated markers, one near 16.3Mb (rs875622; p=0.00008) and a second at 18.1Mb (rs273506; 0.0004). Analysis in the subset of families (N=38) showing positive linkage throughout the region targeted the region at 16.3Mb as the most likely location of an autism risk gene (rs4808047; p=0.0003) in the *EPS15L1* gene (epidermal growth factor receptor pathway substrate 15-like 1). The protein encoded by *EPS15L1* is thought to be a component of clathrin-coated pits that is required for receptor-mediated endocytosis. In summary, we have found evidence for significant association in a linkage region for autism risk and have targeted several genes including *EPS15L1* for follow-up and additional analysis.

Autism Genome Project: Linkage Analyses. *B. Devlin* Dept Psychiatry, Univ Pittsburgh, Pittsburgh, PA. for the Autism Genome Project (AGP).

The genetic architecture of autism spectrum disorders (ASD) is undoubtedly complex, and identification of risk loci requires large samples. For this reason the Autism Genome Project (AGP) Consortium was formed from 4 consortia in North America and Europe: Autism Genetics Cooperative, Autism Genetics Resource Exchange, Collaborative Programs of Excellence in Autism and International Molecular Genetic Study of Autism Consortium. For linkage analysis, DNA from MPX families was genotyped using the 10K Affymetrix SNP microarray. After quality control analyses, 1168 MPX families had genotypic and diagnostic data useable for linkage analysis. These families were partitioned by diagnosis into narrow, broad and hASD, depending on the distribution and severity of ASD in the MPX families. Narrow is a subset of broad, but hASD for this analysis is considered an independent set of families, which are more heterogeneous for diagnosis and possibly etiology. Thus we focus on the narrow and broad analyses. Families were further subdivided into whether they contained affected females (FC) or only affected males (MO), because the male:female ratio for those diagnosed with autism is 4:1, and on the basis of inferred parental ancestry into all families or European. Multipoint linkage analyses were performed using the exponential S-all statistic and MERLIN. Linkage information, as reported by ALLEGRO, averaged 95% over the genome. Its minimum occurred at telomeres (> 71%). The information for linkage is large because of good coverage of markers and because parental genotypes were typically available. There were 522 MPX families meeting narrow diagnostic criteria (329 MO and 193 FC) and 731 meeting broad criteria (458 MO and 273 FC). The most compelling linkage statistics emanate from the FC families, consistent with standard quantitative genetics theory of threshold models. Results from FC families nominate regions of Chromosome 11p, 9p and 5p for targeted follow-up analysis: 11p is genome-wide significant, whereas 9p and 5p meet suggestive linkage. Two other regions that drew support from previous linkage studies, namely 7q and 2q, continue to draw support from the enlarged sample.

Chronic subdural hematomas in a male with ornithine transcarbamylase deficiency. *M. Gucsavas-Calikoglu[1], J. Koepke[1], M. Tennison[2], R. Greenwood[2], J. Muenzer[1]* 1) Dept Pediatrics, Univ of North Carolina, Chapel Hill, NC; 2) Dept Neurology, Univ of North Carolina, Chapel Hill, NC.

Ornithine transcarbamylase (OTC) deficiency is a rare X-linked urea cycle disorder with hyperammonemia and devastating neurological outcomes in affected males. Neuroradiological findings consist of diffuse cortical edema with compression of third and lateral ventricles during hyperammonemic episodes and bilateral low-density white matter lesions and diffuse cortical atrophy with ventriculomegaly in survivors. Subdural hematomas, however, have not been reported in OTC. Here we present a 22 month-old male who developed multiple and recurrent subdural hematomas. He was diagnosed at less than 24 hours of age because of a positive family history and had an initial ammonia of 75 mol/L. He was adequately treated with ammonia scavenger drugs and low protein diet. Mutation analysis revealed a donor splice site error in intron 5 at IVS5 + 2(T>C), previously reported in severe neonatal disease. He remained neurologically intact until 5 months of age when his ammonia rose to 448 mol/L with a viral illness. A CT scan within 48 hours of treatment showed diffuse cerebral edema with effacement of the sulci, Sylvian fissures and lateral ventricles. He became spastic and regressed in development after this episode. At age 7 months, he presented with partial-complex seizures and MRI showed large bilateral subdural hematomas and marked frontotemporal cortical atrophy. Investigation for child abuse was negative. He continues to experience multiple hyperammonemic episodes with further neurological deterioration and recurrent acute, subacute and chronic subdural hematomas. He has spastic quadriplegia and cortical blindness. It is likely that stretching of bridging veins secondary to severe cortical atrophy resulted in susceptibility to recurrent bleeding in our patient. In conclusion, subdural hematomas should be considered among the neurological complications of OTC. In addition, OTC should now be included among the inborn errors of metabolism associated with the development of subdural hematomas such as glutaric academia type I, Menkes disease, and D-2-hydroxyglutaric aciduria.

A Genome-Wide Study for Determinants of Type 2 Diabetes Mellitus in the Pima Indians. *L. Baier, R. Hanson, W. Knowler, C. Bogardus* PECRB, NIDDK/NIH, Phoenix, AZ.

The Pima Indians of Arizona have the highest reported prevalence of type 2 diabetes mellitus (T2DM). To identify genes that contribute to this disease, we have recently completed a genome-wide association (GWA) study using the Affymetrix Mapping 100k SNP chip. This study was done in two phases. Phase 1 was designed to detect potential associations with young-onset T2DM, by genotyping 300 early-onset T2DM subjects (onset age<25 yrs) and 329 non-diabetic controls (age >45 yrs), and 271 additional subjects who were diabetic and non-diabetic siblings of the selected subjects. Associations for T2DM were calculated using both a case/control analysis (N= 629) and a within-family analysis (482 siblings from 169 sibships), and SNPs that had the strongest association for the combined associations were prioritized. Phase 2 of the GWA was designed to detect associations with pre-diabetic traits (% body fat, insulin action as measured by the hyperinsulinemic euglycemic clamp technique, and the acute insulin response to an intravenous bolus of glucose). Six hundred non-diabetic subjects who had been metabolically phenotyped for these predictors of T2DM were genotyped using the 100k chip. SNPs were prioritized that were associated with a pre-diabetic trait and had also been associated with early-onset T2DM in GWA Phase 1. Prioritized SNPs from the GWA analyses are being further genotyped, by the method of SNPplex, in a large population-based sample of full-heritage Pima Indians (N= 3600) to identify variants that also have an effect on a population level. To date, 124 SNPs have been genotyped in the population sample and 12 of the 124 SNPs are associated with early-onset T2DM, a pre-diabetic trait, and T2DM at the population level. All of these 12 SNPs map to distinct chromosomal regions. Follow-up studies within these regions are ongoing.

Infant with Vascular Malformations most closely resembling Klippel-Trenaunay Syndrome with Hypoplastic Thumbs and Extensive Mongolian Spots. *C.A. Bay, S. Morrill-Cornelius, R.G. Cadle, B.D. Hall* Division of Genetics, Department of Pediatrics University of Kentucky, 740 S. Limestone Dr. Lexington, KY 40536.

We have observed a female Hispanic/Caucasian infant at age 8 days and at FU at 3 1/2 months with striking cutaneous findings and dysmorphic features including extensive capillary malformations and mongolian spots of the full face, thorax, and extremities; subtle extremity hemihypertrophy, and bilateral hypoplastic thumbs. We feel she is most consistent with the diagnosis of Klippel-Trenaunay(KT) syndrome, and broadens the clinical spectrum to include thumb anomalies. We wonder if her extensive mongolian spots may be due to differences in parental pigmentation. She was the 44.5 cm (<10 centile), 3.09 kg (25-50 centile) product of a 39 week gestation to a 28 year old G1P1 pale caucasian female. Father was 48 years old; of Hispanic ancestry with reported dark skin pigmentation. Pregnancy was unremarkable. Teratogens: Insulin; good control for DM; 1 PPD cigarettes; antibiotic for UTI. Family history: negative for vascular malformations, hemihypertrophy; positive for mongolian spots. At age 8 days: length: just below 5th centile, weight: 25-50 centile, and OFC 25 centile. She had striking flat capillary malformations and blue-gray color of the full face, thorax, abdomen, and all extremities; subtle hemihypertrophy of the right leg; bilateral hypoplastic thumbs. Brain MRI unremarkable, echocardiography: mild LVH, mild hypoplasia transverse aorta, flow acceleration across MV. Ophthalmic exam: mild increase in ocular pressures. At age 3 1/2 months skin showed less blue-gray discoloration and red areas, but they were still clearly visible. Same degree of leg hemihypertrophy, but fullness of right face was seen for first time. Development is age appropriate. We feel that the diagnosis is most consistent with Klippel-Trenaunay syndrome. Malformations of the hands including polydactyly and oligodactyly have been reported in KT syndrome, but to our knowledge thumb anomalies have not been reported previously. One of us (BDH) has seen a case of KT with triphalangeal thumbs. This extends the spectrum of anomalies in KT syndrome to include thumb anomlies.

Mis-expression of Zic2 and dominant-negative Zic2 in mouse ES cells results in abnormal proliferation and axon growth. *S. Brown, L. Brown* Dept. Ob/Gyn, University of Vermont, Burlington, VT.

Our previous work has shown that that the holoprosencephaly-associated transcription factor, Zic2 is highly expressed in the inner cell mass of the blastocyst as well as in ES cells. As ES cells differentiate, expression of Zic2 diminishes, suggesting that Zic2 is a marker of pluripotentiality. This observation has led us to attempt probe the role of Zic2 in ES cell biology by manipulating the expression in ES cells. To this end, we have developed several systems for mis-expression of wtZic2 and dominant-negative Zic2 (dnZic2)in ES cells. These include cell lines with a Zic2 transgene that is regulated by tamoxifen inducible Cre recombinase as well as cell lines in which Zic2 alleles are regulated by tetracycline.

Studies with these cell lines show that, in the undifferentiated state, mis-expression of wtZic2 or dnZic2 is tolerated and that the cells continue to grow apparently normally, although transgene expression is down-regulated over time. During in-vitro differentiation, mis-expression of either wtZic2 or dnZic2 has profound effects on ES cell growth. In one neural differentiation paradigm, in which cells are allowed to form embryoid bodies and are then physically disrupted to form single cell suspensions and are plated on glass, mis-expression of Zic2 results in a near complete failure of the cells to proliferate. Lower levels of Zic2 misexpression results in cells that proliferate and differentiate into neurons but exhibit very abnormal axon growth. In another differentiation paradigm, in which cells are allowed grow as embryoid bodies that are then fixed and sectioned, Zic2 mis-expression results in abnormal apoptosis as well as abnormal embryoid body morphology. *Based on the fact that Zic2 is normally expressed in ES cells and that perturbations in its expression result in abnormal growth and differentiation of ES cells, we conclude that Zic2 is likely to have an important role in regulating early neural differentiation events. Current work is aimed at understanding the molecular mechanisms underlying Zic2 action.

Amyotrophic lateral sclerosis (ALS) and superoxide dismutase (SOD1) mutations: an intronic perspective. *R.M. Game, J. Lynes, N.J. Schisler* Biology, Furman University, Greenville, SC.

ALS, a fatal neurodegenerative disease, currently affects 30,000 people in the United States with 5,000 new cases annually. About 10 percent of cases of ALS are familial; of these 15 to 20 percent are associated with mutations in the SOD1 gene on chromosome 21q22.1. Sporadic cases of ALS may also be due to novel mutations in the SOD1 gene.

Lindberg et al. (2002) assessed folding-related defects by comparing the unfolding behavior of 5 mutants: A4V at the interface between the N and C termini, C6F in the hydrophobic core, D90A at the protein surface, and G93A and G93C, which decrease backbone flexibility. With the exception of A4V and C6F, these mutations only marginally affected the stability of the native protein, but showed pronounced destabilization of the metal-free apo state of the enzyme which was correlated with lower mean survival time for ALS patients. Hough et al. (2004) also suggested a subset of mutations located close to the dimeric interface can lead to a major destabilization of the mutant enzymes.

The primary medical literature inventories 418 mutations in SOD1-associated ALS patient that are non-randomly distributed: 404 missense, 5 deletions, 6 insertions and 3 nonsense. The vast majority of missense mutations were found in exon 1 (111) and 4 (222), which include the folding-related defects described above. Exons 2, 3, and 5 had the fewest missense mutations (29, 8, and 34 respectively).

The present study seeks to determine if intronic elements play a role in the origin of ALS-associated mutations. The mutation free/reduced regions that included exon 2 and 3 were found to be associated with repeated elements located in introns 1 and 2. The relative importance of these intronic sequences is assessed using phylogenetic comparisons with other species including mice.

Exploration of 100 cardiovascular candidate genes across different ethnic groups in the INTERHEART study.

J.C. Engert[1], S. Yusuf[2], B. Keavney[3], G. Paré[4], D. Serre[4], A. Montpetit[4], M. McQueen[2], H. Cordell[3], T.J. Hudson[1,4], S. Anand[2] 1) Depts Med and Human Genetics, Royal Victoria Hospital, McGill Univ, Montreal, Quebec, Canada; 2) Population Health Research Institute and McMaster Univ, Hamilton, Ontario, Canada; 3) University of Newcastle upon Tyne, Institute of Human Genetics, United Kingdom; 4) McGill University and Genome Québec Innovation Centre, Montreal, Quebec, Canada.

With the advent of high throughput genotyping technologies, it has become increasingly practical to do association studies on large numbers of genes and SNPs in well-powered study samples. The ability of tagging SNPs developed from the HapMap data set, to capture unassayed SNPs has been well characterized within specific ethnic groups, but their performance in a truly global study remains to be determined. The INTERHEART myocardial infarction (MI) case/control sample consists of >27,000 individuals recruited from 52 countries around the world. In a sample of three ethnicities (~10,000 European, South Asian and Arab individuals) from this study population, we have genotyped 1536 SNPs from ~100 candidate loci to determine their association with myocardial infarction and multiple associated measured risk factors. All three HapMap ethnic groups were used to select tSNPs from the gene regions to capture common variation (minor allele frequency > 5%). Hardy-Weinberg Equilibrium was tested for all SNPs, but only SNPs that failed a visual quality control inspection were eliminated from further analysis. In addition, we have used the program STRUCTURE to identify ethnic outliers and miscategorized individuals. We performed separate analyses within each ethnic group, but also analyzed all samples together (cases and controls were matched by ethnicity and geographical region). Significant differences in the allele frequencies of some SNPs in candidate genes were observed between ethnic groups, which may affect their role in the pathogenesis of MI.

Evaluation of the aryl hydrocarbon receptor nuclear translocator (ARNT) gene on 1q21 as a type 2 diabetes (T2DM) susceptibility gene in the Old Order Amish. *M. Fu[1], C. Damcott[1], M. Sabra[1], S. Ott[1], X. Shi[1], A.C. Naj[1,2], L. Reinhart[1], J. Gunton[3], T. Pollin[1], J. O'Connell[1], B.D. Mitchell[1], C.R. Kahn[3], A.R. Shuldiner[1,4]* 1) Dept Med, Div Endocrinology, Univ Maryland, Baltimore, MD; 2) Dept Epid, Johns Hopkins Univ, Baltimore, MD; 3) Joslin Diab. Cent., Harvard Med School, Boston, MA; 4) GRECC, VAMC, Baltimore, MD.

ARNT is a member of the basic helix-loop-helix Per/AhR/ARNT/Sim (bHLH-PAS) family of transcription factors. Its expression is markedly decreased in pancreatic islets of humans with T2DM. Decreases in expression of ARNT in cultured cells using siRNA and in mice with a cell-specific deletion of ARNT causes significant abnormalities in insulin secretion and glucose metabolism. ARNT is located within a region on chromosome 1q21-q24 linked to T2DM in the Old Order Amish and six other populations. We hypothesized that variation in ARNT may affect glucose-stimulated insulin secretion, thus increasing susceptibility to type 2 diabetes. We sequenced the 22 exons, the surrounding intronic sequences, and the proximal regulatory region of ARNT in 24 Amish samples. We identified 16 polymorphisms, including a missense mutation (D511N) and a silent mutation (V174V). Sixteen haplotype-tagging SNPs and the cSNPs were genotyped in more than 1,300 subjects of the Amish Family Diabetes Study for association analysis. None of the SNPs showed evidence of association with T2DM in the Amish. However two SNPs (rs10847 and rs11204737) significantly associated with insulin levels at 30 and 60 min following a 75 gram oral glucose tolerance test in nondiabetic individuals (P = 0.004-0.039). Individual homozygous for the rare alleles of D511N were found in one diabetic patient and in two subjects with impaired glucose tolerance but not in any normoglycemic subjects. Our data do not suggest that ARNT can explain the linkage of T2DM to this region, but polymorphisms in ARNT may play a modest role reducing early insulin secretion in response to glucose. Whether homozygosity for the D511N missense mutation affects T2DM susceptibility in a small number of subjects merits further investigation.

Regulation of endogenous L1 expression by splicing and polyadenylation. *V.P. Belancio, P.L. Deininger*
Epidemiology, Tulane University, New Orleans, LA.

LINE-1 is the only active autonomous non-LTR human retroelement. The expression and activity of these elements contribute to human genomic instability. LINE-1 elements comprise 17% of the human genome, which translates into about 5x105 L1 copies, the majority of which are defective due to truncations at their 5 end. We have demonstrated that LINE-1 mobility is significantly attenuated by the presence of functional polyadenylation signals throughout the coding region of L1 genomes. Additionally, we reported a number of functional splice donor and acceptor sites present throughout the L1 sequence. These splice and polyadenylation signals are utilized by both transiently transfected and endogenously expressed L1 elements. Typical polyadenylation and splice sites are composed of highly conserved as well as variable cis-elements that are recognized by the cellular proteins for processing. The strength and usage of any given signal may vary between tissues as demonstrated by the presence of tissue-specific alternatively spliced and/or polyadenylated transcripts of numerous mammalian genes. We have analyzed the expression profiles of endogenous L1 elements from different human cell lines. We find that some of the splice and polyadenylation signals are used constitutively, while others seem to be utilized differentially, resulting in significant variation in the amount of specific spliced and polyadenylated L1-related RNA species among these cell lines. This apparent regulation of the post-transcriptional processing events brings up the possibility that L1 elements have differential impacts in different tissues based on both the level and nature of the specific transcripts expressed.

Psoriasis-associated alleles from the haplotype block harboring PSORS1 within the HLA class I interval have differential enhancer activity compared to wild type alleles. *L. Cao[1], Y. Liu[1], C. Helms[1], M. Fernandez[2], A. Bowcock[1]* 1) Dept Genetics, Washington Univ, St Louis, MO; 2) Dept. Laboratory Medicine, University of Texas M.D. Anderson Cancer Ctr, Houston, TX.

Psoriasis is a complex inflammatory disease of the skin affecting 1-2% of the Caucasian population. Associations with alleles from the histocompatibility locus antigen cluster (HLA) class I region (now known as PSORS1), particularly HLA-Cw*0602, were described over 20 years ago. However, extensive linkage disequilibrium (LD) within this region has made it difficult to identify the true susceptibility allele from this region. We recently performed a comprehensive case/control and family-based association study on 242 Northern European psoriasis families and two separate European control populations. With all tests, association was strongest with single markers and haplotypes from a block of LD harboring HLA-C and SNP n.9, a locus lying 4kb upstream from HLA-C. HLA-Cw*0602 is commonly over-transmitted to affected members, but rare over-transmitted haplotypes also harbor HLA-Cw*12 alleles. HLA-Cw*12 family members are closely related to HLA Cw*0602, sharing identical sequences in their alpha-2 domains, peptide binding pockets A, D and E and all 3 introns. The introduction of a potential binding site for the RUNX/AML family of transcription factors in intron 7 (CINT), is also specific to these HLA-C alleles. Genotyping of CINT in patients revealed that it is in complete LD with SNP n.9 and SNP n.7 that lies 10kb from the start of HLA-C. Transient transfections with luciferase reporter constructs of alleles from these loci revealed that they harbor enhancer activity that differentiates psoriasis-associated and wt alleles. This suggests that several HLA-C alleles may be responsible for psoriasis susceptibility, or that PSORS1 is an enhancer affecting expression of genes within the class I region such as HLA-C itself, or skin-specific genes such as corneodesmosin (CDSN) that lies ~150kb downstream from HLA-C. This is consistent with our observation that CDSN is up-regulated in patients with predisposing HLA class I alleles.

Genomewide association with eleven intermediate phenotypes for CVD in women. *D.I. Chasman[1,5,8], D.T. Miller[3,5,6,8], P. Kozlowski[3,5,8], J. Suk Danik[1,5,8], R. Lazarus[4,5,8], R.Y.L. Zee[1,5,8], N.R. Cook[1,5,7,8], D.J. Kwiatkowski[3,5,8], P. M. Ridker[1,2,5,8]* 1) Preventive Medicine; 2) Cardiovascular Medicine; 3) Hematology; 4) Channing Laboratory; 5) Brigham and Women's Hospital; 6) Children's Hospital; 7) Harvard School of Public Health; 8) Harvard Medical School, Boston, MA.

The biological processes underlying cardiovascular disease (CVD) may be understood through analysis of intermediate phenotypes corresponding to disease etiologies based in lipid metabolism, inflammation, and thrombosis/hemostasis. Among 28,000 participants from the Womens Health Study (WHS), a prospective cohort of initially healthy women with over 10 years of follow-up observation and over 600 accrued CVD events, we had previously measured 11 plasma-based intermediate phenotypes thought to be associated with disease risk including: LDL, HDL, triglycerides, total cholesterol, apoA1, apoB, and Lp(a) for lipid-based etiologies, C-reactive protein (CRP), soluble ICAM-1, and fibrinogen for inflammation-based etiologies, and homocysteine and fibrinogen for thrombosis/hemostasis-based etiologies. We have now collected Affymetrix GeneChip 100K single nucleotide polymorphism (SNP) data on a subset of about 1000 Caucasian, non-smoking WHS participants who were also not using hormone replacement therapy at enrollment. In preliminary analysis, we have identified significant candidates for association with the 11 plasma biomarkers. The initial candidates include previously known associations with the biomarkers, e.g. CRP gene SNPs with CRP and an MTHFR gene SNP with homocysteine, as well as potentially novel genes and SNPs that may contribute to the risk and progression of CVD. To validate the initial candidates, we are re-examining the associations through replication in additional subsets of 1000 subjects from the WHS. Once validated, candidate associations with the intermediate phenotypes will be tested for association with incident CVD in the WHS and in other prospective cohorts.

Identification of Down syndrome heart defect candidate genes. *L.J.H. Bean[1], K.J. Dooley[2], S.B. Freeman[1], T.C. Rosser[1], C. Oxforc-Wright[1], C. Kohler[3], G. Capone[3], S.L. Sherman[1]* 1) Dept Human Genetics, Emory Univ, Atlanta, GA; 2) Sibley Heart Center, Dept Pediatrics, Emory Univ, Atlanta, GA; 3) Div Neuro and Dev Med, Kennedy Kreiger Institute, Baltimore, MD.

Individuals trisomic for chromosome 21 exhibit a wide range of phenotypes known as Down syndrome (DS). DS is mainly characterized by mental retardation and clinical features that include hypotonia, abnormalities of the face, hands, and feet. Other variable phenotypes associated with DS may include congenital heart disease (CHD), digestive tract abnormalities, congenital cataracts, or leukemia. The prevalence of DS, approximately 1 in 600 to 1 in 1000 live births, makes this syndrome the most commonly identified form of mental retardation. Compared to the general population, individuals with DS are at a 2000-fold increased risk for complete atrioventricular septal defects (AVSDs), a severe form of CHD. The purpose of this study is to identify genetic variants on chromosome 21 that contribute to CHD susceptibility using a candidate gene approach in a DS population with complete AVSDs. We have ascertained the largest reported collection of DNA on complete AVSD DS cases and their parents. Studies done in the mouse indicate that several genes within the candidate region are differentially expressed during heart development. We hypothesize that genes expressed in fetal, but not adult, heart may play a role in the etiology of congenital heart defects. The human heart expression pattern of several interesting candidate genes in a 10Mb DS CHD critical region on chromosome 21 has been determined. Interestingly, the PDE9A gene has specific splice variants whose expression is developmentally regulated. Combining this expression data with the vast array of genomic resources available for chromosome 21, we will use molecular approaches to identify candidate genes for DS CHDs within a 10 Mb heart defect critical region. We are in the process of genotyping 384 SNPs within this region in our population of approximately 125 Caucasian cases(DS with AVSD) and controls (DS with no heart defect) and 40 African-American cases and controls. Parent samples will also be genotyped.

Patterns of gene flow between human populations. *D. Garrigan[1], Z. Mobasher[2], S.B. Kingan[1], M.M. Pilkington[2], M.F. Hammer[2]* 1) Organismic & Evolutionary Biol, Harvard University, Cambridge, MA; 2) Genomic Analysis & Technology Core, University of Arizona, Tucson, AZ.

It is widely recognized that human populations exhibit low levels of genetic differentiation, as evidenced by low observed values of summary statistics such as FST. However, summary statistics do not take full advantage of all the information in DNA sequence data and it is difficult to know whether low FST values are due to recent population divergence, high rates of gene flow, or both. To overcome this limitation, we estimate parameters of a general isolation-with-migration (IM) model of population structure with re-sequenced data in large samples from 10 human populations. A Markov chain Monte Carlo (MCMC) technique is used to infer simultaneously divergence times, rates of directional gene flow, and changes in population size. The results indicate that sub-Saharan African populations diverged at least 100 thousand years ago (kya), while most non-African populations diverged as recently as 7-25 kya. The divergence time between African and non-African populations is estimated at 40 kya, suggesting that non-African populations descend from a genetically structured ancestral African population. There is no support for radical historical changes in African population sizes, while non-African populations appear to have experienced a strong, recent bottleneck(s). Rates of gene flow, both within and between continents, are estimated to be highly asymmetrical and implicate Asia as the primary source for most recent human gene flow. Lastly, the estimated locus-specific rates of gene flow, including mitochondrial DNA and Y chromosome data, reveal complex patterns of sex-specific gene flow.

Association analyses of common SNPs in *TCF7L2* with type 2 diabetes covariates, gender, age of onset and BMI, and the interaction with other diabetes risk alleles. *L. Gianniny[1], R. Saxena[1,2], N. Burtt[1], V. Lyssenko[3], C. Giuducci[1], M. Sjögren[3], J.C. Florez[1,2,4], P. Almgren[3], B. Isomaa[5], M. Orho-Melander[3], U. Lindblad[3,6], M.J. Daly[1,2,4], T. Tuomi[5], J.N. Hirschhorn[1,4,7], K. Ardlie[1,8], L. Groop[3], D. Altshuler[1,2,4]* 1) Medical & Population Genetics, Broad Institute of Harvard and MIT, Cambridge, MA; 2) Center for Human Genetic Research, Massachusetts General Hospital, Boston, M; 3) Department of Clinical Sciences- Diabetes & Endocrinology, University Hospital MAS, Lund University, Malmö, Sweden; 4) Department of Medicine, Harvard Medical School, Boston, MA; 5) University of Helsinki, Helsinki, Finland; 6) Skaraborg Institute, Skövde, Sweden; 7) Divisions of Genetics and Endocrinology, Childrens Hospital, Boston, MA; 8) Genomics Collaborative Inc., Cambridge, MA.

Common non-coding variants in the *TCF7L2* gene are reproducibly associated with increased risk of type 2 diabetes. We genotyped 13 SNPs across *TCF7L2* in 8,310 individuals from Scandinavia, Poland and the US. We examined the correlation of *TCF7L2* genotypes with gender, body mass index (BMI) and age of onset, to test if *TCF7L2* variants contribute to risk of type 2 diabetes through an effect of these covariates. It was observed that *TCF7L2* risk associated with type 2 diabetes was stronger in men than in women (men n=3389, OR 1.50 (95%CI 1.35-1.67), $P = 5.9 \times 10^{-14}$ vs. women N= 3529, OR 1.33 (1.19-1.47), $P = 8.8 \times 10^{-8}$). We found that diabetic individuals with the risk allele(s) are leaner than diabetic individuals who do not carry the risk allele ($P = 0.01$). Additionally, no significant association of *TCF7L2* variants with BMI were observed in controls or with age of onset ($P = 0.64$) in cases. Finally, we tested for epistatic interactions between *TCF7L2* rs7903146, *PPAR* P12A, and *Kir6.2* E23K and found no significant evidence of epistasis. Our results indicate that *TCF7L2* variants may exert sex-specific effects, and may interact with BMI to influence the risk of type 2 diabetes.

Genomic Approaches for Pathway Identification in Regenerating Sensory Epithelia of the Inner Ear. *D. Alvarado, K. Powder, D. Hawkins, S. Bashiardes, V. Bhonagiri, R. Veile, J. Speck, M. Warchol, M. Lovett* Washington Univ, St Louis, MO.

The cochlea and the utricle of the vertebrate inner ear utilize sensory hair cells as mechano-electric transducers of sound and balance respectively. In mammals if these sensory hair cells are damaged they cannot regenerate. In contrast, avian sensory hair cells of the utricle and cochlea maintain their regenerative ability. To identify genes involved in hair cell regeneration, avian sensory epithelia (SE) from the cochlea and utricle were separately damaged by laser or chemical ablation and changes in gene expression were measured at recovery time points on a custom transcription factor (TF) gene microarray. Approximately 90 of the ~500 differentially expressed genes are involved in known genetic signaling pathways such as *Pax, Tgf*, Hedgehog, Notch, *Ap1, Igf* and NfkappaB pathways. To identify TFs that are required for early events in SE regeneration, 20 were knocked down by targeted siRNA in a high throughput proliferation assay. Knockdowns of components of the *Pax* pathway, of *JunD* and *Cebpg*, as well as chemical inhibition of the Jun kinase (*JunK*) pathway, significantly inhibited SE proliferation. Many of these TFs specifically affect inner ear SE proliferation, as evidenced by failure of RNAi treatments to inhibit proliferation in retinal epithelia. We identified novel epistatic relationships by conducting gene expression profiles on the SE of targeted siRNA knockdowns. For example, the TF *Cebpg* was placed in this way as being downstream of the *Ap1* (*JunK, JunD*) pathway. Five more genes were positioned downstream of *Cebpg*: *IRX4, LRP5, TAF-172, RARA,* and *ZNF44*. Three of these are specifically required for proliferation in the utricle SE. Interestingly, one of these, *LRP5*, is a co-receptor for *Wnt* signaling. Six known components of *Wnt* signaling are also differentially expressed during the SE regenerative process. Taken together our observations indicate that the *Ap1* pathway may directly intersect with *Wnt* signaling during the early stages of hair cell regeneration and that this pathway is necessary and important for early events in the regenerative process.

SNP correlates with LDLR splicing efficiency in pre-menopausal women. *K.E. Grear[1], H.M. Tucker[2], H. Zhu[1], S. Estus[1]* 1) Department of Physiology, Sanders-Brown Center, University of Kentucky, Lexington, KY; 2) Department of Pediatrics, University of Kentucky, Lexington, KY.

The liver is an important organ in cholesterol homeostasis. Seventy percent of low-density lipoprotein (LDL) is removed from circulation by the liver via low-density lipoprotein receptors (LDLRs). Excess LDL in the circulation can lead to a myriad of health problems, including myocardial infarctions, strokes and peripheral vascular disease. Our laboratory has found a single-nucleotide polymorphism (SNP) that modulates splicing efficiency differences in LDLR in vitro. This SNP also associates with higher LDL levels in pre-menopausal women. To test our findings in vivo, I obtained 30 pre-menopausal female liver samples from the Brain and Tissue Bank for Developmental Disorders at the University of Maryland and extracted RNA via the phenol-chloroform method. Primers were designed that flanked the exon containing the SNP and resided in exon 10 and exon 14 of LDLR. I performed RT-PCR, separated the products on an 8% polyacrylamide gel and quantified the ratio of full-length LDLR to total LDLR. Each of the samples were then genotyped for the SNP using a TaqMan Custom SNP Genotyping Assay. LDLR splicing efficiency was found to correlate in vivo with SNP genotype ($p=0.02$). These results support the hypothesis that the SNP correlates with splicing efficiency differences in pre-menopausal female liver. Future experiments will include evaluating LDLR splicing efficiencies in male and post-menopausal female liver tissue.

Genetic basis of Hirschsprung disease in Down syndrome. *S. Arnold, A. Chakravarti* McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.

Hirschsprung disease (HSCR), a condition characterized by aganglionosis along varying lengths of the colon, manifests as an intestinal obstruction in neonates and children. The risk of HSCR among individuals with Down Syndrome (DS) is fifty fold greater than in the general population. Mice heterozygous for loss of function mutations in both the receptor tyrosine kinase RET and a second HSCR susceptibility gene, EDNRB, phenotypically recapitulate most of the characteristics of HSCR and display disregulation of nine genes in the DS critical region. Interestingly, there is evidence to suggest that one of the upregulated genes, superoxide dismutase (SOD1), interferes with RET dimerization when overexpressed in cultured cells. Taken together, these results suggest that an additional copy of a gene (or genes) on Chromosome 21, likely in an over-represented, mutant form, combines with a mutation in RET to elevate the incidence of HSCR in the DS population.
In accord with this hypothesis, a recently described enhancer mutation in Intron 1 of RET is overtransmitted in individuals with HSCR and DS to the same extent as in individuals with HSCR alone. However, the involvement of Chromosome 21 in increased HSCR risk for DS individuals is less clear. Microsatellite genotyping of individuals with HSCR and DS and comparison to results obtained from DS controls shows no relationship between reduction to homozygosity and increased incidence of HSCR. This weakens support for the contribution of an over-represented Chromosome 21 mutation to disease. In addition, no Hirschsprung phenotype was detected in mice overexpressing SOD1 and heterozygous for the RET null mutation, though the possibility remains that the combined overexpression of multiple Chromosome 21 genes is essential for the development of HSCR in this context. To this end, the introduction of the RET null mutation into mice overexpressing genes orthologous to one-third of the genes on human Chromosome 21 (the Ts65Dn mouse) is underway.

The National Coordinating Center (NCC) for the Genetics and Newborn Screening Regional Collaborative (RC) Groups: A national resource for enhancing services in local communities. *J. Benkendorf[1], M. Lloyd-Puryear[2], M. Mann[2], J. Shuger[2], M. Watson[1]* 1) American College of Medical Genetics, Bethesda, MD; 2) MCHB/HRSA, Rockville, MD.

In 2004, the ACMG entered into a cooperative agreement with MCHB/HRSA to serve as the NCC for the 7 RCs. Because genetic services are multifaceted, multidisciplinary, but best delivered locally the NCC enhances abilities of the RCs to strengthen and support genetics and newborn screening (NBS) capacities of States and address maldistribution of genetics providers. The NCC provides infrastructure, coordination and technical assistance, and it generates materials to address issues of common interest, avoiding duplication of efforts and allowing the RCs to focus on their unique needs. The NCC also facilitates collaborations between the RCs and national projects, using local communities to pilot materials and programs for policymakers, health professionals and families. 16 national organizational partners contribute additional resources. NCC initiatives include building national capacity in the use of telegenetics; establishing a searchable national network of genetic service and subspecialty providers experienced in diagnosis and management of infants detected in NBS programs; collecting and disseminating data that establish the value of genetic services to payers and policymakers; developing disaster preparedness measures to assure that NBS programs and treatment of patients with metabolic conditions are not interrupted; bridging NBS and genetic testing laboratories with the medical home through development and distribution of management guidelines, including the transition of patients from pediatric to adult care; and developing resources for State policymakers. National data collection efforts include tracking pilot NBS programs, and establishing and maintaining a patient follow-up database useful in rare disease research. Maximizing collaboration between the genetic services, primary care, NBS and public health communities is critical to the success of each effort and the collective impact of the NCC and the RCs. Mechanisms for local, national, and organizational involvement will be emphasized.

Follow up genome-wide SNP assay reveals additional structural variation and confirms extended homozygosity and cell-line induced alterations in normal individuals. *A.F. Britton[1], J. Simon-Sanchez[1, 2], S. Scholz[1], H.-C. Fung[1], M. del Mar Matarin[1], D. Hernandez[1], J.R. Gibbs[1], F. Wavrant de Vrieze[1], E. Peckham[3], K. Gwinn-Hardy[4], A. Crawley[4], J.C. Keen[5], J. Nash[5], D. Borgaonkar[1], J. Hardy[1], A. Singleton[1]* 1) Laboratory of Neurogenetics, NIA, Bethesda; 2) Unidad de Genética Molecular, Departamento de Genómica y Proteómica, Instituto de Biomedicina de Valencia-CSIC, Valencia, Spain; 3) Human Motor Control Section, NINDS, Bethesda; 4) Neurogenetics Branch, NINDS, Bethesda; 5) Coriell Institute for Medical Research, Camden.

Previous genome-wide SNP assay of 109, 365 SNPs in 276 neurologically normal North American Caucasian individuals revealed extended homozygosity (contiguous tracts > 5 Mb) in 6.9 % (19/273) and gross chromosomal abnormalities in 8.8 % (24/276) of DNA samples assessed. These data suggest that structural genomic variation is a common occurrence in the general population. It is also believed that individuals with a least one large region of homozygosity are likely to harbor other large regions of homozygosity, indicating parental consanguinity. Given this, a follow-up assay of 317, 511 SNPs was conducted in the same subjects. There are 18, 072 SNPs in common between the two arrays, providing data on 408, 803 unique SNPs. DNA for both assays was extracted from the Epstein-Barr virus (EBV) immortalized lymphocyte cell lines (LCLs) and directly from the blood sample used for each immortalization. Data was visualized by two metrics; B allele frequency (which determines the ratio of alleles at each genotype) and log R ratio (which provides the copy number of each SNP), derived from Genome Viewer within Beadstudio v2.2.22 (Illumina Inc. San Diego, CA). The raw genotype data from these efforts are available on the Coriell website (http://ccr.coriell.org/ninds/catalog). Based on the greater accuracy of assaying 317, 511 SNPs, we identified additional structural variation and confirmed extended homozygosity and chromosomal abnormalities in individuals from the previous assay. These data confirm the strength of identifying chromosomal abnormalities in the general population using genome-wide SNP assays.

Genome-wide copy-number analysis of parathyroid carcinomas by single nucleotide polymorphism arrays. *J. Costa-Guda[1], N. Kawamata[2], H.P. Koeffler[2], A. Arnold[1]* 1) Molecular Medicine, University of Connecticut Health Center, Farmington, CT; 2) Hematology/Oncology, Cedars-Sinai Medical Center, Los Angeles, CA.

Parathyroid cancer is a rare, clinically aggressive cause of primary hyperparathyroidism. Inactivation of the *CDC73* (*HRPT2*) tumor suppressor gene, encoding parafibromin, is a central contributor to the molecular pathogenesis of parathyroid cancer, but other genes essential to parathyroid carcinogenesis remain unknown. To identify regions of allelic imbalance potentially harboring oncogenes or tumor suppressor genes central to the development of parathyroid carcinoma, we performed genome-wide copy-number and loss of heterozygosity (LOH) analysis using Affymetrix 50K SNP Mapping Arrays on 13 parathyroid carcinomas, local recurrences or distant metastases and matched normal control DNA from 9 individuals. Copy number analysis was performed using Copy Number Analyzer for GeneChip software and LOH was determined by direct comparison of genotype calls from each patient's normal and tumor DNA. Recurrent regions of allelic loss were observed on chromosomes 1p, 3, 13q, and 14, including instances of copy-number neutral LOH, strongly suggesting that tumor suppressor genes important to parathyroid carcinogenesis are located in these chromosomal locations. Recurrent allelic gain was seen on chromosome 16, suggesting the likely presence of a key parathyroid oncogene(s) on this chromosome. While recurrent changes on 1p, 3, 13q and 16 have been described in prior studies using comparative genomic hybridization and molecular allelotyping (Agarwal, *Cancer Genet Cytogenet*, 1998 and Imanishi, *J Bone Miner Res -Suppl. 1*, 1999), our results narrow the target regions on 1p and 13q and recurrent allelic loss on chromosome 14 in parathyroid cancer is a novel finding. Further studies are needed to determine which of the candidate genes in these chromosomal locations are important to the molecular pathogenesis of parathyroid cancer.

Interactions Between Candidate Genes, Nutritional and Lifestyle Factors in Osteoporosis. *S.L. Ferrari* Geneva University Hospital, Geneva, Switzerland.

Osteoporosis is a bone fragility disorder caused by low bone mass and deterioration of skeletal microarchitecture. The major nutritional and lifestyle factors implicated are poor dietary calcium intake, vit.D insufficiency and low physical activity. However genetic effects account for 60 to 80% of the population variance in bone mass. Numerous quantitative trait loci for bone density have been identified as well as dozens of candidate genes that are more or less strongly associated with bone mass and/or fracture risk. Among them, vit.D receptor gene 3'-UTR (Bsm1) alleles have been associated with small differences in bone density and an independent risk of osteoporotic fractures. Some reported interactions between calcium intake and VDR alleles, both in children and the elderly, seemingly with a modulation of intestinal calcium absorption and mineral homeostasis by VDR genotypes. Consistent with the known role of IL-6 on mediating the effects of estrogen-deficiency on bone resorption and bone loss, allelic variants in the IL-6 gene promoter have been associated with bone turnover, bone density and wrist fracture risk. In 1574 unrelated men and women from the Offspring Cohort of the FHS, the relationship between IL-6-174 genotypes and bone mineral density in women was influenced by significant interactions with years since menopause, oestrogen status, dietary calcium and vit.D intake. Recently described non-synonymous SNPs in the LDL-receptor related protein 5 gene (LRP5), implicated in the regulation of bone formation, have been related to bone density, vertebral bone size and spinal fracture risk with a net predominance in males. Some data suggest this gender-related association is mediated by LRP5 interaction with physical activity which effects on the skeleton could therefore be partially transduced by the Wnt-LRP5 canonical signaling pathway. Eventually Ppar gamma polymorphisms, a member of the nuclear receptor superfamily of transcription factors that plays an essential role in the selective differentiation of mesenchymal stem cells towards the adipogenic vs osteogenic lineage, have been associated with bone density. Work in progress suggests interactions with the lipid content of the diet.

Translocation breakpoint mapping in four 1p36 subjects with der(1)t(1;22)(p36;q11 or q13). *M. Gajecka[1], C.D. Glotzbach[1], K.L. Peterson[1], R. Saadeh[2], K. Spodar[3], M. Iliszko[4], D. Chitayat[5], B.C. Ballif[6], L.G. Shaffer[1,6]* 1) Hlth Res and Educ Ctr, Washington State Univ, Spokane, WA; 2) McKusick-Nathans Inst of Genet Med, Johns Hopkins Hospital, Baltimore, MD; 3) Dept of Med Genet, Children's Hospital Memorial Health Inst, Warsaw, PL; 4) Dept of Biol and Genet, Medical Univ, Gdansk, PL; 5) Dept of Pediatr, The SickKids, Univ of Toronto, Toronto, ON; 6) Signature Genomic Laboratories, LLC, Spokane, WA.

Although terminal deletions of 1p36 are relatively common, occurring in about 1 in 5,000 individuals, unbalanced translocations of 1p are rare, and little is known about their mechanism of formation. Of the 132 1p36 deletion cases ascertained by our laboratory to date, 22 (16.7%) are derivative chromosomes. Here we present the mapping and cloning of the breakpoints in four subjects with der(1). Two der(1)t(1;22)(p36;q13.3) are paternally inherited and one is maternally inherited, whereas the der(1)t(1;22)(p36;q11.2) is maternally inherited. To determine the sizes of the 1p36 deletions and 22q partial trisomies, we performed array CGH using the SignatureChip (Version 4.0) with cell lines derived from each of the four subjects. This microarray contains 55 BACs representing the most distal 11 Mb of 1p36 and 52 BACs from chromosome 22 targeted mainly to the cat eye syndrome region, DiGeorge syndrome region, breakpoint cluster region, and the subtelomere. Array CGH and metaphase FISH analyses showed that the 1p36 deletions and 22q duplications vary in size. To identify the breakpoints, we generated somatic cell hybrids from the subjects and/or their carrier parents containing the derivative chromosomes 1 or 22 segregated from the normal chromosomes 1 or 22 and performed PCR and STS marker walking. After narrowing the regions containing the junctions, we used the TOPO Walker protocol to amplify across the derivative chromosome junctions. Junctions were identified and analyses of the DNA sequence surrounding the breakpoints were performed. Alignment of the derivative chromosome junctions revealed a lack of sequence similarity between the breakpoints implicating nonhomologous end joining in stabilizing these broken chromosomes.

Novel intraoral phenotypes in hyper-immunoglobulin E syndrome. *D.L. Domingo[1], S.M. Holland[2], A.F. Freeman[2], J. Davis[3], T.C. Hart[1]* 1) NIDCR, National Institutes of Health, Bethesda, MD; 2) NIAID, National Institutes of Health, Bethesda, MD; 3) NHGRI, National Institutes of Health, Bethesda, MD.

OBJECTIVE: Hyperimmunoglobulin E syndrome (HIES) is a multi-systemic primary immunodeficiency characterized by eczema, recurrent skin and lung infections with pneumatocele formation, and extremely elevated serum immunoglobulin E. The genetic etiology is unknown, but most cases are sporadic or inherited in an autosomal dominant manner. Due to variable clinical expressivity, the familial nature may be overlooked in some cases . Non-immunologic findings include characteristic facial features (prominent forehead, broad nasal bridge, fleshy nasal tip and increased interalar distance) and skeletal involvement (pathological fractures, scoliosis and craniosynostosis). Retention of primary teeth and delayed eruption of permanent teeth have been described. This study aims to characterize the intraoral soft tissue features in HIES patients. METHODS: 56 HIES patients (4-54 years; 25 males; 31 females) received comprehensive intraoral examinations and craniofacial radiographic evaluations. Soft tissue anomalies of the hard palate, tongue, buccal mucosa and lip mucosa were characterized. Chronological dental development was also assessed. The association of intraoral lesions and two previously noted HIES features - (1) characteristic facies and (2) retention of primary teeth - were evaluated. RESULTS: 55% manifested asymptomatic irregularities of the hard palate and/or dorsal tongue. Palatal lesions ranged from a generalized surface keratosis to a midline sagittal fibrotic bridge in varying degrees of severity. Dorsal tongue lesions consisted of multiple fissures and pyramidal midline clefts. Lip and buccal mucosal lesions, consisting of keratotic plaques and/or surface fissures, were found in 21% and 7% of patients, respectively. 75% of patients manifested at least one intraoral soft tissue finding, of which 92% also exhibited characteristic facial features and 79% had delayed primary tooth eruption. CONCLUSIONS: Alterations of oral mucosa and gingiva were present in the majority of HIES patients. These novel intraoral findings may facilitate the diagnosis of HIES.

Experts' ethical concerns about a genetic screen for nicotine addiction. *M.J. Dingel, B.A. Koenig* Biomedical Ethics Research, Mayo College of Medicine, Rochester, MN.

Studies in behavioral and addiction genetics can give insight into the ethical issues of behavioral genetic research more broadly. For example, genetic studies of smoking can serve as a test case for analyzing the practicality of public health genetics in the realm of behaviors. If there is a genetic susceptibility to nicotine addiction, might this information be used for prophylactic interventions such as a nicotine vaccine (a product currently under development)? In our study, we interviewed 54 expert stakeholders (17 research scientists, 17 clinicians, and 20 prevention workers) to discover their ethical and practical concerns with a hypothetical genetic screen for smoking behaviors. These experts do not wholeheartedly support genetic screening for susceptibility to nicotine addiction. In general, while these experts did not believe screening would be a silver bullet to keep people from smoking, they also did not think it would promote fatalism in individuals. They were split between thinking that genetic knowledge would help people make better choices, and thinking that it would make no difference in peoples actions. Scientists were the only group to express concern about how the genetic information was communicated to the public. Clinicians and prevention workers, but not scientists, argued that it was important to have a course of action suggested by a genetic test. Though a course of action, the nicotine vaccine, is under development, significant resistance exists among experts for using the vaccine in concert with public health genetics to prevent smoking behaviors. Though some would advocate widespread vaccination of susceptible individuals, in general our experts have reservations about the safety and ethics of giving this vaccine to people who have not yet started smoking, and see its best use instead to be as a cessation device. The experts concerns highlight barriers to incorporating genetic screening for behaviors into clinical settings. Specifically, for testing to be adopted, it appears that there must be a safe therapy, and there must be a clear way of accurately communicating risk.

Characterization of the ubiquitin hydrolase activity of mouse ataxin-3 and of its subcellular localization in skeletal muscle. *M.C. Costa, A.-J. Rodrigues, P. Maciel* ICVS, School of Health Sciences, Univ. Minho, Portugal.

Machado-Joseph disease (MJD) is a neurodegenerative disorder, caused by the expansion of the $(CAG)_n$ tract in the *ATXN3* gene, encoding ataxin-3 (ATX3)- a protein with deubiquitinase (DUB) activity. The *ATXN3* mouse homologue gene (*Mjd*) is very similar to its human counterpart, and is highly regulated by MyoD (a muscle-specific transcription factor). Mouse ataxin-3 (mATX3) is ubiquitously distributed, and particularly abundant in the neurons of the affected regions of the MJD patients, in all types of muscle, testis and in ciliated cells, suggesting a role in cell structure and/or motility. Although the ubiquitin proteasome system plays an important role in mediating skeletal muscle protein wasting, the function of mATX3 in this tissue is unknown. We have expressed the recombinant His:mATX3 that, similarly to human ATX3, had a DUB activity, towards both K48 and K63 polyubiquitin chains *in vitro*. His:mATX3 was used for the production of a polyclonal antibody, anti-mATX3, which revealed to be specific for this protein. In mouse skeletal muscle protein extracts, both the anti-mATX3 and the anti-humanATX3 antibodies recognized mainly a band of 33 KDa; however the first detected another band at 55 KDa and the second at 42 KDa, suggesting that these antibodies may recognize different protein species. To gain insight into the subcellular localization of mATX3 within mouse skeletal muscle, we have performed immunoelectron microscopy, using anti-mATX3 and anti-ATX3. Interestingly, both antibodies revealed similar labeling patterns. Mouse ATX3 was present both in the nucleus (with a higher concentration in the heterochromatin), and in the cytoplasm, where it was mostly localized inside vesicles, in the endoplasmic reticulum, and in the mitochondrial matrix. In the sarcomeres, mATX3 was mostly present in the A band, and to a minor extent in the I band, being also present in a vestigial content in both M and Z bands; it was present inside the sarcoplasmic reticulum and in the mitochondrial matrix. In conclusion, mATX3 is not exclusively associated with a specific cellular structure of skeletal muscle; its enzymatic activity may regulate different cellular pathways.

A multimarker method to localize signals of differential selection by analyzing SNP frequency differences between two populations. *M.J. Barber[1,2], H.J. Cordell[3], J.A. Todd[2]* 1) Department of Statistics, University of Washington, Seattle, WA; 2) Diabetes & Inflammation Lab, Department of Medical Genetics, Cambridge, UK; 3) Institute of Human Genetics, University of Newcastle upon Tyne, International Centre for Life, Newcastle upon Tyne, UK.

Three problems exist if signals of differential selection are localized by the F_{ST} metric of genetic differentiation for each SNP individually. Firstly, the distribution of the F_{ST} metric is unknown. Secondly, any localization is biased towards regions of high SNP density. Thirdly, any localization is insensitive to the neighboring per SNP F_{ST} metrics. The method applied here overcomes all three problems by defining a multi-SNP localizing statistic that relies on the property of exchangeability of locations under the null hypothesis of no differential selection. Specifically, the multi-SNP localizing statistic is calculated from using linear regression on the quantiles of the per SNP F_{ST} metrics observed within a sliding window of genetic distance from a test location given an expected correlation matrix between the quantiles, which is estimated as the average correlation between all pairs of quantiles across the genome that are separated by a similar genetic distance. The multi-SNP localizing statistic is ignorant of the phase information between the SNPs but has three beneficial properties. Firstly, it can be assumed to follow a standard normal distribution under the null hypothesis of no differential selection, irrelevant of how differentiated the two populations are. Secondly, from use of genetic distance within the sliding window the multi-SNP localizing statistic is a property of the test location and not just of the test region. Thirdly, inference can rapidly be made on a genome-wide level with a dense genotyped set of SNPs (e.g. phase II of the HapMap project) without the need for any simulations. Application to the different SNP frequencies observed for the four HapMap populations has shown two things, firstly, that the multi-SNP localizing statistic follows a standard normal distribution for almost all test locations, and secondly, that there are six regions of interest with a *P*-value of less than 10^{-8}.

Renal phenotype of heterozygous Lmx1b knockout mice (Lmx1b+/-) after uninephrectomy. *S.U. Endele[1], S. Klein[1], T. Molter[1], S. Richter[1], B. Klanke[2], A. Winterpacht[1]* 1) Institute of Human Genetics, University of Erlangen-Nuremberg,Erlangen, Germany; 2) Department of Medicine IV, University of Erlangen-Nuremberg, Erlangen, Germany.

The Nail-Patella Syndrome (NPS) is a rare autosomal-dominant disorder characterized by dysplastic nails, absent or hypoplastic patellae, dysplasia of the elbows and, in some cases (~50%), nephropathy. Nephropathy is the most serious aspect of NPS and often results in complete renal failure. NPS is caused by heterozygous loss-of-function mutations in the transcription factor gene *LMX1B*. Despite of identical or very similar mutations only 50-60% of the NPS patients develop a renal phenotype, which suggests genetic modifiers in the outbred human genetic background. $Lmx1b^{-/-}$ knockout mice (Chen et al., 1998) show an NPS-like phenotype including renal dysplasia but die within a few days after birth, while $Lmx1b^{+/-}$ mice seem to be phenotypically normal. We started to evaluate the Lmx1b knockout mouse as a possible model for the mapping of genetic modifiers of LMX1b activity in the kidney. This requires the identification of a renal phenotype in the heterozygous $Lmx1b^{+/-}$ knockout animals. Since we were not able to identify a renal phenotype in C57BL6 or different mixed genetic backgrounds by conventional histological examination, we tried to induce renal damage and thus provoke a renal phenotype in $Lmx1b^{+/-}$ mice. In total, 30 $Lmx1b^{+/+}$ and $Lmx1b^{+/-}$ mice (23 in C57BL6 and 7 in JF1 genetic background) underwent unilateral nephrectomy and received a salt diet for 6 weeks. Renal damage was evaluated following measurements of organ weights, urine albumin and total protein excretion as well as glomerular volume, mesangiolysis and glomerulosclerosis indices. Although we identified a slight, strain specific difference in kidney weight gain, no significant differences between the $Lmx1b^{+/-}$ and $Lmx1b^{+/+}$ mice could be detected. We therefore assume a compensatory mechanism in $Lmx1b^{+/-}$ mice explaining the missing phenotype in heterozygous Lmx1b knockout mice.

Genetic Heterogeneity of Meckel Gruber Syndrome. *L. Deda[1], S. Herd[1], D. Chitayat[3,4], E. Héon[1,2]* 1) Genetics and Genomic Biology, Sick Kids Hospital, Toronto, Ontario, Canada; 2) Dept of Ophthalmology and Vision Sciences, Sick Kids Hospital, Toronto, ON, Canada; 3) Prenatal Diagnosis and Medical Genetics Program, Mt. Sinai, Toronto, ON, Canada; 4) Dept of Clinical and Metabolic Genetics, Sick Kids Hospital, Toronto, Ontario, Canada.

Meckel Gruber Syndrome (MKS) is a severe autosomal recessive disorder characterized by bilateral renal cystic dysplasia, developmental abnormalities of the central nervous system, hepatic ductal dysplasia and polydactyly. Although incidence rates range from 1:3,400 to 1:140,000 amongst different populations, MKS represents the most common form of syndromic neuronal tube defects (NTDs) and shares phenotypic similarities with Bardet-Biedl syndrome (BBS). MKS is genetically heterogeneous with three documented loci MKS1 (17q21-24), MKS2 (11q13), MKS3 (8q21.13-q22.1) and two genes identified: *MKS1* and *MKS3* (*TMEM67*). Genotype analysis with highly heterozygous STRP markers was performed for all three loci on a non-consanguineous Portuguese family of 5 affected and 3 unaffected individuals. Suggestion of linkage was identified with the *MKS3* (*TMEM67*) locus on chromosome 8, between markers D8S167 to D8S1774 (~30Mbp interval). No pathogenic sequence changes in *TMEM67* were identified through mutational analysis of genomic and cDNA samples. This work further supports the genetic heterogeneity of MKS.

Mitochondrial Haplogroup U and Prostate Carcinogenesis: Possible Roles in Both Prostate Cancer and High-Grade Prostatic Intra-epithelial Neoplasia (HGPIN). *J.A. Canter, A.R. Kallianpur, J.L. Haines, J.H. Fowke* Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN.

Mitochondria, with their maternally inherited DNA, are critically important in free radical generation, apoptosis and cellular energy production. Accumulating evidence suggests that variation in the mitochondrial genome plays a role in carcinogenesis. We recently reported an association between the mitochondrial DNA G10398A polymorphism and invasive breast cancer in two separate populations of African-American women, and the same polymorphism was subsequently reported to be associated with prostate cancer in African-American men. (Mims et al, Cancer Res 2005;65: 8028-33) Recently, Booker et al (J Urol. 2006;175: 468-72) reported an association between Haplogroup U and prostate cancer risk in Caucasian men (OR=1.95, p=0.02). Haplogroup U is defined in part by a polymorphism (A12308G) that alters the structure of one of the tRNAs for leucine encoded by the mitochondrial genome. In this study, we determined the Haplogroup U status of 268 Caucasian participants in the Nashville Mens Health Study: 71 with prostate cancer, 69 with high-grade intra-epithelial neoplasm (HGPIN), a prostate cancer precursor, and 128 controls without cancer or HGPIN on biopsy. We first found that Haplogroup U was over twice as common in prostate cancer cases compared to controls (26.7% vs. 11.7%). The crude and age-adjusted odds ratios for the association of haplogroup U with prostate cancer in this study population were 2.75 (95% CI 1.2-6.3, p=0.007) and 2.79 (95% CI 1.3-6.0, p=0.008), respectively. Next, we extended the investigation to include HGPIN and found a suggestive positive association between Haplogroup U and this previously unstudied phenotype (age-adjusted OR =1.56, 95% CI 0.70-3.58). This finding is consistent with an etiologic role for this mitochondrial DNA variant in the progression to prostate cancer. A larger study is currently underway to accurately determine the magnitude of this risk. Prostate cancer remains a leading cause of cancer-related mortality. Further research focusing on the mitochondrial genome may uncover new factors important in prostate carcinogenesis.

Rapid prenatal detection of partial chromosome imbalance by Quantitative Fluorescent PCR (QF-PCR). *V. Cirigliano[1,2], G. Voglino[3], E. Ordoñez[1], M.P. Cañadas[1], A. Marongiu[3], E. Lloveras[4], A. Plaja[4], C. Fuster[2]* 1) Dept Molecular Genetics, General Lab, Barcelona, Spain; 2) Unitat de Biologia. Departament de Biologia Cellular, Fisiologia i Immunologia. Universitat Autònoma de Barcelona. E-08193 Bellaterra. Barcelona, Spain; 3) Molecular Genetics and Cytogenetics Lab. Promea-Day Surgery, 1026 Turin. Italy; 4) Departament de Citogenètica. General Lab. 08021 Barcelona, Spain.

The Quantitative Fluorescent PCR (QF-PCR) assay allows performing prenatal diagnoses of common chromosome aneuploidies in a few hours after sampling. Highly polymorphic STR markers are needed on chromosomes X, Y, 21, 18 and 13 to minimise uninformative samples; STRs selection along the examined chromosomes may also allow detecting partial trisomies. We developed a QF-PCR assay including 4 markers on each of the examined chromosomes and two sequences for sexing that has been applied to screen over 30.000 clinical cases. Samples found homozygous for all markers on one chromosome as well as all aneuploid cases were than retested using chromosome specific assays developed to amplify 10 markers on the X and Y, 7 on both chromosomes 21, 18 and 6 markers on chromosome 13. All 1106 aneuploidies were readily detected with 100% sensitivity and specificity. In the course of this study it was also possible to detect 12 fetuses with partial trisomies resulted from unbalanced translocations, duplications, insertions and other structural rearrangements. Chromosome 18 was involved in 4 cases, 6 fetuses had either X or Y derived extra sequences and in two more samples partial trisomies 21 or 13 were also readily detected as trisomic patterns for two or more markers. In 6 cases QF-PCR was crucial to achieve the correct diagnosis. In this study we demonstrate that an appropriate marker selection for QF-PCR assays also allows the identification of partial chromosome imbalance. The main advantages of the assay are its low cost, speed and automation enabling a single operator to analyse up to 60 samples per day. QF-PCR reaches the purposes of relieving anxiety of most parents within 24 hours from sampling or to accelerate therapeutical interventions in case of abnormal result.

Hyperprolinemia is a risk factor for cognitive and psychotic symptoms in VCFS patients. *D. Campion[1], E. Bumsel[1], B. Hecketsweiler[1], T. Van Amelsvoort[2], A. Swillen[3], A. Vogels[3], V. Drouin-Garraud[1], S. Le Gallic[1], S. Manouvrier-Hanu[4], C. Fantini[4], D. Heron[5], N. Philip[6], M. Carlier[6], A. Gerard[6], P. Sarda[7], A. Philippe[8], D. Lacombe[9], O. Boespflug-Tanguy[10], T. Frebourg[1]* 1) Inserm U614, Faculty of Medicine and Departments of Genetics and Biochemistry, University Hospital, Rouen, France; 2) Dpt of Psychiatry, University of Amsterdam, The Netherlands; 3) Center for Human Genetics, University Hospital, Leuven, Belgium; 4) Dpt of Genetics, University Hospital, Lille, France; 5) Dpt of Genetics, La Pitié-Salpétrière, Paris, France; 6) Dpt of Genetics, University Hospital, Marseille, France; 7) Dpt of Genetics, University Hospital, Montpellier, France; 8) Dpt of Genetics, Necker, Paris, France; 9) Dpt of Genetics, University Hospital, Bordeaux, France; 10) Dpt of Genetics, University Hospital, Clermont-Ferrand, France.

The Velo Cardio Facial Syndrome (VCFS) results from heterozygous deletions of the 22q11 region. About one third of VCFS patients present mild mental retardation, a small subset severe mental retardation, and a high rate of psychotic illness has been reported in VCFS. We had previously shown that heterozygous alterations of *PRODH*, causing moderate hyperprolinemia, were a weak risk factor for schizoaffective disorders whereas homozygous alterations, causing hyperprolinemia type I (HPI), were associated with mental retardation, seizures and behavioural disorders. Since 50% of VCFS patients have hyperprolinemia, we determined if hyperprolinemia is a risk factor for cognitive and/or psychotic symptoms in VCFS patients. Ninety two VCFS patients aged 15 years or older were evaluated for psychiatric and cognitive symptoms, plasma proline levels were measured and the *PRODH* and *COMT* genes were analyzed, since an epistatic effect between both genes has recently been reported. We found that (i) a subset of VCFS patients with severe hyperprolinemia has a phenotype distinguishable from that of other VCFS patients and reminiscent of HPI, and (ii) hyperprolinemia associated with the low activity *met-COMT* allele is a joint risk factor for psychosis in VCFS patients, as predicted by the *Pro/Re* mice model.

Normal telomere length in 5p- with a telomerase reverse transcriptase *TERT* deletion. *HY. Du[1], R. Idol[1], S. Robledo[1], J. Ivanovich[2], D.B. Wilson[3], A. Londono-Vallejo[4], P.J. Mason[1], M. Bessler[1]* 1) Internal Medicine; 2) Surgery; 3) Pediatrics, Washington University School of Medicine, St. Louis, MO63110; 4) Institut Curie, 75248 Paris, France.

Telomerase, which maintains the ends of chromosomes, consists of two core components, the catalytic subunit (TERT) and the telomerase RNA component (*TERC*). We have recently shown that haploinsufficiency for *TERC* leads to progressive telomere shortening and disease of autosomal dominant dyskeratosis congenita. Here, we investigated the consequences of *TERT* deletion in patients with 5p- syndrome. The 5p- or Cri du chat syndrome is caused by a partial deletion of the short arm of chromosome 5. 33 individuals with 5p- were investigated during the Annual Meeting of the 5p- Society in St. Louis, 2005. The median age was 7 (1-39). 57 family members aged from 12 to 68 years old with median of 39 served as controls. All individuals with 5p- had or have clinical features characteristic of the syndrome. Individual probands had ridged fingernails, atrophic skin on palms and early graying, indicative of premature aging. Peripheral blood cell analysis showed normal values for hemoglobin (12.891.10g/dl) white blood cell (8.542.83×K/mm3) platelets (277.3880.66×K/mm3) and mean corpuscle volume (86.264.89fl). 4 individuals with 5p- had slightly decreased hemoglobin levels (11.2, 11.2, 12.7, 13g/dl respectively). All participants with 5p- had a deletion of the *TERT* as confirmed by Q-PCR and FISH analysis. Telomere length was measured in peripheral blood cells using flow-FISH. In 29 individuals with 5p- (88%) telomere length was between the 10th and 90th percentile of the healthy controls. In 4 individuals telomeres were below the 10th percentile. While telomere length in 54 family members (95%) were within the 10th to 90th percentile, 3 were below the 10th percentile. There was no correlation between telomere length, blood cell counts or signs of premature aging. Our results demonstrate that the majority of individuals with 5p- including a *TERT* deletion have telomere lengths similar to healthy controls, indicating that premature shortening of telomeres does not contribute to the clinical manifestations in 5p-.

New Insights into Mechanisms and Consequences of Small Marker (Ring) Chromosomes. *E.L. Baldwin, L.F. May, C.L. Martin, D.H. Ledbetter* Dept. Human Genetics, Emory University, Atlanta, GA.

Supernumerary marker chromosomes occur in 1/1000 prenatal diagnostic studies and in 4/1000 individuals with mental retardation. The mechanisms of formation and clinical consequences of marker chromosomes are poorly understood. We developed a panel of 871 unique BAC clones in the pericentromeric regions of each human chromosome in order to evaluate the euchromatic makeup of marker chromosomes and to investigate their mechanism of origin. Seven small non-satellited markers derived from acrocentrics were studied, and 5 were found to be negative for the most proximal unique BAC clones, consistent with a normal phenotype. Two cases contained 1.6 and 5.9 Mb of euchromatin, including 11 and 20 predicted genes. Of 8 cases of non-acrocentric small ring marker chromosomes, 6 contain euchromatic material from either the p- or q-arm, but not both arms. This is consistent with a breakpoint within the centromeric alpha-satellite which can produce two functional chromosomes (ring and deletion), as a major mechanism of marker chromosome formation. Normal individuals may be balanced carriers of complimentary ring and deletion chromosomes, and therefore at high risk for genetically unbalanced offspring. Only 2 markers (both derived from chromosome 8) contain euchromatic material from both the p- and q-arms. Chromosome 8-derived markers are the most frequently occurring non-acrocentric ring marker chromosomes. Since breakage in both the p- and q-arms would produce only one centric chromosome product (small ring) and two acentric arms, it is possible that this mechanism is associated with a trisomy 8 conception followed by trisomy rescue by marker formation. For the above cases, marker sizes range from less than 1 Mb (containing only 3 known genes) to 15 Mb (containing over 170 known genes). Large markers can be readily detected and sized using array CGH, but small marker chromosomes containing only segmental duplications can be problematic. Although these small markers do not contain euchromatic DNA, the individual may be at risk for UPD of the normal homologs. In addition, array CGH cannot detect balanced carriers containing complimentary ring and deletion chromosomes.

PhenCode: Connecting Genome and Phenotype. *B. Giardine[1], W.J. Kent[2], R.C. Hardison[1], PhenCode Consortium* 1) Center for Comparative Genomics and Bioinformatics, Huck Institutes of the Life Sciences, Penn State University, University Park, PA; 2) Center for Biomolecular Science and Engineering, University of California, Santa Cruz, CA.

PhenCode (Phenotypes for ENCODE) is a collaborative project to better understand the relationship between genotype and phenotype in humans. The initial project connects human phenotype and clinical data in various locus-specific databases (LSDBs) with data on genome sequences, evolutionary history, and function in the UCSC Genome Browser. Detailed data on naturally-occurring human mutations and the phenotypes they cause tend to be scattered among literature articles and/or individual databases dedicated to a specific gene or disease. PhenCode aims to connect these databases and make it easy to compare their data with the kinds of genotypic and functional data available at the browsers. With PhenCode, Genome Browser users can find interesting mutations and follow links back to the LSDBs for more detailed information. Alternatively, LSDB users can start with queries on mutations or phenotypes and then display the results at the Genome Browser to view complementary information, such as chromatin modifications and protein binding from the ENCODE consortium. Furthermore, users can query on phenotype across loci (e.g. all mutations that cause anemia, regardless of gene). PhenCode is not a new database, but a new conduit among existing resources. It is implemented as a Human Mutations track at the Genome Browser, and is updated regularly from the source LSDBs, who continue to curate their data.

URLs: genome.ucsc.edu, www.bx.psu.edu/phencode/.

Universal Detector for Single Sample SNP Genotyping. *A. Broomer, D. Merrill, R. Koehler, C. Chen* Applied Biosystems, Foster City, CA.

A conventional TaqMan SNP Genotyping Assay interrogates one SNP from one gDNA sample with a specific forward primer, a specific reverse primer, and two allele-specific TaqMan probes. However, multiple gDNA samples must be assayed in order to provide definitive allelic clustering and accurate allelic calls. Here, we present a new method termed Universal Detector, which overcomes the issues of specific PCR oligos and the required use of multiple gDNA samples. The two-step method first encodes 48 SNPs from one gDNA sample in one 48-plex OLA reaction. The resulting OLA products are then decoded in 48 singleplex PCR reactions with one pair of universal TaqMan detectors and 48 pairs of universal ZipCode PCR primers. These universal oligos are used for all SNPs ever to be tested. Statistics of design rate, call rate, conversion rate, and accuracy (concordance with conventional TaqMan SNP genotypes) will be presented. Universal Detector should prove valuable in cases of human identification and clinical diagnostics where SNP profiling of one individual is desirable and obtainable without positive assay controls. Also, the method may be expanded to include studies of gene expression, gene copy number, and DNA methylation.

Turbo Genomic Control. *W.J. Astle, D.J. Balding* Centre for Biostatistics, Department of Epidemiology and Public Health, Imperial College London.

In the analysis of association studies, Genomic Control[1] (GC) adjusts the Armitage trend statistic to correct the type I error for the effect of population substructure, but it does not maximise power. We generalise GC to incorporate co-variation of relatedness and phenotype, retaining control over type I error while improving power. For example, a closely-related pair of subjects with concordant phenotypes should carry less weight in the test statistic than either (a) a distantly-related pair with concordant phenotypes or (b) a closely-related pair with discordant phenotypes. We derive appropriate weights from an approximation to the score statistic of a mixed-model likelihood. Our approach is similar to that of Yu *et al*.[2], but we extend to binary (case-control) in addition to quantitative phenotypes, we implement improved estimation of relatedness coefficients, and we derive an explicit statistic that is simple and fast to compute.

The problems of population structure and cryptic relatedness are essentially the same: if patterns of shared ancestry differ between cases and controls, whether distant (coancestry) or recent (cryptic relatedness), false positives can arise and power can be diminished. With large numbers of widely-spaced genetic markers, coancestry can now be measured accurately for each pair of individuals via patterns of allele-sharing. Our method dispenses with the notion of subpopulation, working instead with a coancestry coefficient for each pair of individuals in the study.

We present simulation studies and real data analyses to illustrate the performance of our method in a range of scenarios incorporating both substructure and cryptic relatedness.

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Alternative Complement Pathway Genes Strongly Associate with Age Related Macular Degeneration (AMD). *B. Gold[1], LS. Hancox[3], JE. Merriam[2], J. Bergeron[5], AJ. Taiber[3], J. Zernant[2], AK. Olsh[1], GR. Barile[2], LI. Hardisty[3], K. Cramer[4], J. Neel[4], RT. Smith[2], JD. Borchardt[3], GS. Hageman[3], R. Allikmets[2], M. Dean[1]* 1) Lab Genomic Diversity, National Cancer Inst, Frederick, MD; 2) Dept of Ophthalmology, Pathology & Cell Biology, Columbia University, New York, NY; 3) Dept of Ophthalmology, University of Iowa, Iowa City, IA; 4) Sapio Sciences, LLC, York, PA; 5) SAIC-Frederick, Frederick, MD.

Age related macular degeneration (AMD) is a complex genetic disorder and is the most frequent cause of vision loss in the elderly. Genetic and biological studies implicated the complement factor H (CFH) gene in AMD. A haplotype containing the Y402H variant in CFH confers a 3-5 fold risk for AMD and in addition, we found haplotypes (H2 and H4) that are protective. As CFH is the major inhibitor of the alternative complement pathway, we examined the role of the major activator, complement factor B (BF). BF is adjacent to the complement 2 (C2) gene in the Class III region of the MHC locus, and SNPs in both BF and C2 confer significant protection from AMD. When we analyzed CFH and C2/BF SNPs in a joint analysis, 74% of affected individuals and 56% of control clinical phenotype can be explained on the basis of their CFH and C2/BF haplotype. Both the CFH and BF loci contain common variants differing significantly among separate racial and ethnic groups suggesting balancing selection, similar to other immune function genes. The variants modifying risk for AMD are common in both African and European populations and are less common in Asian and Native Americans. Recently a third locus on chromosome 10q26 containing the PLEKHA1 and hypothetical LOC387715 genes has been implicated in AMD. We have confirmed a strong association between the S69A SNP in LOC387715 in cohort of 643 cases and 368 controls from Columbia University. The effect of the LOC387715 locus is much more prominent in late stage AMD than in early AMD. Combined analyses of these three loci will enhance pharmacogenetic studies of AMD. Our data are consistent with a major role of the alternative complement pathway in the etiology of AMD.

Integrating personalized medicine into the U.S. health care system: A comprehensive study of key stakeholder groups. *P. Deverka[1], T. Doksum[2], S. Shoemaker[2], R. Carlson[3]* 1) Inst Genome Sciences & Policy, Duke University, Durham, NC; 2) Abt Associates Inc., Cambridge, MA; 3) University of Washington, Seattle, WA.

Scientific advances in personalized medicine (PM), including pharmacogenetics, have potential to positively impact health, yet reveal many hurdles in the translational pathway to clinical integration. The objectives of this study were to characterize the current and future landscape for PM, describe perceived risks/benefits of PM and barriers/enablers to the integration of PM, and elicit recommendations to ensure that the potential benefits of PM are realized. METHODS: During Spring 2006, 60 in-depth telephone interviews were conducted with leaders from government agencies, payers, health care delivery organizations, professional societies, consumer groups, academic institutions, venture capital firms, and industry. Interviewees were selected based on their reputations as experts in their field and because they were involved or could become involved in PM. Interviews were recorded, transcribed, and analyzed using a qualitative data analysis package. RESULTS: Most stakeholders agreed that PM is likely to improve health outcomes, but felt that there are few examples where this is occurring in clinical practice today. The reasons identified for the limited integration of PM were varied and complex, and included typical issues facing many medical innovations (e.g., scientific, financial, regulatory), but also encompassed challenges specific to genetics and the goal of personalization. Stakeholders called for further evidence of clinical utility of PM, as they expressed concerns about the system cost impacts of adding complex new tests to predict risk. In addition, enabling technologies such as linked electronic health records were identified as rate-limiting to the integration of PM. A number of pilot PM projects provided insights into potential solutions. CONCLUSION: PM has broad support but will require changes in how personalized technologies are evaluated, how health care is financed and delivered, and how clinicians and consumers are prepared. The recommendations from the stakeholders in this study will be instrumental in devising strategies to appropriately integrate PM.

Gene Repair in SMA Using Single-stranded Oligonucleotides. *D.M. DiMatteo, L. Ferrara, E.B. Kmiec* Biology, University off Delaware, Newark, DE.

Spinal muscular atrophy (SMA) is a recessive, neuromuscular disease characterized by symmetrical muscle wasting, caused by the death of motor neurons, leading to muscle weakness. It is the leading genetic cause of death in children under 5 years. The causal gene is the survival motor neuron (SMN) gene. It is located on chromosome 5q13 in two nearly identical copies- SMN1 and SMN2. The SMN1 gene produces full-length, functional protein and it is this copy that is deleted or mutated in SMA patients. The SMN2 gene produces approximately only 10% of full-length protein due to a single base pair change in exon 7. This base change from a C in SMN1 to a T in SMN2 causes exon 7 to be skipped, leading to a non-functional protein that is quickly degraded. Current clinical trials include drugs that increase transcription or activate splicing and, subsequently, increase the amount of functional protein. Our lab has pioneered a method for introducing single, base changes into dysfunctional and/or mutant genes. With this method, short, single-stranded DNA oligonucleotides (ODNs) are introduced into a cell where they utilize both DNA repair and replication mechanisms to catalyze the base exchange. The resulting product of this activity is a corrected gene that can make the normal protein. In effect, the cell uses the ODN as a template and endogenous repair activities to correct the mutant gene. This approach avoids the problems of long-term drug treatment. ODNs are well known to exhibit low toxicity in humans and have been used extensively in clinical setting for other purposes. We chose to study the disease SMA since making a single base pair change would create a functional gene. This is possible because SMA patients have only the SMN2 gene. We have used ODNs to change a C in exon 7 to create an SMN1-like gene able to make the full-length functional protein. In patients, more protein would lead to milder symptoms. Currently, the drugs used to treat SMA are highly toxic and have many side-effects. Many patients discontinue use of these medications and fail to realize the potential benefits of the drug. Our method would eliminate the need for life-long for drug treatment.

The clinical utility of enhanced subtelomere coverage in array CGH. *B.C. Ballif, S.G. Sulpizio, R.M. Lloyd, S. Gaskin, S.L. Minier, K. Sundin, M. Lincicum, E.A. Rorem, C.D. Kashork, B.A. Bejjani, L.G. Shaffer* Signature Genomic Laboratories, Spokane, WA.

Telomeric chromosome abnormalities are a substantial cause of mental retardation and birth defects. Although subtelomeric FISH probes have been widely used to identify submicroscopic telomeric rearrangements, array-based comparative genomic hybridization (array CGH) has emerged as a more efficient and comprehensive approach to the identification of chromosomal aberrations. Due to the clinical relevance of telomeric abnormalities, it has been proposed that array CGH using panels of BAC clones which map to regularly spaced intervals along the length of each telomere could be used to more precisely characterize subtelomeric aberrations in a single experiment. We have constructed a CGH microarray that includes expanded coverage for 41 subtelomeric regions using 1120 FISH-mapped BAC clones. Contigs of clones were selected in increments of ~0.5 Mb, beginning with the most distal unique sequence for each telomere and extending on average 5.7 Mb. We have used this microarray to analyze 62 cases with known subtelomeric aberrations previously characterized using an array with only a single contig of 3-6 clones at each telomere. The expanded telomere coverage was sufficient to define the breakpoint regions of over half (55%) of the chromosome abnormalities. However, 45% of the subtelomeric aberrations extended beyond the size of our expanded coverage suggesting that many subtelomeric abnormalities are 5 Mb in size and that representation extending to 10 Mb may be of even greater value. The enhanced coverage not only clarified the size of the telomeric rearrangements, it also identified at least one case with a more complex subtelomeric rearrangement which may shed light on its mechanism of formation. In addition, we have identified 6 cases of interstitial deletions that would have been missed by both subtelomere FISH and limited array CGH using clones only to the most distal unique sequences. These data suggest that increased coverage at the subtelomeres improves the diagnostic usefulness of array CGH and detects unsuspected complexity of these clinically significant regions.

Congenital Erythropoietic Porphyria: High Affinity Purification, NMR Resonance Assignments, and Localization of the Active Site of Human Uroporphyrinogen III Synthase. *L. Cunha[1], M. Kuti[2], D.F. Bishop[1], M. Mezei[2], L. Zeng[2], M.-M. Zhou[2], R.J. Desnick[1]* 1) Department of Human Genetics; 2) Department of Molecular Physiology and Biophysics, Mount Sinai School of Medicine, New York, NY.

Congenital erythropoietic porphyria (CEP) results from the deficient activity of uroporphyrinogen III synthase (URO-synthase), the fourth enzyme in the heme biosynthetic pathway. This 29.5 kDa monomeric enzyme catalyzes the cyclization and D-ring isomerization of hydroxymethylbilane to form uroporphyrinogen (URO'gen) III. Previously, the X-ray crystal structure of the enzyme was determined, but the active site was only inferred, due to the inability to co-crystallize the enzyme with substrate analogues. To determine the URO-synthase three-dimensional solution structure, its possible interaction in a cytosolic complex with the third and fifth enzymes in the pathway, hydroxymethylbilane synthase (HMB-synthase) and uroporphyrinogen decarboxylase, and to characterize its active site, milligram quantities of stable-isotope-labeled active human recombinant enzyme were expressed and purified for NMR studies using an 800 MHz instrument with a cryoprobe. NMR analyses permitted 100% assignment of the URO-synthase backbone ^{1}H and ^{13}C resonances, 94% of the ^{1}HN, and non-proline ^{15}N resonances, and 85% of the side chain ^{1}H and ^{13}C resonances. The absence of chemical shift changes in the ^{15}N spectrum of URO-synthase when it was mixed with the human recombinant HMB-synthase apoenzyme and/or URO-decarboxylase precludes the occurrence of a stable two or three enzyme cytosolic complex. *In silico* docking experiments using the reaction product URO'gen III as a flexible ligand, localized the putative enzymatic active site to a refined cleft region between the enzymes two major domains. Localization of the active site region was confirmed by NMR analyses of URO-synthase titrated with the competitive inhibitors UROgen III and N_D-methyl-1-formylbilane revealing resonance perturbations of residues lining the cleft. These studies will facilitate prediction of genotype/phenotype correlations and decisions for early bone marrow transplantation in this porphyria.

Study of disease-relevant polymorphisms in the TLR4 and TLR9 genes: a novel method applied to the analysis of the Portuguese population. *A. Carvalho, P. Maciel, F. Rodrigues* Life and Health Sciences Research Institute (ICVS), School Of Health Sciences, University of Minho, Braga, Portugal.

Toll-like receptors (TLRs) are cellular receptors that mediate recognition of microbial challenges and the subsequent inflammatory response. Mutations within the TLR4 gene at the 299 and 399 residues have been shown to be associated with blunted physiological responses to inhaled lipopolysaccharide. Furthermore, the Asp299Gly TLR4 mutation was proposed to underlie an increased risk of gram-negative septic shock, whereas the variation within the promoter region of TLR9 at position -1237 has also been associated with an increased risk for asthma. We developed a simple and rapid method based in the bi-directional PCR amplification of specific alleles (Bi-PASA) for genotyping known sequence variants in TLR4 (Asp299Gly and Thr399Ile) and TLR9 (T-1237C) genes. This method allows the genotype determination in a single reaction and is amenable to high throughput analysis. We used this methodology to characterize the distribution of these polymorphisms in the Portuguese population. Two hundred randomly selected blood donors of Portuguese origin (107 females and 93 males) were genotyped and allele and genotype frequencies were determined. Among the tested individuals, 11.5% were heterozygous for both the Asp299Gly and Thr399Ile polymorphisms. None of the individuals was a homozygote for the TLR4 polymorphisms. In what concerns the T-1237C variation in TLR9, 25.7% were heterozygous and 3.2% were homozygous for this polymorphism (none of the polymorphisms deviated from the Hardy-Weinberg equilibrium; D=1.000). These data provide valuable information on the distribution of TLR polymorphisms in the Portuguese population that can be used to stratify risk patients with increased susceptibility to infection. * Carvalho, A. was the recipient of a fellowship from Fundação para a Ciência e Tecnologia (FCT), Lisbon, Portugal (SFRH/BD/11837/2003) This study was partially supported by Fundação para a Ciência e Tecnologia, Portugal (POCI/SAU-ESP/61080/2004) and by Fundação Calouste Gulbenkian, Serviço de Saúde e Desenvolvimento Humano, Portugal (Proc/60666-MM/734).

The ciliary proteome database: An integrated community resource for the genetic and functional dissection of cilia. *E.E. Davis, A. Gherman, N. Katsanis* McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD.

In vertebrates, cilia have been recruited in a virtually ubiquitous fashion to carry out a menagerie of diverse tasks. Not surprisingly, dysfunction of the cilium and its anchoring structure, the basal body, have been associated with a number of phenotypes that include defects in neural tube development, retinal dystrophy, renal cystic disease and situs inversus. These observations have highlighted the existence of the ciliopathies, an expanding group of diverse but overlapping clinical entities, and have suggested that an improved understanding of ciliary structure and function has the potential to inform the genetic and cellular basis of such phenotypes. To facilitate these efforts, we assembled and manually curated the recent results of ten unique, yet overlapping proteomics investigations to decipher the repertoire of proteins required for ciliary biogenesis and function in eukaryotes. Our database (available online at http://www.ciliaproteome.org) consists of some ~1200 non-redundant human ciliary, basal body, and centrosomal proteins and provides the means to probe the ciliary proteome with user-defined stringency criteria through basic search, study-based search, or BLAST query options. In addition, we are populating this dataset with useful technical reagent information (ORF and shRNA vectors) and corresponding cell localization data for each putative ciliary protein as well as other phenotypes, (through overexpression or suppression). Preliminary analyses of the human ciliary proteome suggest that our integrated effort markedly improved the saturation of the ciliary proteome, revealing the importance of data amalgamation from diverse sources to compensate for the strengths and weaknesses of each individual proteomics method. Currently, the greatest challenges are to confirm experimentally and characterize further the proteins in the database. As our comprehension of ciliary biology evolves, we are committed to appending new information and encourage community-wide participation in the continued curation of this resource.

Infertility and reproduction failure in autosomal translocations carriers. *N.B. Abdelmoula[1], A. Amouri[2], M. Meddeb[3], A. Sallemi[1], T. Rebai[1]* 1) Laboratory of Histology, University of Medicine, Sfax, Tunisia; 2) Laboratory of Cytogenetics, Pasteur Institute, Tunis, Tunisia; 3) Laboratory of Genetics, Tunis, Tunisia.

Cytogenetic investigations were performed in 242 infertile men because of severe male infertility with low sperm count and in 47 couples because of recurrent miscarriages. Out of the 131 oligospermic and 111 azoospermic men, 32 had an abnormal karyotypes (13.2% : 20,7% in azoospermic and 6,87% in oligospermic men). Chromosome aberrations observed were 22 sex chromosomal aberrations [comprising 46,XX (n=1), 47,XXY(n=18), 47,XYY(n=1) and 46,X,del(Yq) (n=2)] and 10 autosomal aberrations including 4 reciprocal translocations in oligospermic men [t(4;9)(p15.3;p21), t(11;22)(q24;q11),t(2;3)(p24;q26) and t(16;22)(q13;q12)] and 6 robertsonian translocations [t(13;14) in 2 oligospermic and 2 azoospermic men and t(14;21) in an oligospermic and an azoospermic brothers]. The incidence of autosomal balanced structural abnormalities in oligospermic men was higher than in azoospermic men especially for reciprocal translocations (44,4 % v/s 0%). For robertsonian translocations carriers, a maternal meiosis transmission is recorded in two cases while for reciprocal translocations, a familial history is noted for 2 cases. In the other hand, chromosomal abnormalities were recorded twice out the 47 couples, in a female partner and in a normozoospermic male partner, who carried reciprocal translocations [46,XX,t(1;4)(q31;q26) and 46,XY,t(4;17)(p16;p12)]. None of theses translocations carriers come to conceive naturally or with assisted conception. In fact, fertilization failures in previous ICSI attempts (one to 5 attempts) were recorded for 6 couples. So, there is interest in preimplantation genetic diagnosis with assisted conception in carriers of autosomal translocations to improve the implantation rate and protect the couple from termination of pregnancy. Unfortunately, this practice is not yet available in our country.

Development of upQMPSF and mutation-specific multiplex PCR for detecting large germline genomic rearrangements. *S. Azrak[1], L. Li[2], W.D. Foulkes[2], P. Liang[1]* 1) Department of Cancer Genetics, Roswell Park Cancer Institute, Buffalo, NY; 2) Department of Human Genetics, McGill University, Montreal, Quebec, Canada.

Large germline genomic rearrangements, mostly heterogyzous deletions or duplication of genomic regions ranging from several hundreds of base pairs to several hundreds of kb in size, constitute a significant portion of the mutation spectrum associating with genetic diseases, such as hereditary non-polyposis colorectal cancer (HNPCC). The detection of these types of mutations has been technically difficult as they tend to escape from the classical mutation screen methods. Recently, a number of new methods, including Multiplex Amplifiable Probe Hybridization (MAPH), Multiplex Ligation-dependent Probe Amplification (MLPA), Quantitative Multiplex PCR of Short fluorescent Fragments (QMPSF), quantitative multiplex polymerase chain reaction (QMPCR), have been developed for the detection of such types of genomic rearrangements. We report here the development of two additional methods. The first method is a modified version of the QMPSF, in which the fluorescent labeling of individual primers are replaced with a universal fluorescent primer (upQMPSF). In comparison with QMPSF, upQMPSF achieves significant cost saving without sacrificing the sensitivity. The second method is the use of Mutation-specific Multiplex PCR (MM-PCR) primer sets designed via a bioinformatics approach for the detection of all known genomic rearrangements in a given gene. MM-PCR is performed as a regular PCR and is analyzed on agarose gels with the samples carrying targeted genomic rearrangements yielding an extra product smaller than the positive control and the normal samples generating only the control product. Therefore, MM-PCR can be used as a very cost-effective and easy-to-use method for genetic screening of known genomic rearrangements. We used a set of HNPCC samples carrying previously characterized rearrangements in MLH1 and MSH2 to demonstrate the efficiency of our methods and compared the results with those obtained using QMPSF and MLPA. (This research is in part supported by NCI grants (CA16056 and CA101515) and grants from Roswell Alliance Foundation and Gynecological Cancer Foundation.).

Generation of a novel mouse model by targeted insertion of a PGK-NEO cassette into murine *Zic3* locus: a mouse model of Goldenhar Syndrome/Oculo-Auriculo-Vertebral (OAV) spectrum? *J.W. Belmont, L. Zhu, J.L. Peng, K.G. Harutyunyan, M.D. Garcia, M.J. Justice* Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.

Goldenhar Syndrome (OMIM164210), also called oculo-auriculo-vertebral (OAV) spectrum, is a rare birth defect characterized by unilateral deformity of the external ear, hemifacial microsomia, epibulbar dermoid, and defects of the vertebral column. Although candidate regions in the human genome are under investigation, no human gene mutations or mouse models for Goldenhar Syndrome are known. In this study we generated a novel allele by insertion of a PGK-neo cassette into the 5 of the murine *Zic3* locus. *Zic3*^{neo} mutant mice exhibited hemifacial microsomia, asymmetric low set ears, unilateral deformity of the external auricle, microphthalmia, mandible underdevelopment, kyphosis and scoliosis; a combination of defects which mimics Goldenhar Syndrome. *Zic3* is a zinc finger transcription factor located in the X chromosome and is the first gene unequivocally associated with human heterotaxy. Mice bearing a null mutation in *Zic3* correctly model the laterality defects found in human patients, but have no apparent abnormalities in tissues derived from neural crest. However, overexpression of *Xenopus Zic3* induces neural crest marker expression, the formation of enlarged and coarse head, and poorly developed eyes. Interestingly, *Zic3* transcript in this novel *Zic3*^{neo} allele was up-regulated in ES cells and in E9.0 embryos, but no ectopic expression was detected. Unlike the *Zic3*^{null} mutation in which only 20% of mutant animals survive to adulthood, there was no evidence of excess fetal death caused by the *Zic3*^{neo} allele. Penetrance of the individual defects observed in the *Zic3*^{neo} allele was relatively low, with the exception of kyphoscoliosis. This study indicates that the dosage of Zic3 is critical for embryonic development. Given the X-linked inheritance and low penetrance observed in Goldenhar Syndrome, we hypothesize that *ZIC3* may be one of the genes contributing to this rare disease and future study will focus on screening both the coding and noncoding sequence of *ZIC3* in Goldenhar Syndrome patients.

A detailed study of the serotonin pathway in autism. *B. Anderson[1], N. Schnetz-Boutaud[1], J. Bartlett[1], H.H. Wright[3], R.K. Abramson[3], M.L. Cuccaro[2], J.R. Gilbert[2], M.A. Pericak-Vance[2], J.L. Haines[1]* 1) Center for Human Genetics Research and Department of Molecular Physiology and Biophysics, Vanderbilt University Medical Center, Nashville, TN; 2) Center for Human Genetics and Department of Medicine, Duke University Medical Center, Durham, NC; 3) W.S. Hall Psychiatric Institute, University of South Carolina, Columbia, SC.

Classic autism is characterized as one of the Pervasive Developmental Disorders (PDDs) of childhood, a spectrum of often severe behavioral and cognitive disturbances of childhood development. The high heritability in autism has driven multiple efforts to identify susceptibility genes, but to no avail. Numerous studies have suggested that deficits in the peripheral and central metabolism of serotonin (5-HT) may play a role in the pathophysiology of autism. We hypothesize that multiple variations in the serotonergic pathway genes are involved in autism. We assembled a dataset collected through the collaborative efforts of Vanderbilt and Duke Universities. Our sample includes 284 total autism families consisting of 155 parent-child trios and 129 multiplex families, a screening subset of our overall dataset of 333 trios and 158 multiplex families. We prioritized our work to include 15 prominent serotonin pathway candidate genes for detailed study within our autism families. Ninety-three SNPs were genotyped within candidate genes involved in serotonergic function: *HTR1A*, *HTR2A*, *DDC*, *TPH1*, *TPH2*, *SLC6A4*, *SLC7A5*, *AANAT*, *MAOA* and *MAOB*. Parametric two-point analyses identified a peak LOD score of 1.38 for marker rs1522307 within the *TPH2* gene when the complete dataset was considered. Stratification of the dataset to include male-affected-only demonstrated a highly significant p-value of 0.006. Another candidate *SLC6A4*, the serotonin transporter, has been associated with autism in other datasets, but no significant signal was observed in our overall dataset. These results suggest that individual polymorphisms within the serotonin pathway may not be important in autism. However, significant effects may arise through tests for gene x gene interactions, which are underway.

LINE-1 expression and retrotransposition in Human Embryonic Stem Cells. *J.L. Garcia-Perez[1], K.S. O'Shea[2], J.V. Moran[1]* 1) Human Genetics, Univ Michigan Med Sch, Ann Arbor, MI; 2) Cell and Developmental Biology, Univ Michigan Med Sch, Ann Arbor, MI.

Long Interspersed Element-1 (LINE-1 or L1) is an abundant retrotransposon that comprises ~17% of human DNA. The average human genome contains approximately 100 retrotransposition-competent L1s (RC-L1s) and their mobility in both germ and somatic cells has resulted in a variety of genetic disorders, including hemophilia A, muscular dystrophy, and colon cancer. The proteins encoded by RC-L1s also are responsible for the mobilization of Alu elements and the formation of processed pseudogenes, which together comprise at least 10% of human DNA. Thus, either directly or by the promiscuous mobilization of cellular RNAs, L1 retrotransposition has had a tremendous impact on human genome evolution. Despite these findings, questions remain about how frequently, in what cell types, and when during development L1 retrotransposes in vivo. Here we show that undifferentiated human embryonic stem cells (hES) express endogenous L1 transcripts and the L1 ORF1-encoded protein. We demonstrate further that these cells also can accommodate retrotransposition of engineered L1 elements in vitro. Analysis of six retrotransposition events revealed hallmarks of L1 integration and indicate that L1 can retrotranspose into genes in hES cells at frequencies cells similar to those reported in both cultured human HeLa cells and mouse models. Although L1 retrotransposition in hES cells appears relatively inefficient when compared to retrotransposition in HeLa cells, the data also demonstrate that small genomic deletions can accompany L1 retrotransposition events. Together, these findings suggest that L1 mobility can lead to genetic variability in hES cells. In addition, we report that cultured embryonic carcinoma cell lines can support L1 retrotransposition in vitro, and that the resultant integration events often undergo epigenetic silencing either during or soon after retrotransposition. We hypothesize that epigenetic silencing may represent a host mechanism to restrict the number of retrotransposition-competent L1 sequences in mammalian cells. Supported by a NIH P20 GM-069985 grant.

AUTISM GENETICS COOPERATIVE: Preliminary results of a combined linkage genome scan. *R. Goedken* On behalf of the Autism Genetics Cooperative.

A genome screen for autism was completed on data collected as part of the Autism Genetics Cooperative (AGC), a consortium established to facilitate the discovery of genes that contribute to autism. The cooperative currently consists of 6 groups that have worked toward a goal of effectively sharing data and continues to assemble a comprehensive database of genotypic and phenotypic genome screen data.

Data originating from independent genome screens carried out by participating groups, each using a different marker set, were gathered for common analyses. Thus far, an ~10 cM genome screen (2593 microsatellite markers total) has been performed on 338 families, primarily affected sib-pairs, incorporating language and IQ phenotypes. The analyses included the Posterior Probability of Linkage (PPL) and the Kong and Cox LOD. For the PPL, the families were grouped by i) contributing site, ii) phrase speech delay (at least two affected children with onset of phrase speech greater than 36 months), and iii) IQ (at least two affected children with IQ less than 50). The PPL uses Bayesian sequential updating to accumulate evidence across the subgroups. Results indicate 2 distinct regions showing non-negligible evidence in favor of linkage, with peak PPLs of 49% and 60%, on Chromosomes 2 (159 cM) and 16 (62 cM), respectively. The Kong and Cox LOD analysis was based on all families as one group and yielded LODs < 3.0 across the entire genome. We will be updating these results with additional follow-up markers in the peak regions. In addition, we are in the process of gathering full ADI information on all families for inclusion into a common database for further analyses.

A systematic HAPMAP-based survey of schizophrenia candidate genes in the German population. *S. Cichon[1], T. Mühleisen[1], A.M. Hillmer[1], R. Fürst[1], R. Abou Jamra[2], J. Schumacher[2], P. Hoffmann[1], M. Alblas[1], S. Kopmann[1], A. Georgi[3], T.G. Schulze[3], P. Propping[2], M. Rietschel[3], M.M. Nöthen[1]* 1) Dept. of Genomics, Life & Brain Center, Bonn, Germany; 2) Institute of Human Genetics, University of Bonn, Bonn, Germany; 3) Dept. of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Mannheim, Germany.

Recent association studies have identified a number of potential schizophrenia susceptibility genes that currently await replication in independent, large samples of schizophrenia. In our present study, we systematically covered 6 promising genes with haplotype tagging SNPs capturing all haplotypes with a frequency >1% using HAPMAP phase I and II data and tested these SNPs for association in a total of 2,230 individuals, comprising a family-based sample of 210 parent-offspring trios with schizophrenia and in an independent sample of 800 schizophrenia patients and 800 controls, all of German origin (all four grandparents and parents German). All diagnoses were made based on DSMIV criteria. Using Illuminas GoldenGate assays, we genotyped a total of 628 SNPs in these samples, covering the genes RGS4 in chromosomal region 1q23.3 (15 SNPs), the DISC1/DISC2/TSNAX locus on 1q42.1 (138 SNPs), ENTH on 5q33 (30 SNPs), DTNBP1 on 6p22.3 (45 SNPs), NRG1 on 8p21 (384 SNPs), and COMT on 22q11.2 (16 SNPs). We have completed genotyping of all individuals. Genotypes are currently under statistical analysis using TDT and FAMHAP. For associated variants/haplotypes detailed genotype/phenotype correlations will be performed.

Glycerol Kinase Expression Increases Metabolic Flux Through The Pentose Phosphate Pathway: Implications for Glycerol Kinase Deficiency. *K. Dipple[1,2,3], L. Rahib[1,3], J. He[1], A.E. Campos[1], J.C. Liao[3,4], G. Sriram[1,4]* 1) Dept. Human Genetics; 2) Dept. Pediatrics; 3) Biomedical Engineering; 4) Dept. Chemical and Biomolecular Engineering, UCLA Los Angeles CA.

Glycerol kinase deficiency (GKD) is an inborn error of metabolism (IEM) that is caused by mutations in the glycerol kinase (GK) gene on Xp21. GK is an important lipogenic enzyme in liver and patients present with metabolic crisis. GKD exhibits complexities that are not explained by lack of the biochemical activity; therefore, there is no correlation between genotype and phenotype in patients with this disorder. We hypothesize that systems dynamics, including flux through metabolic pathways, can play a significant role in explaining the lack of genotype-phenotype correlation. Therefore, we investigated comparative flux analysis of wild type and GK-overexpressing (GK transgenic) H4IIE rat hepatoma cell lines. H4IIE cells were cultured on a mathematically designed mixture of U-^{13}C, 1-^{13}C, and naturally abundant glucose. Gas chromatography-mass spectrometry was used to measure mass isotopomer abundances in protein hydrolysates from the cells. Fluxes were evaluated from the isotopomer data using a comprehensive isotopomer balancing. The GK transgenic cell lines exhibited significantly reduced cell growth and lactate production rates compared to the wild type, while consuming carbon sources at nearly the same rates as the wild type, thus suggesting a futile cycle. Principal component analysis revealed that carbohydrate metabolism in the GK transgenic cell lines was significantly different from that in the wild type. Metabolic flux analysis revealed that in the presence of transgenic GK, there was significantly increased flux through the pentose phosphate pathway (2.04 0.10 times that of wild type, $p <$ 0.01). We hypothesize that this is due to the increased requirement of cytosolic NADPH needed for increased triglyceride synthesis. This shows that cellular metabolism is sensitive to dosage of metabolic genes and that the metabolic pathways with altered flux are not always predicable. These findings will be important to understanding the complex nature of GKD and other IEM.

Association between iNOS and Age-related Macular Degeneration: Potential effect modification by cigarette smoking. *G.E. Byfield[1], S. Schmidt[1], P. Gallins[1], E.A. Postel[2], W.K. Scott[1], A. Agarwal[2], K. Spencer[3], J.L. Haines[3], M.A. Pericak-Vance[1], M.A. Hauser[1, 2]* 1) Duke Center for Human Genetics, Durham, NC; 2) Ophthalmology, Duke University Medical Center, Durham, NC; 3) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN.

Age-related macular degeneration (AMD) is one of the most common causes of blindness in older adults in the United States. Genetic and environmental factors have been strongly implicated in this retinal degenerative disease which causes impairment of central vision. Cigarette smoking is the most consistently identified non-genetic risk factor for AMD. Inducible nitric oxide synthase (iNOS) is the primary source of nitric oxide (NO) and smoking has been associated with decreased production of NO. Polymorphisms in the iNOS gene have been previously linked to neurodegenerative diseases such as Parkinson disease and multiple sclerosis but not AMD. It is against this background that we tested associations between polymorphisms in the iNOS gene and AMD while controlling for smoking. We genotyped 13 single nucleotide polymorphisms (SNPs) across a 60kb region on chromosome 17 in a data set of 610 cases and 259 controls. Genotyping used TaqMan Allelic Discrimination Assays (ABI) and association analysis of each SNP utilized logistic regression. A comparison between the grades 3, 4, & 5 AMD vs controls revealed very significant association of the intronic SNP rs8072199 in the dominant model (p=0.0007). Subtype analysis of individuals with grade 5 vs 1 AMD (International Classification Criteria, Bird et al. 1995), revealed significant association with AMD risk in 367 grade 5 cases (p=0.0024). This association was even more significant (p=0.0013) when the same SNP was analyzed in smokers with grade 5 AMD compared with grade 1 controls. Analysis of this SNP in non-smoking individuals in the equivalent groups was not significant (p=0.39). Other markers exhibiting significant association with AMD risk and smoking included rs2255929 and rs3794766. The data support our previously noted association of the iNOS SNP rs8072199 with AMD and further implicate smoking is as an integral environmental component in AMD susceptibility.

Localisation of a gene for Human Premature Hair Greying on chromosome 9q34. *J.-L. Blouin[1], O. de Lacharrière[2], E.T. Dermitzakis[3], C. Deloche[2], P. Galan[4], P. Bastien[2], G. Duriaux Sail[3], M. Gagnebin[3], C. Gehrig[3], A. Christen[3], B. Bernard[2], S. Hercberg[4], S.E. Antonarakis[1,3]* 1) Medical Genetics, Univ Hosps Geneva, Switzerland; 2) LOREAL Recherche, Clichy, France; 3) Genetic Medicine and Development, University of Geneva School of Medicine, Switzerland; 4) UMR INSERM-INRA-CNAM-ParisXIII, CRNH Ile de France, France.

Hair greying is one of the most common traits linked to aging process in humans. To identify the genomic basis of this trait, we investigated premature hair greying (PHG) which is known to have a familial clustering and is linked with some autoimmune diseases. We first carried out a genome-wide linkage analysis in 12 multigeneration families and found 2 intervals with significant LOD scores (3.52, 3.37) on chr. 6p12-p21 and 9q31-qter respectively. We then performed a case-control association study (187 cases/186 controls) matched for sex, hair color at 18 yrs, geographical origin, ethnicity, with a dense set of SNPs within the 2 candidate intervals. The association analysis with SNPs under the linkage peaks confirmed the previous results. On 6p, the association was quite diffuse on the entire HLA region. However on 9q, the association was localized to a 100 Kb region at 9q34. A further study with additional SNPs (5-Kb ave. interval) revealed a highly significantly associated haplotype (defined by SNPs rs3739902, rs2583805, rs377090) with PHG, centered around genes *GTF3C4* and *DDX31*. The highest $p=6x10^{-5}$ was obtained with SNP rs3739902 in intron 3 of *DDX31*. Mutation search in coding regions, splicing sites of *DDX31* and *GTF3C4*, and intronic CNCs, showed a few variants with unclear functional significance, not present or present under significant different frequency in controls. Interestingly, yeast two-hybrid experiments revealed a functional link between DDX31 and PAX3 (involved in the pigmentation pathway since pathogenic mutations cause Waardenburg syndrome). DDX31 which belongs to helicase family and its genomic region appears to be a strong candidate for harbouring variability that predisposes to PHG. *Authors JLB, OL had equal contribution Acknowledgements to the SUVIMAX team and volunteers for their high involvement in the study*.

Predominant Ashkenazi BRCA1/2 mutations in families with pancreatic cancer. *E. Dagan[1,2], M. Haimi[1], R. Gershoni-Baruch[1,3]* 1) Departement of Human Genetics, Rambam Medical Center, Haifa, Israel; 2) Departement of Nursing, the faculty of health and social studies, University of Haifa, Israel; 3) The Ruth and Bruce Rappaport faculty of medicine, Technion, Haifa.

To study the frequency of the three predominant BRCA1 and 2 Ashkenazi mutations in patients with pancreatic cancer, we evaluated 1014 families recruited at our high risk breast-ovarian cancer onco-genetic clinic. Twenty three families with either personal or family history of pancreatic cancer were identified. In nine families the probands themselves presented with pancreatic cancer and two of them (22%) were found to carry a BRCA mutation (185delAG in one case and 6174delT mutations in BRCA1 and BRCA2, respectively). In the other 14 families, only a family history of pancreatic cancer was reported. Of these, seven families segregated either the 185delAG (3 families) or the 6174delT (4 families) mutation. Pedigree analysis shows that four of the seven pancreatic cancer cases were obligatory carriers. In the other seven families, without mutations in BRCA1 and 2, according to the family pedigrees, it seems that the patients with pancreatic cancer were non-carriers. In summary, in the 23 families with either a personal or family history of pancreatic cancer, reported in this study, six (26%) BRCA1 /2 mutation carriers were identified. In Ashkenazi Jews, mutations in BRCA1 and 2, may constitute a major cause for pancreatic cancer as is the case with ovarian cancer.

Large-scale genomewide association analysis of multiple disease phenotypes: The Wellcome Trust Case-Control Consortium Study. *P. Donnelly for the Wellcome Trust Case Control Consortium* Dept Statistics, Univ Oxford, Oxford, United Kingdom.

Efforts to identify genome sequence variants underlying common complex diseases have met with limited success thus far. All too often, poorly-designed, underpowered studies have generated false positive findings that cannot be replicated. Completion of the International HapMap project and advances in genotyping technologies now allow implementation of systematic, well-powered genomewide studies of complex trait susceptibility. The Wellcome Trust Case Control Consortium (www.wtccc.org.uk) represents a collaborative network of 24 UK laboratories established to examine questions relevant to the design, execution and analysis of disease association studies, as well as to deliver etiological insights through susceptibility gene discovery. The study is principally based around a series of UK-wide case samples (2000 each of type 1 and 2 diabetes, coronary heart disease, bipolar disorder, rheumatoid arthritis, hypertension, Crohn's disease) each compared to 3000 common UK controls (equal numbers from the 1958 Birth Cohort and a national blood donor collection). All are being typed with the Affymetrix 500k chip. A parallel analysis of tuberculosis susceptibility is underway in Gambian case-control samples. In addition, we are typing 1000 cases each of ankylosing spondylitis, breast cancer, multiple sclerosis and autoimmune thyroid disease and the 1500 birth cohort controls using a custom-made chip (Illumina) based around 15000 non-synonymous SNPs. First stage genotyping for the main study (1000 from each case set and all 3000 controls) will be completed during June 2006 and second stage genotyping (the balance of each case sample) during August. Data release of genotypes for the 1500 birth cohort controls is imminent. The WTCCC represents one of the largest studies of complex trait susceptibility yet undertaken (in terms of disease range, sample size and marker density): it is expected to generate important clues to the etiopathology of complex traits, and to inform on a range of issues pertinent to the implementation of large-scale association studies.

Prenatal detection of absent nasal bone predicts malformation syndromes. *N. Blagowidow[1], M. Ferguson[1], A. Kimball[1], M. Cunningham[2], A.D. Kline[1]* 1) Harvey InstituteFor Human Genetics, Greater Baltimore Medical Center, Baltimore, MD; 2) Seattle Children's Hospital, University of Washington, Seattle, WA.

Detection of absent or hypoplastic nasal bone by ultrasound is known to be associated with possible fetal aneuploidy, particularly Down syndrome, more often in a high risk population. Its utility in the general population has been questioned, and there is ethnic variation. Few other chromosomal abnormalities and malformation syndromes have been described in association with absent nasal bone. We report our series of 11 patients with absent or hypoplastic nasal bone detected in pregnancy. Case 1 is a Hispanic female with bilateral cleft lip and palate and normal chromosomes, who was postnatally found to have lobar holoprosencephaly. Case 2 is a mixed White and Asian female with prenatal choroid plexus cysts, echogenic focus and normal chromosomes. After birth marked dolichocephaly, a huge posterior fontanel, and a duplicated 5th toe were noted. She, her mother and maternal grandmother appear to have the parietal foramina syndrome. Case 3 presented with polyhydramnios and pyelectasis in the 3rd trimester. The newborn was noted to have dysmorphic features, natal teeth, pectus and hypotonia. Chromosomal analysis revealed 46,XX,der(22)t(14;22)(q24;q13)[95]/46,XX[5]. Three of the other 8 cases had trisomy 21, including one in which the absent nasal bone was the only ultrasound abnormality. Of the remaining 5 cases, three patients have delivered normal newborns, and 2 are pending delivery, including one fetus with post-axial polydactyly. The nasal bone, detectable by prenatal ultrasound between 11 and 13 weeks gestation, is formed from the frontonasal prominence. An interruption of normal neural crest migration, as is seen in a number of malformation syndromes, could lead to absence of the nasal bone. Because the nose continues to grow throughout life, the postnatal consequences may be less dramatic. Although it is challenging to acquire expertise in recognition of this finding, it could prove to be useful in early detection of multiple malformation syndromes.

Curry-Jones syndrome: A new case associated with trichoblastoma of the skin and a review of the literature.

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We report a case of Curry-Jones syndrome in a 6 month-old male. At birth, he had abnormal skin, iris colobomas and polydactyly. At 3 months, MRI of the brain showed hydrocephalus, agenesis of the corpus callosum (ACC) and Chiari I malformation. A VP shunt was required. He has mild plagiocephaly without craniosynostosis, bilateral iris colobomas, bilateral pre-axial polydactyly and syndactyly of the hands and left foot. Pale white to yellowish, slightly raised, streaky and swirling skin plaques partially follow lines of Blaschko on the trunk and extremities. There are brownish, atrophic lesions on the soles, linear scar-like lesions around the nails and raised pearly lesions on the left eyelid. Skin biopsy showed trichoblastoma, not previously reported in Curry-Jones syndrome. Karyotype and chromosome microarray analysis were normal. Temple (1995) reported five cases of Curry-Jones syndrome, 2 of which were originally described by Curry and Jones. Two additional cases were described by Mingarelli (1999) and Thomas (2006). Features include craniosynostosis, coloboma or microphthalmia, and pre-axial polydactyly and syndactyly of hands and/or feet. Intestinal abnormalities include malrotation, dysmotility and myofibromata. ACC, dilated ventricles and developmental delays may occur. Streaky skin lesions following lines of Blaschko are often hypopigmented or atrophic. Skin biopsies in previous patients were normal, or showed findings of sebaceous or epidermal nevi. Patches of abnormal hair growth, including the periocular region, may be present. Trichoblastoma is a benign skin tumor of the hair germ cell, composed of primordial epithelium associated with collagenous stroma, with occasional sebaceous or primitive follicular differentiation, and is the most common tumor arising in sebaceous nevi. The unusual skin lesions in Curry-Jones syndrome have shown characteristics of sebaceous and epidermal nevi, and now in our patient, trichoblastoma. Further investigation of the molecular pathogenesis of these skin lesions may provide clues to the genetic basis for Curry-Jones syndrome.

Primary Care Physicians Knowledge of Race, Ethnicity, Human Genetic Variation and Health. *V. L. Bonham[1], L. Cooper[2], D. Frank[3], T. Gallagher[3], A. Odunlami[1], E. Phillips[1], E. Price[4], S. Sellers[5]* 1) NHGRI, NIH, Bethesda, MD; 2) Johns Hopkins Univ., Baltimore, MD; 3) Univ. of Washington, Seattle, WA; 4) Tulane Univ., New Orleans, LA; 5) Univ. of Wisconsin-Madison, Madison, WI.

While physicians are assuming a key role in translating genomic knowledge to the public, few studies have explored physicians attitudes and knowledge regarding human genetic variation, race and ethnicity. A mixed-method study was conducted to assess primary care physicians knowledge of race, ethnicity and genetics. Ninety self identified Black and White general internists participated in 10 race-concordant focus groups in 5 geographic areas in the country. The physicians discussed their knowledge of human genetic variation, their beliefs regarding the genetic basis of racial and ethnic differences in disease morbidity and mortality, and the clinical applicability of race-based therapeutics. Focus group discussions were audio taped, transcribed and coded using grounded theory. Two investigators independently reviewed each transcript and identified distinct themes. Participants: 43% of the physicians self-identified as Black, 53% as White; 73% of the physicians were males; 88% had been practicing internal medicine for at least 10 years; 48% are academic physicians; 73% of the physicians rated their exposure level to genetics as low, 22% as medium and 3% as high. Considerable confusion was present among physicians regarding basic concepts of human genetic variation. Limited consensus existed regarding key topics, such as the relationship between race, human genetic variation and health disparities or the role of race and ethnicity in clinical decision-making. The physicians opinions varied widely about the medical relevance of racial, ethnic and ancestral information. White physicians expressed more concern about talking directly with patients about their racial, ethnic and ancestral background. Developing a deeper understanding of physicians knowledge of and attitudes about human genetic variation, and use of medical application of race, ethnicity and ancestry will be instrumental for successfully translating genomics into clinical practice.

A Novel Form of Autosomal Recessive Lethal Congenital Arthrogryposis Mapped to Chromosome 19p13 Using SNP microarrays. *G. Narkis[1,2], D. Landau[2], E. Manor[2], R. Ofir[1], K. Elbedour[2], O.S. Birk[1,2]* 1) MK Lab. of Human Molecular; 2) Genetics Institute, Soroka Medical Center, Ben-Gurion University, Beer-Sheva, Israel.

We have recently described a novel autosomal recessive syndrome, lethal congenital contractural syndrome type 2 (LCCS2), in a large Israeli Bedouin kindred. Genome-wide linkage analysis demonstrated a region of homozygosity on chromosome 12q13. In the present study we set out to determine the disease-associated locus in another non-related Bedouin family with a similar phenotype of LCCS. We excluded linkage to the LCCS2 locus on chromosome 12q13 and to two other known candidate regions associated with autosomal recessive arthrogryposis (5q35 related to arthrogryposis multiplex congenita in a large Israeli-Arab inbred family, and 9q34 associated with the Finnish type of lethal arthrogryposis). We performed genome-wide linkage analysis using the Affymetrix 10K SNP arrays. A region of homozygosity containing 5 SNPs (5.3 Mb interval) was identified on chromosome 19q13. Using microsatellite markers, the interval harboring the disease-associated locus was confirmed and narrowed down to a chromosomal region spanning 4.5 Mb (based on recombination events). Two point linkage analysis using SUPERLINK program demonstrated maximum LOD score of [Zmax] = 4.0 at recombination fraction [] = 0.0. The linkage established allows for molecular prenatal diagnosis of the disease in the affected families. Identification of the disease-associated mutated gene is underway. It should be noted that in nine other non-related Israeli Bedouin families affected with LCCS we have now ruled out linkage to the new locus or to any of the previously known loci associated with the disease. Thus, we suggest that there are at least 3 genes related to LCCS in the Israeli Bedouin population.

Association of a common interferon regulatory factor 5 (*IRF5*) variant with increased risk of systemic lupus erythematosus (SLE). *F.Y. Demirci[1], S. Manzi[2], R.L. Minster[1], F. Bontempo[3], P.S. Shaw[2], A.H. Kao[2], M.I. Kamboh[1]*
1) Dept. of Human Genetics, GSPH, Univ. of Pittsburgh, Pittsburgh, PA; 2) Division of Rheumatology and Clinical Immunology, Lupus Center of Excellence, Univ. of Pittsburgh, Pittsburgh, PA; 3) Dept. of Medicine, School of Medicine, Univ. of Pittsburgh, Pittsburgh, PA.

Systemic lupus erythematosus (SLE) is a systemic inflammatory autoimmune disease caused by a complex interaction of genetic and environmental factors. The serum interferon (IFN)- levels and the expression of the type I IFN-inducible genes have been shown to be correlated with SLE activity and severity, suggesting the involvement of the type I IFN system in the pathogenesis of SLE. *IRF5* belongs to a family of transcription factors that controls the trans-activation of the type I IFN system-related genes, as well as the expression of several other genes involved in immune response, cell signaling, cell cycle control and apoptosis. Two recent studies reported significant association between the *IRF5*/rs2004640 T allele and SLE in cohorts from Sweden, Finland, Argentina, Spain and the US. The T allele creates a consensus GT donor splice site for an alternative exon (exon 1B) of *IRF5*, allowing the expression of transcripts containing this alternate exon. The purpose of this study was to replicate the reported rs2004640 SNP association in our independent Caucasian American SLE case-control cohort. DNA samples from 308 women with SLE and 407 healthy control women were genotyped using the TaqMan Assay-on-Demand (ABI) for the rs2004640 SNP. The genotype frequencies were in Hardy-Weinberg equilibrium in both case and control groups. The frequency of the T allele was significantly higher in cases than controls (55.8% vs. 50.0%; p=0.028). The age-adjusted odds ratio (OR) for T allele carriers was 1.68 (95% CI: 1.16 - 2.43; p=0.0057), which is within the range of the reported ORs (1.31 - 1.84). Our results in an independent case-control sample confirm the robust association of the *IRF5*/rs2004640 T allele with SLE risk and support the important role of the type I IFN system in the pathogenesis of SLE and autoimmunity.

Chemokine Levels and Their Receptors are Increased in Hermansky Pudlak Syndrome Type I. *H. Dorward*[1], *T. Markello*[2], *W.A. Gahl*[1] 1) MGB, NIH/NHGRI, Bethesda, MD; 2) Children's National Medical Center, Washington, DC.

All patients with Hermansky Pudlak syndrome (HPS) have oculocutaneous albinsim and a platelet storage pool deficiency due to absent platelet dense bodies. There are 8 different genetic subtypes of HPS, each representing a putative defect in intracellular trafficking of lysosome-related organelles; these organelles include the dense bodies of platelets, the melanosomes of melanocytes, and the lamellar bodies of type II pneumocytes. For unknown reasons, up to 70% of patients with type I HPS have a fatal pulmonary fibrosis. This process appears homologous to that of idiopathic pulmonary fibrosis (IPF), i.e., Usual Interstitial Pneumonitis, or UIP. A current hypothesis regarding the etiology of IPF/UIP involves dysregulation of the chemokines that are involved in wound healing. We measured the levels of the chemokines MCP-1 and TGF-beta in bronchioalveolar lavage fluid (BALF) from patients with HPS. Compared to normal BALF, the HPS fluid exhibited increased levels of MCP-1 and TGF-beta on Western Blotting. Preliminary estimates suggest 3 to 5 fold increases. We also assayed CCR2, the receptor of MCP-1, in paraffin fixed autopsy lung sections of: 1 IPF, 3 HPS and 1 normal control patient(s) using immuno-histochemical staining. The level of CCR2 in HPS lung was dramatically upregulated compared to that in normal lung, and was indistinguishable from control IPF lung sections. Staining for CCR2 colocalized with fibrotic sections of the autopsy lung tissue HPS lung pathology appears to represent a pathologic process equivalent to that of IPF. This allows studies of lung fluid and lavage cells in presymptomatic HPS patients who are at high risk for pulmonary fibrosis, and to test current models of the role of specific cytokines and chemokines to initiate some of the pathologic changes seen in pulmonary fibrosis.

Recurrent Interstitial Deletions of Proximal 18q: Description of a New Syndrome Involving Expressive Speech Delay. *J.D. Cody, A. Malik, C. Sebold, P. Heard, A. Duran, E. Carter, D.E. Hale* Dept Pediatrics, Univ Texas Health Sci Ctr, San Antonio, TX.

The majority of individuals with deletions of chromosome 18q have deletions involving the distal 30 Mb of the chromosome. We have identified 5 individuals with cytogenetically diagnosed interstitial deletions that are all proximal to the most commonly deleted region. The extent of the deletions was characterized using molecular and molecular cytogenetic techniques. In addition, each patient was assessed under the comprehensive clinical evaluation protocol for the Chromosome 18 Clinical Research Center. Three of the five individuals were found to have identical interstitial deletions between 37 Mb and 42 Mb. One individuals deletion was much larger and extended from a more proximal breakpoint of 23 Mb to a more distal breakpoint at 43 Mb. The fifth individual had an identical proximal breakpoint, but a distal breakpoint at 43.5 Mb. The medical and developmental histories of these individuals suggest a clinical phenotype that is distinct from that of the terminal 18q deletions. In general, proximal interstitial deletions appear to be associated with relatively minor dysmorphic features. Two of the five patients had congenital defects requiring intervention (bilateral Hutch diverticuli, cryptorchidism). Hypotonia and seizures as well as vision problems and recurrent ear infections were present in two of the five patients. Two of the patients also had agenesis/hypoplasia of the corpus callosum. Of note, some of the features common in individuals with terminal deletions were absent in this patient population. None of the patients with proximal interstitial deletions had growth hormone deficiency, stenotic ear canals, or cleft lip/palate, although one had delayed myelination. All individuals exhibited developmental delays and mental retardation. However, not all aspects of development were affected to the same degree. Receptive language skills were universally better preserved than expressive language. This leads us to hypothesize that there are genes in this region of chromosome 18 that are specific to the neural and motor planning domains necessary for speech.

Method for analyzing markers in linkage disequilibrium using polychoric correlation. *S. Bacanu, M. Nelson, L. Li, M. Ehm* GlaxoSmithKline, RTP, NC.

Datasets pertaining to association scans with dense sets of markers are coming online at a rapid pace. However, there are a lot of theoretical questions that remain at least partially unanswered. Do we analyze markers in high linkage disequilibrium (LD) singly or jointly? What analysis methods do we employ? What measure of LD should we use to cluster markers to be analyzed jointly? How to identify which combinations of markers to analyze jointly? What is the influence of missing genotype rates on the power of different methods? For validation purposes, how to efficiently simulate a large number of markers in high LD? To answer these questions we estimated power via simulation for a host of univariate and multivariate tests. Genotypes for markers in high LD were obtained by first simulating a latent multivariate normal observation. Gnotypes at each locus in the cluster are obtained from latent variables using thresholds based on allele frequencies and assuming Hardy-Weinberg equilibrium. In our simulations, the (polychoric) correlation structure of latent variables associated with disease marker and observed markers was assumed to be either exchangeable or autoregressive. Cases were obtained via rejection sampling. Simulations, requiring hundreds of years of CPU time, were carried on the GSK parallel computing GRID. Simulation results showed a poor performance of traditional multivariate methods, especially with realistic missing data rates. A near optimal test was a Bonferroni-adjusted minimum p-value of two different types of tests. The first test employs a minimum p-value of univariate tests for additive, dominant and recessive models at each marker in the cluster. The second test is a multivariate chi-square test obtained from the first two principal components of the Pearson correlation matrix of univariate tests above. For complete genotype data, optimal LD threshold for obtaining marker clusters were estimated to be 0.4-0.5 on a square polychoric correlation scale; it is even lower than 0.2 for realistic missing genotype rates. Theoretical results are compared with simulation results using LD structure derived from an Affymetrix 500K genome scan containing 500 subjects.

Development and validation of a Real Time PCR assay for detection of *MECP2* gene rearrangements. *D. del Gaudio, B.B. Roa, C.M. Eng, P. Fang* Department of Molecular and Human Genetics , Baylor College of Medicine, Houston, TX.

Mutations in the *MECP2* gene cause Rett syndrome, a progressive neurodevelopmental disorder that affects ~ 1 in 10,000 females. In addition, *MECP2* mutations are found in females and males with a wider spectrum of neurodevelopmental phenotypes. Diagnostic sequencing of the *MECP2* coding region detects ~86% of mutations in classic Rett syndrome. Our laboratory previously implemented a dosage-sensitive Southern analysis for deletions and duplications in *MECP2*, which detects an additional ~10% of mutations in classic Rett syndrome. However, heterozygous duplications in females are difficult to assess by Southern analysis. We recently developed and validated a quantitative real-time PCR assay (qRT-PCR) for *MECP2* gene dosage analysis. This assay uses multiple Taqman probes covering the entire *MECP2* gene coding region (exons 1-4), with each target amplicon co-amplified with an internal control (RNaseP). Amplicon copy number in patients versus sex-matched controls is determined by the comparative cycle threshold method (ddCt). The assay was validated using 12 blinded control samples (10 females and 2 males) that were previously tested by Southern analysis and multiplex ligation-dependent probe amplification (MLPA). Our qRT-PCR assay correctly identified *MECP2* heterozygous partial gene deletions in 7 females, and whole gene duplication in 1 male and in his heterozygous carrier mother. In comparing several methods, a female patient with a heterozygous deletion in *MECP2* exon 3 was identified by Southern analysis and by qRT-PCR using Taqman probes located at the 5 and 3 ends of exon 3, but not by MLPA. Confirmation of the deletion was provided by long-range PCR, followed by sequencing of the junction amplification product (205_IVS3+287del460). Our data demonstrate the utility of quantitative real time PCR analysis to detect *MECP2* gene rearrangements with high sensitivity and highlight the value of incorporating multiple probes in a gene dosage assay used in a clinical diagnostic setting.

Analysis of the expression of *mRib72-1/Efhc1* gene during mouse brain development. *F.F. Conte, P.A.O. Ribeiro, L. Sbragia Neto, R. Gilioli, F. Cendes, I. Lopes Cendes* Medical Genetics, UNICAMP, Campinas, Brazil.

Missense mutations in the *mRib72-1/Efhc1* gene have been identified to co-segregate with juvenile myoclonic epilepsy (JME) patients. In addition, functional studies have demonstrated that expression of *mRib72-1/Efhc1* gene induces apoptosis in neurons in culture. However, the exact relationship between *mRib72-1/Efhc1* function and epileptogenesis is still unclear. The objective of this study was to determine the expression pattern of Missense mutations in the *mRib72-1/Efhc1* have been identified to co-segregate with juvenile myoclonic epilepsy (JME) patients. In addition, functional studies have demonstrated that expression of *mRib72-1/Efhc1* gene induces apoptosis in neurons in culture. However, the exact relationship between *mRib72-1/Efhc1* function and epileptogenesis is still unclear. The objective of this study was to determine the expression pattern of *mRib72-1/Efhc1* gene in mouse brain in order to investigate its possible role in programmed cell death during development. Balb/c mice were submitted to programmed mating to obtain animals in different developmental stages. The brains of embryos aged 15, 17 and 18 days old and of neonates aged 1, 7, 14 and 28 days old were subsequently removed. We used brains of three animals for each age studied. After RNA extraction, the relative gene expression of *mRib72-1/Efhc1* was determined by the real-time PCR technique using TaqMan assay. *GAPDH* gene expression was used as an endogenous control. Preliminary results demonstrate that the expression of *mRib72-1/Efhc1* is higher in embryos than in neonates (p=0.001), with a progressive decrease from embryos aged 18 days to neonates. The highest expression of the *mRib72-1/Efhc1* gene in embryo brains in comparison to neonates suggests that the putative pro-apoptotic role of this gene may be necessary for the correct organization of the central nervous system during its initial phases of development. Supported by CAPES.

Congenital myotonic dystrophy presenting as unexplained neonatal death. *T. Friedberg[1], M. Thomas[1], D. Chitayat[1,2]* 1) The Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Toronto, Ontario, Canada; 2) Division of Clinical and Metabolic Genetics, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada.

Myotonic dystrophy type I (MDI) is an autosomal dominant disorder with intrafamilial and interfamilial variability in the clinical findings ranging from mild and late onset to severe and congenital depending on the size of the CTG repeat in the DMPK gene. Congenital myotonic dystrophy is characterized by infantile hypotonia, mental retardation, and respiratory deficits, which may lead to early death. Here, we report on two families whose babies died shortly after birth, presumably of an unknown cause, and were subsequently found to be affected with congenital myotonic dystrophy based on maternal examination. Case 1: Fetal ultrasound done at 20 and 28 weeks gestation showed mild bilateral ventriculomegaly and mild polyhydramnios and at 30 weeks gestation a distended abdomen and polyhydramnios were noted. Delivery was at 33 weeks gestation and the baby died 10 minutes after birth. On autopsy, no significant abnormalities were identified apart from megalencephaly (OFC of 38 GA). The mother had features of DMI not noted before and on Analysis had 14 and 100 CTG repeats. The baby had 11 and 3800 CTG repeats. Case 2: The parents were first cousins and had five pregnancies in the 1980s and 1990s in Iran which resulted in children who died in the first two weeks of life. They had two daughters diagnosed as having hydrocephalus, one daughter with IUGR, and two sons with macrocephaly. The cause of death in any of the children was not known and was suspected to be an AR condition. Investigations were initiated on the mother due to this history and identified a DMPK gene expansion of 13 and 700 CTG repeats, thus she is affected with DMI and the early death of her children is most likely due congenital myotonic dystrophy. Unexplained neonatal death can be the result of congenial DM and this should taken into consideration in the forensic investigation.

Differential effects of DRB1*0301 and DQA1*0501-DQB1*0201 on the activation and progression of islet cell autoimmunity. *E. Eller[1], P. Vardi[2], K.K. McFann[1], S.R. Babu[1], L. Yu[1], G.S. Eisenbarth[1], P.R. Fain[1]* 1) Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences Center, Aurora, Colorado, USA; 2) Felsenstein Medical Research Center (Beilinsen Campus), Tel Aviv University, Petah Tikva, Israel.

We have found an unusual DR3 haplotype, DRB1*0301-DQA1*0102-DQB1*0502, in a Bedouin Arab kindred that is characterized by a high prevalence of autoimmune diseases including type 1 diabetes (T1D) and celiac disease. While the usual DR3 haplotype DRB1*0301-DQA1*0501-DQB1*0201 is frequent in this family and occurs in 23 of the 24 relatives with T1D, the novel 0301-0102-0502 haplotype occurs disproportionately in individuals who are positive for islet cell autoantibodies but do not have T1D. This kindred provides a unique opportunity to investigate the effects of the two DQ loci conditioned on DRB1*0301. We show that the 0301-0102-0502 haplotype is overtransmitted to autoantibody-positive relatives without T1D, suggesting that the DQA1*0102-DQB1*0502 haplotype creates conditions for autoantibody positivity but decreases the probability of progression to type 1 diabetes.

Estimating the split time of Human and Neanderthal populations. *G. Coop[1], S. Kudaravalli[1], J.P. Noonan[2,3], D. Smith[2], J. Krause[4], J. Alessi[2], D. Chen[2], D. Platt[2], S. Pääbo[4], J.K. Pritchard[1], E.M. Rubin[2,3]* 1) Human Genetics, University of Chicago, Chicago, IL; 2) US DOE Joint Genome Institute, Walnut Creek, CA; 3) Genomics Division, Lawrence Berkeley National Laboratory, Berkeley, CA; 4) Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany.

Previous genetic studies of Neanderthal ancestry have used mtDNA and thus have been limited in their conclusions on the relationship of humans and Neanderthals. We present here the first use of Neanderthal genomic DNA to assess the joint history of human and Neanderthal populations. Our data consist of 37kb of short fragments of genomic DNA sequenced in Neanderthal. By studying the degree to which modern human diversity is shared with Neanderthal we can assess the time at which the human and Neanderthal populations split. We use a flexible simulation based approach that demonstrates the power of using human variation data in such analyses. We find that the two populations split ~400,000 years, predating the emergence of modern humans. Our best fitting model predicts that the Neanderthal lineage will be outgroup to the human population ~52% of the time.

A High Density Linkage Screen for POAG: Evidence for Different Loci by Race. *R.R. Allingham[1], J.L. Wiggs[2], F.L. Graham[1], K.R. LaRocque-Abramson[1], C. Santiago-Turla[1], A. Ventura-Viray[1], J.L. Haines[3], M.A. Pericak-Vance[1], M.A. Hauser[1]* 1) Duke Univ Medical Ctr, Durham,NC; 2) Harvard Medical School, Boston,MA; 3) Center for Human Genetics Research, Nashville,TN.

Primary open-angle glaucoma(POAG) is a common complex inherited disorder. Here we report a major SNP-based genome-wide screen on a large POAG family dataset, consisting of 142 multiplex POAG families (including 87 Caucasian and 37 African-American families), each containing 2 or more affected family members. The marker panel consisted of 5067 single nucleotide polymorphisms (SNPs)and was performed utilizing the Illumina Bead Station platform. Two-point and multipoint heterogeneity lod scores were calculated using Allegro. For multipoint analyses a single SNP was selected from each bin of SNPs in linkage disequilibrium (r2 0.16). Genotype data were analyzed *in toto* and after stratification by Caucasian and African-American race. The two-point analyses was performed on the total dataset. For multipoint analyses the Caucasian and African-American family subsets were also analyzed. Peak two-point MLOD scores for chromosomes 3, 6, 14, and 15 were 4.4, 2.6, 4.4, and 3.5, respectively. Peak multipoint(parametric or non-parametric)lod scores for chromosomes 3, 6, 14, and 15 were 2.6, 1.7, 2.1, and 2.6, respectively. Additional multipoint lod scores 2.0 were found on chromosomes 12 (2.2) and 17 (2.0). Multipoint lod scores for the loci on chromosomes 3,14, and 15 were primarily obtained from the African-American subset. The lod score for the locus on chromosome 12 was derived from the Caucasian subset. Contributions from both racial subsets contributed to the lod scores on chromosomes 6 and 17. We have performed the most definitive genome-wide linkage screen for POAG described to date, using over 5000 markers in one of the largest reported multiplex family datasets. For the first time we report known and novel chromosomal loci for POAG that appear to be derived primarily from either Caucasian or African-American family subsets. These findings suggest that different genetic loci may in part explain variations in the prevalence and phenotype of POAG among races described in population-based studies.

The impact of folic acid fortification on the prevalence of congenital anomalies other than neural tube defects. *K. Godwin[1], B. Sibbald[2], T. Bedard[2], B. Lowry[2], B. Kuzeljevic[3], L. Arbour[1]* 1) Medical Genetics, Children's and Women's Health Centre of BC, Vancouver, BC, Canada; 2) Alberta Congenital Anomalies Surveillance System, Department of Medical Genetics, Alberta Childrens Hospital, Calgary AB; 3) BC Research Institute for Childrens and Womens Health, Vancouver BC.

INTRO: There is evidence for a reduction in NTDs in Canada where mandatory grain fortification with folic acid was initiated in 1998. However, there has been little exploration as to whether the fortification program is also impacting the rates of other congenital anomalies (CAs). METHODS: Using the ACASS, we examined changes in birth prevalence of select CAs between 1992-1996 (pre-fortification) and 1999-2003 (post-fortification). Changes in birth prevalence between the two time periods were assessed by calculating odds ratios and 95% confidence intervals for each CA. RESULTS: We found a statistically significant decrease in the prevalence of spina bifida (OR 0.51, 95%; CI 0.36-0.73) and ASDs (OR 0.80, 95%; CI 0.69-0.93). However, between those time periods there were also significant increases in the prevalence of obstructive urinary tract defects (OR 1.45, 95%; CI 1.24-1.70), abdominal wall defects (OR 1.40, 95%; CI 1.04-1.88), particularly gastroschisis (OR 1.91, 95%; CI 1.29 - 2.84) and pyloric stenosis (OR 1.49, 95%; CI 1.18-1.89). CONCLUSION: The results of this study are consistent with the reductions in birth prevalence reported elsewhere for NTDs after fortification of the Canadian grain supply. As previously suggested with multivitamin studies, folic acid fortification may also modestly decrease the risk of ASDs; while increasing the risk of obstructive urinary tract defects. The increase in abdominal wall defects, most notably gastroschisis, is likely related to a pre-existing trend, documented in several regions around the world. The increase in prevalence of pyloric stenosis has not been seen in other studies related to folic acid/multivitamin consumption and perhaps represents a general increasing trend in this CA. Similar studies by other CA surveillance systems are needed to provide further confirmation of these trends.

The GATOR program for association analysis on quantitative traits with and without censoring. *J.C. Curry*[1], *Y.W. Li*[1], *E.R. Martin*[1], *A.S. Allen*[2], *Y.J. Li*[1] 1) Ctr for Human Genetics, Duke Univ Med Ctr; 2) Dept Biostatistics and Bioinformatics, Duke Univ.

Mapping loci contributing to quantitative traits is recognized as an important component in dissecting the etiology of complex diseases. Although many statistical methods for linkage and association studies have been proposed for quantitative traits, most of them require assumption of the distribution of the trait(e.g. normal distribution). Furthermore, censored data can be found in some quantitative phenotypes. For instance, age-at-onset data is censored in the unaffected individuals. Therefore, there is a need to develop a method that utilizes the additional information from censored individuals (e.g. unaffected individuals for studying age-at-onset). We recently proposed a novel family-based association method, genetic association tests based on ranks (GATOR), for analysis of quantitative traits both with and without censoring (Allen et al. 2006). We have examined the power and efficacy of the GATOR method through a serious of simulation studies. We are currently developing a computer program for GATOR method in order to analyze real data. The input files include a file with pedigree and marker information and a phenotype file that contains information on quantitative phenotypes and censoring status for up to 20 traits. This new program is able to perform the GATOR test for four different genetic models: general, dominant, recessive, and additive models. Our current program development plan focuses on biallelic markers. However, it is possible to extend to multi-allelic markers in the future. As described in our previous study, this method can handle parent-offspring (triads), sibships with and without parents, and extended multi-generation pedigree data. In order to accommodate future whole genome association studoes, this program will have the capacity to read in up to 500K markers. We will test the program by both simulated and real data. We will use this program to analyze SNP data generated from an ongoing project to detect genes associated with age-at-onset of Alzheimer and Parkinson diseases. The final GATOR program will be freely distributed from our website.

Initial Un-biased Identification of Genes for Exceptional Longevity in Humans. *G. Atzmon[1], K. Ye[2], N. Barzilai[1]* 1) Institute for Aging Research, Department of Medicine; 2) Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, NY 10461.

We reasoned that the rare selected survivors who became centenarians are enriched with benefactor alleles compared to elderly persons in their 70s. We have previously identified several genotypes that are over presented in this population based on a candidate approach. In order to have an un-biased approach, we reasoned that genome association studies to identify loci that underlie complex traits are feasible and cost-effective as a result of development in technologies. Here we pooled DNA from 250 Ashkenazi Jews centenarians (Age 98.2 +/- 0.5, Mean +/- SE) and 250 unrelated individuals (Age 69.1 +/- 1.4), and subjected these pools to Affymetrix GeneChip Mapping array. We established 5 DNA pools (50 samples each) of centenarians and 5 DNA pools (50 samples each) of control groups. These ten DNA pools were subjected to high throughput genomic genotyping using the STY set of the Affymetrix GeneChip Mapping 500K. There were no significant differences within the 5 pools of centenarians and controls. Comprehensive statistical analysis was performed to detect differences of allele frequency estimates from the pooled DNA. As a reference for significant, the flanking SNPs of the previously shown longevity associated genes (CETP and APOC3) were use. Out of ~240k possible SNPs, 8435 (3.5%) passed the significant threshold. Detected SNPs were distributed evenly on the chromosomes with average of 390 SNPs per chromosome (0.2%) and average distance of 344Kbp. One and a half percent (3686 SNPs) were either different genes or unassigned SNP with average distance of 600Kbp. This broad based approach provide us with loci that can be further validated with individual genotyping, establishing effects of heterozygosis and homozygosis, and use for association studies for prevalence of age-related disease, and with longevity according to genotype. Whether longevity is a polymorphic trait modulated by numerous genes or few genes will become apparent only after such individual assessment.

Whole Genome Scans for HDL-C in French Canadian Families Confirms a QTL on Chromosome 16q. *Z. Dastani[1, 3], M. Lemire[2], J. Faith[2], T. Hudson[1, 2, 5], D. Gaudet[4], M. Marcil[3], J. Genest[3, 4], J. Engert[1, 3]* 1) Human Genetics, Mc Gill University, Montreal, Quebec, Canada; 2) McGill University and Genome Quebec Innovation Centre, Montreal, Canada; 3) Cardiovascular Genetics Laboratory, McGill University Health Centre, Royal Victoria Hospital, Montréal, Canada,; 4) Dyslipidemia, Diabetes and Atherosclerosis Group and Community Genomics Research Center, Université de Montréal and Complexe hospitalier de la Sagamie, Chicoutimi, Canada; 5) Department of Medicine, McGill University, Montreal, Canada.

HDL cholesterol (HDL-C) is a well-known independent risk factor for atherosclerotic vascular disease. To identify genes that contribute to HDL-C levels, we performed a genome-wide scan with 485 microsatellites (an average spacing of ~6 cM) on 362 individuals from 12 large multiplex French Canadian families (QUE) with an average size of 39 individuals. The estimated heritability of HDL-C in these families was 62%. Variance components methods implemented in SOLAR identified several regions of linkage with HDL-C, including a locus on chromosome 16q with a LOD score of 2.30 (P=0.00057) at 95 cM. This linkage was replicated in a cohort of large sibships from the Saguenay-Lac-Saint-Jean (SLSJ) region of Quebec (410 individuals from 61 families). The heritability of HDL-C in this cohort was also high (56%). A LOD score of 2.55 (P=0.00030) was observed at 85 cM (10 cM from the QUE peak). Previous studies on Mexican Americans and Finnish and Dutch cohorts resulted in linkage peaks that are less than 20 cM from the two peaks presented here. 183 SNPs were genotyped in a sub sample of the SLSJ cohort in candidate genes from the region under the linkage peak (29.7 cM; 23 Mb). While several SNPs demonstrated significance ($p<0.05$), none of these survived a correction for multiple testing. Affected members in four families of the QUE cohort share a 2 microsatellite haplotype. In a subset of families with positive LOD scores and visible segregation, 38 genes were selected for sequencing. One gene contained an amino acid change that appeared to segregate in four families, but this variant was also found in unaffected individuals.

Gaucher Disease: Novel Null and Hypomorphic Alleles in Chitotriosidase, a Diagnostic and Therapeutic Marker.
M. E. Grace, M. Balwani, R. J. Desnick Department of Human Genetics, Mount Sinai School of Medicine, New York, NY.

Chitotriosidase (CHITO), a chitinase secreted at high levels by activated macrophages of patients with Gaucher disease (GD), is a useful diagnostic and therapeutic biomarker for this lysosomal disorder. Of the >340 Type 1 GD patients [86% Ashkenazi Jewish (AJ)] screened for CHITO genotype and plasma enzyme levels at the Mount Sinai Comprehensive Gaucher Disease Treatment Center, 36% were found to be carriers of the common CHITO null allele (dup24), with 4% being homozygous. Of note, 5 patients with no plasma CHITO activity were dup24 negative. Sequencing identified three novel CHITO alleles: two missense, G102S and E74K, and one complex (G354R+K385K+c.1168+4delGATT). Expression of the mutant cDNAs in COS-7 cells demonstrated that, relative to the wild type allele, G102S and E74K had ~ 23% and 51% reduced catalytic activity, respectively. Constructs containing the G354R mutation alone or with K385K did not express detectable CHITO activity or protein in cell lysates. In addition, RNA studies indicated that the complex G354R null allele caused significant missplicing. Screening 250 unrelated GD patients for these novel CHITO alleles demonstrated that the E74K allele was rare (1.2%), and found only in AJ patients. The complex G354R allele was found in two patients, both of Dominican descent. Surprisingly, the G102S allele was present in ~31% of our AJ GD patients, including 7 who were homozygous. Recognition of these lesions, particularly G102S, will facilitate the interpretation of disease severity and permit more informed monitoring of disease therapy.

Compound heterozygous mutations in the diastrophic dysplasia sulfate transporter gene (DTDST) in a family with intermediate SEMD phenotype between MED and diastrophic dysplasia. *M. Czarny-Ratajczak[1, 2], K. Kozlowski[3], A. Latos-Bielenska[2], D.J. Prockop[1]* 1) Center for Gene Therapy, Tulane University, New Orleans; 2) Department of Medical Genetics, Poznan University of Medical Sciences, Poznan, Poland; 3) Department of Medical Imaging, New Childrens Hospital, Sydney, Australia.

DTDST mutations cause a spectrum of diastrophic dysplasia disorders characterized by a defect of the proteoglycans sulfation. Reduction of a sulfate transporter activity is manifested by lower sulfate uptake and depends on a combination of mutations in the DTDST gene. DTDST mutations can result in four autosomal recessively inherited chondrodysplasias: two lethal forms, achondrogenesis type 1B and atelosteogenesis type 2, a severe form, diastrophic dysplasia and a mild form, multiple epiphyseal dysplasia.We analyzed a family from the Polish population with an autosomal recessive form of spondyloepimetaphyseal dysplasia (SEMD). Three affected and six unaffected family members were included in the studies. A detailed clinical and radiological examination was performed on all affected family members and most of the unaffected family members. Molecular analysis of the DTDST gene revealed that three affected brothers from this family are compound heterozygotes for C653S/A715V mutations. We classified the phenotype of the patients as a new form of SEMD, characterized by short stature, club foot, brachydactyly and clinodactyly of the fifth fingers. Radiographs showed platyspondyly most marked in the lower thoracic and upper lumbar spine, epiphyseal dysplasia affecting predominantly the femoral heads and widening of the metaphyses. Other distinctive features were prominent trochanters minor and ischial spines, grossly normal carpal/tarsal bones and early onset, severe osteoarthritis. Clinical and radiological analysis revealed that the patients have a new border phenotype between diastrophic dysplasia and multiple epiphyseal dysplasia. Supported by grants from: The Louisiana Gene Therapy Research Consortium and The Polish State Committee for Scientific Research (grant 3P05E00722 to M.C-R.).

Patient and Provider Readiness for Pharmacogenomics: A User Survey. *A. Ginsberg[1], T. Brown[2], P. Billings[1], M. Lai-Goldman[2]* 1) Strategic Planning, Laboratory Corporation of America, Burlington, NC; 2) National Office of Science and Technology, Laboratory Corporation of America, Research Triangle Park, NC.

Adverse drug reactions were the 5th leading cause of death in 2004 in this country, and account for 4% of hospitalizations, and approximately $200B in healthcare expenses each year. Pharmacogenomic tests have been developed to reduce morbidity and mortality related to adverse drug events and improve adherence and compliance with proven therapies. We surveyed physician providers and patients on their readiness to adopt these tests in to current use. We utilized mixed quantitative and qualitative survey strategy using both telephonic and web input. 250 physicians, part of the ePocrates registry, randomly selected, responded to a structured questionnaire on the internet with opportunities for open ended response. They came from a range of specialties and parts of the country. 2,000 patients, representative of the U.S. population, were also contacted as part of GfK NOPs OmniTel phone survey and queried with a similarly constructed interview vehicle. Key questions addressed providers and patients level of interest in predictive laboratory testing to determine drug therapy efficacy, safety, and dosing guidance. The data were analyzed for statistically significant differences as well as qualitative themes. Significant findings from the physician survey include widespread acceptance of pharmacogenetic testing, with reduced interest depending on the use of the test or type of physician: for example, primary care physicians interest in a predictive test used for dosing guidance. Overall, although physicians were interested in the concept, relatively few were familiar with examples of predictive tests. Approximately 88 percent of patients would allow their physician to order a pharmacogenetic test, regardless of its application to efficacy, safety, or dosing. While significant barriers exist to the adoption of pharmacogenomic testing in routine clinical use, our survey suggests that a significant number of patients and providers are now ready to use pharmacogenomic tests if offered in routine clinical settings.

A founder mutation of *MYH* associated to adenomatous polyposis and colorectal cancer in North Africa. *S. Baert-Desurmont[1,2], A. Rouquette[1,2], J. Mauillon[1], E. Bessenay[1], N. Soufir[3], I. Ratbi[4], A. Sefiani[4], H. Chaabouni[5], T. Frebourg[1,2]* 1) Department of Genetics, University Hospital, Rouen, France; 2) Inserm U614, Faculty of Medicine, Rouen, France; 3) Department of Genetics, Bichat-Claude Bernard Hospital, Paris, France; 4) Laboratory of Genetics, Faculty of Medicine, Rabat, Morocco; 5) Department of Congenital and Hereditary diseases, Charles Nicolle Hospital, Tunis, Tunisia.

MYH-associated polyposis (MAP) has been initially characterized as an autosomal recessive disease predisposing to variable number of colorectal adenomas with high risk of degenerescence. Numerous studies have indicated that 2 missense mutations (p.Tyr165Cys in exon 7 and p.Gly382Asp in exon 13) account for about 80 % of *MYH* allelic variants in Caucasians. During the systematic screening of *MYH* in patients with colorectal adenomas and / or cancer, we detected in 3 unrelated patients a homozygous germline mutation c.1186_1187insGG, p.Glu396fsX42, within exon 13. The first patient was a woman presenting at 36 years a colorectal adenocarcinoma associated with 6 adenomas. The second subject presented, at the age of 42 years, 2 synchronous colorectal adenocarcinomas and about 20 adenomas. The third patient, a man of 49 years of age, had approximately 30 colorectal and 2 duodenal adenomas. All these individuals originated from North Africa. Analysis of intragenic SNPs and 6 microsatellite markers revealed a common 1.65 cM haplotype between *D1S451* and *D1S322*, suggesting a founder effect. Screening for the c.1186_1187insGG *MYH* mutation in 150 unrelated healthy individuals from Morocco and Tunisia allowed us to estimate the allelic frequency of this *MYH* mutation to 0.33%. Considering the high level of consanguinity in North Africa, it is likely that *MYH* biallelic mutations may contribute to the remarkable frequency of colorectal cancer observed in young patients. In particular, the presence of the c.1186_1187insGG founder mutation should be considered in young patients originated from North Africa presenting sporadic colorectal cancer even with a limited number of adenomas.

Use of HapMap Data and TagZilla to Design Large Scale Multiplex Assays for Candidiate Gene And Gene Pathway Analyses. *L. Burdett[1], K. Jacobs[1,2], R. Welch[1], M. Beerman[1], B. Staats[1], L. Qi[1], T. Li[1], Z. Wang[1], S. Chanock[3], M. Yeager[1]* 1) SAIC-Frederick, NCI, DCEG, CGF, Gaithersburg, MD; 2) Bioinformed Consulting Services, Gaithersburg, MD; 3) Pediatric Oncology Branch, CCR, NCI, Bethesda, MD.

The National Cancer Institutes (NCI) Core Genotyping Facility (CGF) is using information derived from the HapMap Project with the goal of designing custom Illumina GoldenGate multiplex genotyping panels to maximize the number of candidate genes covered without sacrificing quality of coverage. Illumina GoldenGate design scores are incorporated in a tag SNP selection process using HapMap genotype data in gene regions (20kb upstream and 10kb downstream) of interest. Tag SNPs are selected with the TagZilla software using data on HapMap Caucasians, an r2 = 0.8, and MAF>5%. SNPs that have unacceptable design scores are given the designation obligate-exclude, though they are still used as information in the binning process. Design scores obtained from the submission of dbSNP IDs are compared to locally generated annotated sequences in order to identify and eliminate potential genotyping errors. Selection of alternative tags or re-binning is done as necessary when tag SNPs with low design scores must be removed. Two panels have been designed, one for genes involved in innate immunity and another for genes in pathways associated with Non-Hodgkin Lymphoma. Both are intended to be used on several thousand samples from large cohort or case/control studies. An initial comparison of genotyping results from these two panels indicates that a design score cutoff of 0.6 results in lower genotyping replication error rate (0.3%) and a higher cluster separation (0.82) compared to the panel that used a 0.4 design score cutoff (143 snps with design scores < 0.6). While the Illumina GenTrain scores and call frequency are not significantly different, the recommended data cleaning steps will significantly lower the overall number of usable genotypes. We plan to further validate the effectiveness of our design process examining genotype concordance with HapMap samples. Funded by NCI Contract N01-CO-12400.

Functional centromere in a stable ring 5q13q34 in a child with mental retardation. *M. Artigas-Lopez, S. Moreno, A. Pérez-Juana, A. Alonso, T. Durá, M.A. Ramos Arroyo* Dept Genetics, Hospital Virgen del Camino, Pamplona, Spain.

We report a 14-year-old male with severe mental retardation, spastic tetraparesis, failure to thrive and dysmorphic features. He is the product of the second and uneventful pregnancy of an unrelated couple from Ucrania. Previous history is remarkable because, allegedly, he had had a normal development until 2 years of age. He started to walk at 9 months and started to talk at 11 months. By 18 months of age a decrease in his head growth was observed. By 2 years he presented frequent falls and regression of acquired skills. At 2 and a half years of age he developed seizures and started stereotypic movements of the hands that have presently disappeared. He has never been toilet trained, and he has lost completely his language and communications skills. He is microcephalic, with weight and height below the 3rd centile, he has spastic quadriplegia and he is wheelchair-bound.

Chromosome analysis showed a mitotically stable supernumerary marker chromosome. The karyotype showed a deleted small chromosome 5, in which a segment between bands q13-q34, was missing in all cells studied. In addition, a supernumerary ring chromosome 5 was also evident in all analysed metaphases: 47,XY,-5,+der(5)(pterq12::q35qter),+r(5)(q13.q34).ish der(5)(5p15.2)(D5S23+, D5S721+)(ptel+,qtel+).ish r(5)(5q31)(EGR 1+). In a few proportion of cells the ring chromosome was duplicated, carrying two centromeres. The neocentromere formation has probably been the mechanism for the mitotic stabilisation of the initially acentric ring chromosome. Parental chromosomes were normal

In 1981 Cote et al proposed the term ring syndrome and it has been suggested that the ring syndrome is caused by a continuous generation of secondary aneuploid cells with increased cell mortality, responsible of the clinical picture.

Genome-wide linkage analysis for aggressive prostate cancer in Utah high risk pedigrees. *G.B. Christensen, N.J. Camp, J.M. Farnham, L.A. Cannon-Albright* Biomedical Informatics, University of Utah, Salt Lake City, UT.

It has been proposed that studying alternative phenotypes, such as tumor aggressiveness, may be a solution for overcoming the apparent heterogeneity that has hindered the identification of prostate cancer genes. We present the results of a genome scan for predisposition to aggressive prostate cancer using the Utah high-risk pedigree resource. We identified 259 subjects with aggressive prostate cancer in 57 extended and nuclear families. Subjects were classified as having aggressive disease if any of the following criteria were met: regional or distant stage, poorly differentiated or undifferentiated grade, or death due to metastatic prostate cancer confirmed by death certificate. Parametric and non-parametric multipoint linkage statistics were calculated for a genome-wide set of 401 microsatellite markers using the MCLINK software package. No significant results were observed at the genome-wide level, but suggestive evidence for linkage was observed on chromosomes 9q (HLOD=2.04), 14q (HLOD=2.08), 6p (HLOD=1.75); several pedigrees showed individual evidence for linkage at each locus (LOD > 0.58). Stratification analyses by the number of affected subjects (less than five, five or more) and the average age at diagnosis of affected subjects (less than 70 years, 70 or more years) were also performed. The subset of pedigrees with earlier age at onset demonstrated suggestive linkage evidence on chromosomes 3q (HLOD=1.79), 8q (HLOD=1.67) and 20q (HLOD=1.82). The late onset subset showed suggestive linkage on chromosome 6p (HLOD=2.37) and the subset of pedigrees with fewer than 5 affected subjects showed suggestive linkage on chromosome 10p (HLOD=1.99). The evidence for chromosomes 6p and 20q support previously reported prostate cancer aggressiveness loci.

Mitochondrial Genetic Regulation of Nuclear Transcription. *J.E. Curran[1], M.P. Johnson[1], H.H.H. Göring[1], T.D. Dyer[1], J.B.M. Jowett[2,3], J.W. MacCluer[1], G.R. Collier[3], E.K. Moses[1], J. Blangero[1,3]* 1) SW Fndn Biomedical Research, San Antonio, TX; 2) International Diabetes Institute, Melbourne, AU; 3) ChemGenex Pharmaceuticals, Geelong, AU.

Mitochondrial biogenesis is responsible for up to 20% of total cellular protein production. Therefore, mitochondria likely play a role in regulation of transcription. Both mitochondrial biogenesis and dysfunction are influenced by sequence variation within the mitochondrial genome. In this study, we test whether mitochondrial genetic variation influences the basal transcriptional machinery of the nuclear genome. As part of the San Antonio Family Heart Study, we obtained genome-wide quantitative transcriptional profiles from 1,240 individuals. Using lymphocytes, we quantitated 20,413 transcripts. For each transcript, we tested for linkage to the mitochondrial genome using the variance components method. Given large extended pedigrees, this test is powerful even in the absence of mitochondrial genetic markers. Seven of ten mitochondrial encoded transcripts showed strong evidence ($p<0.0001$) for mitochondrial linkage including *MTC01*, *MTND1* and *MTATP6*, suggesting that mitochondrial variants are involved in mitochondrial biogenesis/function. Examination of nuclear gene transcription revealed that 1,525 transcripts showed nominally significant ($p<0.05$) evidence for regulation by a mitochondrial genetic component. When we utilized a false discovery rate of 0.33 across the whole experiment, we identified 349 transcripts exhibiting evidence for linkage to the mitochondrial genome ($p<0.005$). Of these several exhibited overwhelming evidence that mitochondrial genetic variation influences their expression levels. These genes included *CBX1* ($p=7\times10^{-29}$), *AP2B1* ($p=9\times10^{-22}$), *C16orf20* ($p=4\times10^{-18}$), *PRPH* ($p=2.8\times10^{-12}$) and *OAZ2* ($p=4.5\times10^{-10}$). Given the small size of the mitochondrial genome, direct resequencing of the available mitochondrial lineages should lead to rapid identification of the functional variants responsible for the observed effects on nuclear gene transcription. Our results show the utility of large-scale transcriptional profiling for the identification of major genetic determinants of gene expression.

Genetic analysis of transcriptional profiles for the identification of genes influencing obesity. *J.C. Charlesworth[1], J.E. Curran[1], M.P. Johnson[1], H.H.H. Göring[1], T.D. Dyer[1], A.G. Comuzzie[1], S.A. Cole[1], M.C. Mahaney[1], J.B.M. Jowett[2,3], J.W. MacCluer[1], G.R. Collier[3], E.K. Moses[1], J. Blangero[1,3]* 1) Southwest Foundation for Biomedical Research, San Antonio, TX; 2) International Diabetes Institute, Melbourne, AUS; 3) ChemGenex Pharmaceuticals, Geelong, AUS.

The identification of candidate genes for human quantitative traits is typically based upon subjective knowledge of a biological pathway that is extrapolated to a particular phenotype. In this study, we propose an objective approach to candidate gene discovery that utilizes large-scale transcriptional profiling to identify novel *cis*-acting genes that correlate with a given quantitative trait. Using RNA extracted from lymphocytes, we obtained genome-wide quantitative transcriptional profiles from 1,240 individuals in the San Antonio Family Heart Study. In this data set, we were able to significantly detect ~20,000 transcripts. Using quantitative trait linkage analysis, we identified over 3,000 autosomal *cis*-acting QTLs for which we have significant evidence for variation at the transcripts genomic location that influences expression levels. To identify potential novel candidate genes involved in obesity, we examined correlations between expression levels of these *cis*-acting genes and two obesity-related indicators, the body mass index and fat mass (as measured by bioimpedance). Using high-dimensional endophenotypic search procedures, we identified 383 autosomal genes (many novel) that correlate with these obesity-related phenotypes. Several known candidate genes for obesity, including *IGBP3* and *CDF* (which encodes adipsin) were confirmed. The novel genes identified vary in their general biological actions, from mitochondrial functions to inflammation and growth factors. Because these genes were chosen to have large *cis*-acting effects on transcription levels, it is likely that some of our findings reflect causal relationships with risk of obesity. Our results point to the utility of large scale family-based transcriptional data bases for identifying human quantitative trait loci.

An exonic point mutation in SBCAD is very frequent in the Hmong ethnic group and causes exon 10 skipping by simultaneous ESE disruption and ESS strengthening. *B.S. Andresen[1], P. Madsen[1], V.C. Dung[2], N.T. Liem[2], N.T. Hoan[2], D. Hougaard[3], T.J. Corydon[1], R.J.A. Wanders[4], J.P.N. Ruiter[4], A. Kar[5], J.Y. Wu[5], L. Schroeder[1], N. Gregersen[1]*
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SBCAD deficiency is a defect in isoleucine metabolism. Recently, numerous newborns with SBCADD have been detected by MS/MS-screening in the US. The vast majority belong to the Hmong ethnic group, who originates from South-East Asia. We have previously identified a missense mutation, c.1165A>G, in SBCAD exon 10 in these children. We have now genotyped 2053 blood spots from Vietnam. The c.1165A>G mutation was not prevalent in the general population, but very prevalent in Ha Giang, where the Hmong in Vietnam live (Carrier freq. of 1:9). Thus, in the Hmong ethnic group >1:300 are homozygous, which explains the high number of Hmong newborns identified with SBCADD in the US. However, only very few individuals homozygous for c.1165A>G have presented clinically. To explore this enigma we investigated the molecular pathology of c.1165A>G. Analysis of patient cells showed that despite its location far from the splice sites, c.1165A>G is associated with complete exon 10 skipping (no activity). However, over-expression showed that the encoded M389V protein has high residual enzyme activity. Therefore, even a low level of correctly spliced c.1165A>G SBCAD mRNA may result in sufficient enzyme activity to avoid disease manifestation. Consequently, we used minigenes and in vitro RNA techniques to study c.1165A>Gs effect on splicing in detail. This showed that it simultaneously destroys an exonic splicing enhancer (ESE) and strengthens an exonic splicing silencer (ESS). Combined mutagenesis of the ESE/ESS motifs showed that splicing efficiency is determined by a fine balance between their relative strengths. We speculate if variations in the abundance of the splicing regulatory proteins that bind to the ESE/ESS may influence SBCAD exon 10 splicing efficiency and if this may contribute to an explanation for the clinical phenotype of c.1165A>G homozygotes.

Role of ATM during L1 retrotransposition. *S.L. Gasior, P.L. Deininger* Department of Epidemiology, Tulane Cancer Center, New Orleans, LA.

Mobile elements represent a source of insertional mutagenesis in all genomes studied to date. The human L1 belongs to the non-Long Terminal Repeat class of retrotransposons and is the only known active autonomous element in the human genome. L1 encodes an APE-like endonuclease and a reverse transcriptase that enable the L1 RNA to initiate its integration into the genome, a process termed Target Primed Reverse Transcription (TPRT). TPRT suggests that a double-strand break (DSB) would be a potential intermediate, and we have demonstrated previously that overexpression of L1.3 induces -H2AX foci and COMET tails under neutral conditions. Both are hallmarks of DSBs.

To further understand the role of cellular DNA repair proteins in response to L1 DSB formation and integration, we are characterizing whether ATM is activated and/or required during L1 TPRT. ATM is an important kinase for the repair of DSBs. After expression of L1.3, ATM autophosphorylates and ATM-P localizes on chromatin as foci similar to how ATM responds to ionizing radiation. We have also tested the requirement for ATM in a colony plating assay in which colonies represent a cell with an L1 integration. Treatment of cells with wortmannin or caffeine, two molecules that inhibit ATM kinase, lowers the frequency of colonies. Further analyses with an ATM dominant negative allele and AT-deficient cells suggest L1 integration specifically requires ATM.

These results impart an important new understanding for sources of DNA damage that leads to genetic instability in humans. Because L1 expression is elevated in many cancer cells, it may be a major contributor of DSBs, which in turn promote chromosomal rearrangements. In cells deficient in DSB repair, there may be additional consequences for genetic instability due to L1 activity.

Evidence for a susceptibility gene for type 2 diabetes on chromosome 11. *M.R. Erdos[1], H.M. Stringham[2], P.S. Chines[1], N. Narisu[1], A.U. Jackson[2], W.L. Duren[2], L.L. Bonnycastle[1], C.J. Willer[2], L.J. Scott[2], K.F. Doheny[3], E.W. Pugh[3], N.L. Riebow[1], T.T. Valle[4], R.M. Watanabe[5], T.A. Buchanan[5], R.N. Bergman[5], J. Tuomilehto[4], K.L. Mohlke[6], M. Boehnke[2], F.S. Collins[1]* 1) NHGRI, NIH, Bethesda, MD; 2) U. Michigan, Ann Arbor, MI; 3) CIDR, Johns Hopkins U., Baltimore, MD; 4) National Public Health Institute, Helsinki, Finland; 5) U. Southern California, Los Angeles, CA; 6) U. North Carolina, Chapel Hill, NC.

The FUSION study of the genetics of type 2 diabetes mellitus (T2D) previously performed microsatellite linkage scans of two samples of Finnish affected sib pair families in an attempt to identify the genetic variants conferring susceptibility to T2D. The strongest genome-wide evidence for linkage was found on chromosome 11 at 82.0 cM (LOD = 2.98). Subsequent SNP genotyping on pools of 748 probands, 228 older controls, and 182 unaffected spouses in the 12.4 Mb linkage region (achieving an average SNP density of 33.6 kb) identified a closely spaced cluster of associated SNPs with an estimated maximum allele frequency difference > .10 ($p = 4.6 \times 10^{-5}$). Follow up genotyping of individual cases, spouses, and controls confirmed three SNPs with allele frequency differences between cases and controls > .054 ($p < .001$). An additional 28 SNPs were typed in the same region; 18 of these identified a block of linkage disequilibrium (LD) of approximately 210 kb showing association with T2D. Located within this block is the GRB-2 associated binding protein 2 (*GAB2*) gene, a plausible candidate for T2D. We are now performing genome-wide association (GWA) analysis using the Illumina HumanHap300 panel, which includes 1,459 SNPs genotyped in this chromosome 11 linkage region on 1171 cases and 1186 controls from the FUSION study and the Finrisk 2002 population-based study. Initial analysis of the GWA data on 885 cases and 885 controls has identified 61 SNPs with T2D association p-value < .05 adjusted for testing three genetic models per SNP in the linkage region. Although no SNPs in the *GAB2* gene show $p < .05$, the best T2D association result ($p = 0.0011$) is located 1.9Mb proximal to *GAB2* and four additional SNPs show association ($p < .003$) within 1.0Mb distal to the gene.

Emergence of new primate genes by retrotransposon-mediated sequence transduction. *M.A. Batzer[1], J. Xing[1], H. Wang[1], V.P. Belancio[2], R. Cordaux[1], P.L. Deininger[2]* 1) Department of Biological Sciences, Biological Computation and Visualization Center, Center for BioModular Multi-Scale Systems, Louisiana State University, 202 Life Sciences Building, Baton Rouge, LA; 2) Tulane Cancer Center SL-66, Department of Environmental Health Sciences, Tulane University Health Sciences Center, New Orleans, LA.

Gene duplication is one of the most important mechanisms for creating new genes and generating genomic novelty. Retrotransposon-mediated sequence transduction (i.e. the process by which a retrotransposon carries flanking sequence during its mobilization) has been proposed as a gene duplication mechanism. L1 exon shuffling potential has been reported in cell culture assays and two potential L1-mediated exon shuffling events have been previously identified in the genome. SVA is the youngest retrotransposon family in primates and is capable of 3 flanking sequence transduction during retrotransposition. In this study, we examined all the full-length SVA elements in the human genome to assess the frequency and impact of SVA-mediated 3 sequence transduction. Our results showed that ~53 kb of genomic sequences have been duplicated by 143 different SVA-mediated transduction events. In particular, we identified one group of SVA elements that duplicated the entire AMAC gene three times in the human genome via SVA-mediated transduction events which happened before the divergence of humans and African great apes. In addition to the original AMAC gene, the three transduced AMAC copies contain intact open reading frames in the human genome and at least two are actively transcribed in different human tissues. The duplication of entire genes and the creation of new gene families via retrotransposon-mediated sequence transduction represent an important mechanism by which mobile elements impact their host genomes.

Comprehensive Genomic Analysis of Unilateral Retinoblastoma Tumors: Old Disease, New Clues. *A. Ganguly[1], C. MacMullen[1], L. Swanson[1], S. Diskin[2], G. Grant[2], E. Rappaport[3], G. Bunin[4], C. Shields[5], A. Meadows[4], K. Nichols[4]*
1) Department of Genetics, University of Pennsylvania, Philadelphia, PA; 2) Department of Bioinformatics university of Pennsylvania; 3) Department of Pediatric Oncology Childrens Hospital of Philadelphia; 4) NAPCORE, CHOP; 5) Wills Eye Hospital, Philadelphia.

Background Retinoblastoma (RB) is a childhood onset ocular tumor associated with compromised vision and death in many cases. Inactivation of both copies of the RB1 gene in a retinal cell is followed by additional genetic changes leading to tumor formation. It is known that inactivations of p53, p107/p130 in addition to pRb proteins are required to initiate RB formation in mice. However, in human RB tumors, TP53 mutations have not been observed. **Methods** Whole genome SNP based assays were performed on DNA isolated from a set of 25 sporadic, unilateral RB tumors and matched blood samples using Affymetrix 10K SNP-Chips. Gene expression analysis was performed using Affymetrix U133 V2.0 chips on a subset of 18 RB tumors to detect correlations with genomic amplification/loss. **Results and Discussion** The results confirmed previous observations and identified 2q33-37, 3p21, 3q25, 11q14, 11q23, 14q22-23, 15q26.3 and 20q13.12, as novel regions of amplification. In addition,1q21.1, 1q32.2 and 1q44, were identified as minimal regions of gain. RB tumor clusters were defined by presence/absence of loss of heterozygosity (LOH) of chromosome 13. The LOH in most cases was due to copy neutral events caused by mitotic recombination or non-disjunction. No correlation to age, gender or mutation at RB1 was observed. However, the gene expression profiles of tumors were variable. In particular, expression of genes present on 1q32 resulted in two clusters of RB tumors defined by ages of onset. Higher expression of MDM4(1q32) was observed among other genes. This observation is significant as MDM4 in conjunction with MDM2 have unique roles in modulating the level of p53. We will discuss the implications of this finding for novel therapeutic options targeting the MDM4/MDM2 molecules.

Genetic Variants in the Farnesyl-Diphosphate Farnesyltransferase Gene (FDFT1) Region is Associated with Susceptibility to Coronary Heart Disease. *R. Do[1], S.D. Bailey[1], A. Montpetit[3], G. Pare[1,3], K. Desbiens[2], J. Faith[3], T.J. Hudson[1,2,3], D. Gaudet[4], J.C. Engert[1,2]* 1) Department of Human Genetics, McGill University, Montréal, PQ; 2) Department of Medicine, McGill University, Montréal, PQ; 3) McGill University Genome Québec Innovation Centre, McGill University, Montréal, PQ; 4) Université de Montréal, Chicoutimi, PQ.

Research has shown that genetic variants may predispose to various cardiovascular diseases. Founder effect populations are useful tools in linkage disequilibrium mapping due to reduced genetic heterogeneity. The Saguenay-Lac St. Jean region of Quebec is a known founder population that is currently being used to map complex traits of genetic disease. Recently, we have performed a genome-wide linkage scan on a Saguenay-Lac St. Jean cohort and have identified a susceptibility loci for coronary heart disease on Chromosome 8 (non-parametric linkage score = 3.53). Within this region, the squalene synthase (farnesyl-diphosphate farnesyltransferase (FDFT1)) gene is a strong candidate. Squalene synthase catalyzes the first committed step leading to cholesterol biosynthesis in the isoprene biosynthetic pathway. It is believed that levels of squalene synthase may correspond to the intracellular production of cholesterol and therefore, plasma cholesterol levels. We have identified SNPs in the exons and intron-exon boundaries of the FDFT1 gene through sequencing of a small subset of our study population and these SNPs were then genotyped in all individuals. Additional genotype data has been generated for 14 SNPs in the FDFT1 region. In a family-based association test, a silent SNP in exon 1 and a SNP in intron 1 of the FDFT1 gene were significantly associated with CHD ($p<0.02$)) in 24 families. In a sliding window haplotype analysis approach, the strongest associations between haplotypes and CHD were observed between exons 1 and 3 in the FDFT1 region ($p<0.004$). These results suggest that genetic variation in the FDFT1 region may be associated with increased susceptibility to CHD and one of the identified SNPs may either be the causative variant or may be in linkage disequilibrium with the causative variant.

Noninvasive screening for aneuploidy also identifies infants at risk for common malformations:Results from the FASTER Trial. *D.W. Bianchi[1], L.M. Sullivan[2], B. MacKinnon[1], J.D. Hoffman[1], J. Collins[1], F.D. Malone[3], M.E. D'Alton[3], FASTER Trial Research Consortium* 1) Tufts-New England Medical Ctr, Boston, MA; 2) DM-Stat, Inc., Malden, MA; 3) Dept Ob-Gyn, Columbia Univ Sch of Med, NY, NY.

The FASTER trial compared 1st and 2nd trimester screening methods for aneuploidy (NEJM 353:2001-11). Research coordinators also recorded pediatric outcome information (36,837 cases, 97% ascertainment). Here we examined relationships between serum and ultrasound (US) markers (AFP, estriol [uE3], human chorionic gonadotropin [hCG], inhibin A, nuchal translucency [NT]) and pediatric anomalies. All cases with a positive serum or NT screening result as well as a 10% random subject sample underwent medical record review. Unaffected controls were selected for each anomaly, matched by enrollment site, maternal age, race, gestational age, and infant gender. Serum and US markers were dichotomized to either or 2 multiples of the median (MoM), or 0.5 or 0.5 MoM. Conditional logistic regression analysis assessed the relationship between each marker and outcome. Only odds ratios (OR)2 are reported here. The most common anomalies (incidence per 10,000 live births)were hydrocele (34.1),undescended testis(12.4), 2-vessel cord (11.8), pyloric stenosis (5.4), and multicystic dysplastic kidney(3.7). Women with inhibin A 2 MoM had significantly increased risk of multicystic dysplastic kidney (OR=27.5) and 2-vessel cord (OR=4.22). Inhibin A 0.5 MoM carried an increased risk of pyloric stenosis (OR=7.88). Women with free hCG 2 MoM had increased risk of pyloric stenosis (OR=2.31) and multicystic dysplastic kidney (OR=2.73). hCG level 2 MoM had increased risk of multicystic dysplastic kidney (OR=19.56) and hydrocoele (OR=2.48). uE3 2 MoM had increased risk of undescended testis (OR=3.64); uE3 0.5 MoM had increased risk of 2-vessel cord (OR=7.78). In this large prospective study statistically significant associations were found between routine maternal screening markers and pediatric anomalies. This suggests that there may be previously unrealized benefits to screening programs that are primarily designed to detect aneuploidy.

Identifying the major genetic contribution of complex diseases using pooling-based genome-wide SNP association studies. *D. Craig, J. Pearson, M. Huentelman, R. Halperin, W. Tembe, V. Zisman, K. Coon, J. Webster, E. Reiman, D. Stephan* Dept Neurogenomics, TGen, Phoenix, AZ.

Genome-wide association (GWA) studies using hundreds of thousands of single nucleotide polymorphism (SNPs) have the potential to revolutionize our ability to identify the genetic cause to complex traits. Unfortunately, these studies are inaccessible to most researchers, and indeed, for many complex disorders, due to the exorbitant costs of genotyping hundreds to thousands of cases and controls. We have developed an analysis software program called GenePool to conduct analysis of pooling-based GWA studies. We report the development of experimental methods, study designs, and analysis tools for completing pooling-based GWA studies on various high-throughput SNP genotyping platforms. We describe the implementation of single marker and multi-marker statistics, and demonstrate their ability to resolve association signals. We apply these methods to case-control population populations and demonstrate successful identification of the correct genetic susceptibly locus for a monogenic disease, a rare complex disease, and a common complex disease.

BDNF Val66Met polymorphism in Bipolar Disorder: Genomic Imprinting or Neuronal Miswiring? *V. De Luca, J. Strauss, D.J. Müller, M. Semeralul, A. Wong, J.L. Kennedy* Dept Neurogenetics, Univ Toronto, Toronto, ON, Canada.

Background We reported the Val66Met and the GT(n) repeat polymorphisms of the BDNF gene to be associated with bipolar disorder (BD). However, nobody has investigated the possibility of genomic imprinting in BDNF gene in conferring risk for BD. Aims To investigate genomic imprinting in the BDNF gene for association with BD we investigate the Parent of Origin Effect (POE) and differential allele expression in BD. Method We performed a family-based association study and ETDT analyses with 312 nuclear families including the Val66Met polymorphism. Results Maternal and paternal transmission analysis were both significant (p=0.003 and p=0.006 respectively). The effect size for the Val66 allele was higher in the maternal transmissions (RR=2.1) suggesting the presence of the genomic imprinting. Conclusions This data suggests the possibility of genomic imprinting in BDNF gene, on the other hand the differential allelic expression in post-mortem brains and lymphoblast cells can be conclusive to confirm the genomic imprinting or support the hypothesis that different BDNF messenger RNA transcripts can be localized at different subcellular locations in cortical neurons of bipolar patients.

Identification and characterization of a dynactin associated protein (Dynapsin) involved in late-onset Alzheimer disease (AD). *J.R. Gilbert[1], S. Zuchner[1], C.A. Browning[1], G.F. Wang[1], C.R. Lu[1], P.G. Bronson[1], C.F. Potocky[1], S.M. Garvey[1], C.C. Kroner[1], J.R. Gibson[1], J.M. van der Walt[1], Y.J. Li[1], P.T. Xu[1], D.E. Schmechel[1], W.K. Scott[1], J.M. Vance[1], J.L. Haines[2], E.R. Martin[1], M.A. Pericak-Vance[1]* 1) Duke University, Durham, NC; 2) Vanderbilty University, Nashville, TN.

To date four genes, PS1, PS2, APP and APOE have been shown to be involved in the etiology of AD. Using ordered subset analysis (OSA) we established linkage (MAXLOD=3.8) on chromosome 2q33.3 in 31 multiplex AD families with >1 individual with age at onset of AD between 50-60 years. To prioritize candidate genes in the linkage region, we used genomic convergence integrating our linkage data with SAGE expression analysis derived from AD hippocampus. One novel gene, dynapsin was identified. Genotyping a dense array of SNPs across the linkage region further supported the involvement of dynapsin in AD with significant evidence for association in multiple SNPs (minimum p=0.008) in the gene. The association was strengthened when the sample size was increased to a total of 73 AD pedigrees meeting the OSA criteria for chromosome 2 (minimum p=0.003). Little is known about dynapsin or its functional role in the CNS. We identified up to 8 different cDNA isoforms and analyzed their relative expression levels in AD and control brains. Isoform 7 was more highly expressed and variable in AD brains versus controls. In AD brain sections, dynapsin is found in neurons and importantly, in AD neuritic plaques. We also demonstrated that dynapsin immunoprecipitates with components of the dynein-dynactin motor complex and was localized to cytoplasmic vesicles in a cell culture of Neuro2A cells. We hypothesize that dynapsin is a newly identified linker protein between vesicles and the dynein-dynactin complex and is involved in endosomal trafficking. Axonal transport and synaptic integrity are key elements of the proposed pathways for AD. Our combined genetic, molecular and functional results strongly suggest a role for dynapsin in the etiology of AD and that variation in dynapsin is responsible for at least some of the risk of developing AD in these families.

The African genetic contribution to the Brazilian genetic pool. *C.M.B. Carvalho[1, 2], V.F. Gonçalves[1], H.J. Bandelt[3], S.P. Bydlowski[4], M.C. Bortolini[5], S.D.J. Pena[1]* 1) Dept Biochemistry, UFMG, Brazil; 2) Dept. of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 3) University of Hamburg, German; 4) Dept. of Hematology, FMUSP; 5) Dept. of Genetics, UFRGS, Brazil.

From 1550 to 1870 approximately four million Africans were brought to Brazil as slaves. Through intense and prolonged interbreeding with Amerindians and Europeans they were an important component in the formation of Brazilian people. We have been studying the details of this process with lineage markers. We have previously shown that while White Brazilians displaying about equal proportions of African, Amerindian and European mtDNA lineages, the vast majority of them carry Y-chromosomes of European origin. This suggested the occurrence of a sexually asymmetric genetic admixture. We have now studied in detail the maternal and paternal lineages from 120 Black males from the city of Sao Paulo, Brazil. Y-chromosome typing was performed hierarchically using biallelic markers. Only 48% of the individuals presented Y-chromosome haplogroups characteristic of sub-Sahara Africans (E1, E2, E3a*, E3a4, E3a7 and B). Sub-lineages E3a* and E3a7 represented 88.3% of the African patrilineages, suggesting a primary Central African origin. Matrilineages were assessed by sequencing of the control region of mtDNA and RFLP typing of the coding region. Eighty five different haplotypes were seen (99.1% diversity). Sub-Saharan African haplotypes were seen in 85% of cases and their careful mapping in Africa again suggested that, consistently with the historical record, Brazilian slaves were predominantly Bantu-speakers from Central-Africa, with a secondary West-African component. The fact that most of the matrilineages, but only 48% of the patrilineages of Brazilian Blacks were from Africa confirms our previous observation of a sexual bias in the formation of the Brazilian people, with a predominant European paternal component and with a majority of matrilineages originating from Amerindian and African women.

Epigenetic factors influence segregation of human artificial chromosomes in cultured mouse cells. *A. Breman, R. Slee, C. Steiner, B. Grimes* Med & Molecular Genetics, IUSM, Indianapolis, IN.

Human artificial chromosomes (ACs) form following transfection of alpha-satellite DNA into human cultured cells. In order to evaluate the potential of human ACs in gene therapy, it will be necessary to incorporate genes into ACs and to test their function in animal models. Towards this goal, we have constructed a human AC (FIXAC) that has assembled a 33kb factor IX (FIX) gene. Factor IX mutations cause Hemophilia B, an inherited bleeding disorder. FIXAC was generated by co-transfection of a 165 kb PAC with an insert spanning the FIX gene and a 99 kb BAC harboring alpha-satellite DNA into human HT1080 cells. FIXAC was detected in most chromosome spreads in 9/21 cell lines and segregated efficiently under non-selective conditions. As a measure of the potential of FIXAC to segregate in mouse cells, FIXAC was transferred into immortal mouse L-A9 cells. In recipient line L1, FIXAC was maintained as an extra chromosome and assembled the mouse centromere protein, cenp-c. However, FIXAC exhibited reduced mitotic stability in L1 cells when cultured without selection (loss rate 2.58%/division). It was hypothesized that only a subset of FIXACs had acquired all necessary epigenetic modifications for mitotic stability, resulting in a high aggregate FIXAC loss rate in L1 cells. To test this possibility, L1 cells were plated at low density to form single colonies. By Southern analysis, the alpha-satellite segments on FIXAC were similar in structure in L1 and in 5 sub-clonal lines, indicating that FIXAC is structurally stable. FIXAC mitotic stability was variable in sub-clonal lines, ranging from 0.67%/division (corresponding to FIXAC retention in 72% of cells after 30 days off selection) up to 2.27%/division. This suggests that epigenetic factors influence FIXAC segregation efficiencies in mouse L-A9 cells. We anticipate that FIXAC will segregate efficiently in primary cells and animals which, unlike L-A9 cells, do not exhibit genomic instability. In future studies we will test the capacity of FIXAC to rescue factor IX deficiency in the mouse.

Complex management of a patient with a contiguous Xp11.4 gene deletion involving the ornithine transcarbamylase gene. *M. Deardorff[1], P. Kaplan[1], J. Ganesh[1], C. Ficicioglu[1], R. Deberardinis[1], M. Yudkoff[1], T. Markello[2], B. Loechelt[3], U. Lichter-Konecki[2]* 1) Section of Metabolic Diseases, The Childrens Hospital of Philadelphia, Philadelphia, PA; 2) Division of Genetics and Metabolism and; 3) Division of Immunology, The Children's National Medical Center, Washington, DC.

A male infant was diagnosed prenatally with a partial Ornithine Transcarbamylase (OTC) deletion secondary to family history. Following an uncomplicated pregnancy, labor and Cesarean section delivery, treatment with protein restriction, appropriate calories and ammonia scavenger drugs was begun within 10 minutes of birth. Postnatal OTC gene analysis confirmed deletion of exons 1-8. He displayed neurological abnormalities despite effective management of serum ammonia (average 34 umol/L, range 1.5-125; lab normals 9-33). He also had hypoalbuminemia (2.1 g/dL) from birth and developed pleural effusions, ascites, and anasarca. Due to features not solely explained by OTC deficiency, further deletion analysis was pursued. PCR analysis of seven genes from Xp11.4-p21.1 (cen-OTC, RPGR, SYTL5, CYBB, PRRG1 and TMEM47, dystrophin-tel) showed OTC exons 9 and 10, and dystrophin exon 3, were present. PCR confirmed the absence of OTC exons 1-8, and the other five genes. RPGR mutations cause retinitis pigmentosa with recurrent respiratory infections (RP3), features reported in the mother. Defects in CYBB cause an X-linked chronic granulomatous disease (CGD), which was clinically proven. SYTL5, PRRG1 and TMEM47 have not been linked to human diseases. Between CYBB and PRRG1 lies the XK (Kell antigen) gene. Defects in XK are the cause of McLeod syndrome, an X-linked disorder with late onset neuromuscular, hematopoietic abnormalities and transfusion complications. CGH array analysis confirmed presence of the dystrophin gene and absence of three anonymous probes in this region. This case illustrates the complex challenges in a contiguous gene syndrome including treatment of a urea cycle disorder (UCD) plus CGD, and potentially RP3 and McLeod syndrome. It also shows the value of detailed evaluations in seemingly isolated gene deletions.

Yeast NDI1 gene improve oxidative phosphorylation capacity and increases protection against oxidative stress in cells carrying a Lebers Hereditary Optic Neuropathy mutation. *Y. Bai, J. Park, Y. Li* Cellular & Structural Biol, Univ Texas Health & Sci Ctr, San Antonio, TX.

Lebers hereditary optic neuropathy (LHON) is a maternally transmitted disease characterized by bilateral loss of central vision with degeneration of retinal ganglion cells. A transition mutation, G to A, at mitochondrial nucleotide 11778 in the ND4 gene of NADH-ubiquinone oxidoreductase is the most common primary mutation found in LHON patients, accounting for more than 50% of LHON cases. The NDI1 gene, which encodes the rotenone-insensitive internal NADH -quinone oxidoreductase in Saccharomyces cerevisiae, was introduced into the nuclear genome of a human cell line, Le1.3.1, carrying the 11778 mutation. Two transformants. LeNDI1-1 and 2 cells were chosen for detail analysis. In these cells, total and complex I-dependent respiration, were largely resistant to complex I inhibitor, rotenone, indicating a dominant role of NDI1 in transfer of electrons from NADH to ubiquinone in the host cells. Whereas the original mutant Le1.3.1 cell grows poorly in medium containing galactose instead of glucose, the LeNDI1-1 and 2 cells have a full restored growth capacity in galactose medium, although the ATP production was not totally recovered. Furthermore, the increased oxidative stress in the cells carrying the 11778 mutation was alleviated in LeNDI1-1 and 2 cells, demonstrated by a decreased reactive oxygen species (ROS) level. Finally, LeNDI1-1 and 2 were also shown to be desensitized to induction to apoptosis, and also exhibited greater resistance to paraquat-induced cell death. It is concluded that the yeast ND11 enzyme can improve the oxidative phosphorylation capacity in cells carrying the 11778 mutation, and it can also protect the mutant cells from oxidative stress and cell death.

Real Time PCR Analysis of Parkin Exon Copy Number Variation in Large a PD and Aged Control Population. *J. Deng, J. Bunch, J. Grimsley, GM. Mayhew, YJ. Li, J. Connelly, SG. Gregory, MA. Hauser, WK. Scott, JM. Vance* Ctr Human Genetics, Duke Univ, Durham, NC.

Parkin is one of the most significant genes identified in which mutations lead to both early- and late-onset forms of Parkinsons Disease (PD). The genomic region in chromosome 6 where Parkin is located is rich in long and short repetitive sequences, which indicates a high level of genomic instability, and is known to have minimal linkage disequilibrium. Various forms of Parkin mutations have been identified in PD patients including point mutations and chromosome aberrations. But few large scale screening studies for PD patients has been made including copy-number evaluations of the Parkin gene, and even less in the control population. Thus, to evaluate exon copy number variation and its potential contribution to the risk and susceptibility to PD, we developed a screening method based on ABI real-time PCR technique. We validated the real-time PCR results using known Parkin exon heterozygous deletions, and XY chromosome mismatches , and utilized other assistant techniques such as denature high performance liquid chromatography (dHPLC) and comparative genomic hybridization (CGH) to evaluate the patients. We screened Parkin exons in over 900 PD patients and 200 unaffected, neurologically-examined older controls, providing a large database to evaluate the occurrence of these changes. We found that real-time PCR correlates well with those of other methods, and is suitable for detection of large genomic deletions in a large sample set. Our preliminary results indicate that there is a higher frequency of exon duplication or deletion in the Parkin gene than that of other regions of the genome in both PD patients and non-PD controls. Further analysis of possible association of the Parkin exon copy number variation with PD and interaction with other susceptibility genes is currently underway.

Clinical and Genetic Phenotype of a Cohort of 34 patients with Micro-Anophthalmia. *P. Bitoun[1], C. Edelson[2], B. Benzacken[3], E. Semina[4], L. Benzacken[5], J.C. Murray[6], J. Gaudelus[7]* 1) Gen Medicine, CHU Paris-Nord, Hopital Jean Verdier, Bondy, France; 2) Fondation Ophtalmologique Rothschild, Ophtalmopédiatrie, Paris; 3) Embryo-Cytogénétique et Biologie de la Reproduction,Hosp Jean Verdier, Bondy; 4) Molecular Genetics, Medical College of Wisconsin, Milwaukee; 5) Hopital Robert Ballanger, Ophtalmologie ,Aulnay s/b 93; 6) Pediatrics & Biological Sciences , University of Iowa, Iowa City; 7) Pédiatrie, Hopital Jean Verdier, Bondy.

Purpose : to study the clinical phenotype and genetic causes of a cohort of 34 consecutive patients with congenital micro/anophthalmia (MA) Material and Methods: Patients where assessed by a pediatric ophthalmologist and an ophthalmic geneticist to define the ocular and extra ocular dysmorphogy phenotype. Brain MRI , karyotype, telomeric screen and CGH array were performed in some patients. Gene mutation analysis were performed by PCR and sequencing and are still ongoing for several genes including PITX2, PITX3, OTX2, SOX2, BCOR, ND, NHS, LRP5, SIX6 and PAX6. Results: The cohort was divided into primary and secondary MA (post infectious, post-surgical, post retinal detachment or post-phthisis) The cohort is comprised of 34 patients 2 months to 38 years (average 7.9 y), 10 (29%) females and 24 (61%) males. 25 patients (74%) had primary MA and 9 (26%) had secondary MA. Primary MA had 36% female while the Secondary had 11% females . Blindness affected 15/25 (60%) patients with primary MA versus 3/9 (33%) with secondary MA. Patients were classified as ocular alone in 10/25 (40%) of primary MA versus 7/9 (78%) with secondary MA or syndromic in 60% of primary MA versus 22% of the secondary MA patients. MA was unilateral in 3/25 (12%) of primary MA and in 5/9 (55%) of secondary MA patients. 2/25 patients with primary MA died and none of the 9 patients with secondary MA. Genetic evaluation showed 11 patients had a familial case (66%) of secondary MA and 20% of Primary MA. Discussion: The excess of males and the paucity of X linked genes involved with primary MA seems to imply that most of the X linked genes involved with MA are still to be uncovered.

X chromosome investigations in X-linked infantile spinal muscular atrophy (*XL-SMA*): New observations and new strategies. *L. Baumbach[1], J. Ramser[2], M.E. Ahearn[1], K.O. Yariz[1], C. Lenski[2], M.M. Barmada[3], A. Meindl[2]* 1) Miller Sch Med, Univ. Miami, Miami, FL; 2) Gynaekologische Tumorgenetik Ismaningerstr, Munchen, Germany; 3) Univ. Pittsburg Grad Sch Public Health, Pittsburg, PA.

We previously reported an X-linked form of infantile lethal motor neuron disease (MIM 30021), which clinically mimics Werdnig-Hoffman disease, with additional features of early onset or congenital contractures/fractures, in a single family that mapped to Xp11.3-q11.2. We have identified 14 unrelated families. Major and minor clinical features have been defined. Seven families have been tested for X-chromosome linkage; each maps to the same region as the first family. Multipoint linkage has narrowed the disease gene interval to between DXS8080-DXS7132. These results strongly support the existence of a major disease locus in this region. Our studies in the past two years have focused in four areas: 1)further narrowing the candidate gene interval using microsatellites and SNPs; 2)mutation screening of all known cDNAs in the region, 3)searching for small chromosomal duplications or deletions using comparative genomic hybridization arrays; and 4)X-inactivation analysis. CGH studies have not detected a chromosome alteration; however, one *XL-SMA* family evidences strongly skewed X-inactivation of the X chromosome containing the presumed disease gene. We are now focusing on new avenues of investigation for gene discovery. SNP analysis using the Affymetrix SNP chip in selected *XL-SMA* families; Fiber FISH to rule out a large pericentromeric inversion; and further investigation of the skewed X-inactivation family. We are also screening for mutations or alterations in expression of microRNA sequences encoded within the region. Lastly, we are beginning to examine SMN and gemin protein/mRNA levels in cell lines from *XL-SMA*, SMA and control subjects, to determine if the SMN/gemin pathway is implicated in *XL-SMA*. We have demonstrated strong evidence for an X-linked form of infantile SMA with a single disease locus. Our data suggest that *XL-SMA* may not be as rare as previously assumed, and patients negative for SMN mutations might be affected by *XL-SMA*.

Broad Institute Genetic Analysis Platform Utilizing Illumina BeadLab Technology. *S. Gupta, J. Moore, A. Camargo, M. Hagar, N. Beattie, Y. Hoshida, M. Nizzari, T.R. Golub, S.B. Gabriel* Genetic Analysis Platform, Broad Institute , Cambridge, MA.

High throughput, high quality, flexible and affordable genotyping platforms are needed to accomplish wide-ranging goals of todays genetic studies. As a national center for genotyping the Broad Institute has integrated three different platforms for SNP genotyping, each suited to different study designs. Genome-wide analysis is carried out on the Affymetrix and Illumina platforms; targeted SNP genotyping for large numbers of selected SNPs is conducted on the Illumina platform (hundreds-thousands of SNPs) or Sequenom platform (fewer than hundreds of SNPs). Here we present our experience specifically with the Illumina platform for a diverse set of genotyping projects. In total we have designed in excess of 100,000 custom assays for SNP genotyping, as part of the Hap Map and a range of disease mapping projects. Assay conversion rates on average exceed 90%. We have implemented rigorous DNA sample quality control measures from sample intake and observe a sample failure rate less than 0.5%. In addition, the oligo-ligation based genotyping assay has been extended to analyze gene expression, specifically targeting formalin-fixed paraffin-embedded (FFPE) tissue samples that have not yielded to standard microarray analysis. Because the assay targets short segments of DNA degraded samples, degradation in FFPE samples can be surmounted. We have developed a panel for 6,000 cancer related genes and demonstrated that it can be used for sample clustering and marker gene analysis on samples over 20 years old. Finally, early experience with the new genome-wide genotyping products shows excellent data quality with high accuracy (>99.5%) and call rates exceeding 99%.

Fetal acrania associated with cardiac defects. *D. Albu, E. Severin, C. Albu* Dept Human Genetics, Carol Davila Univ Med & Pharm, Bucharest, Romania.

Background: Acrania is a lethal cranial abnormality characterized by complete absence of the skull, with complete but abnormal development of the cerebral hemispheres. It can occur isolated or associated with other fetal abnormalities.**Objectives:**To specify the role of 3D ultrasonography as a screening tool for the early prenatal detection of acrania; to describe and analyze the severity of congenital malformations associated with acrania. **Patient:**A 24-year-old pregnant Caucasian female was referred at 37 weeks gestation for prenatal ultrasound diagnosis. The patient was send to our department for evaluation of fetal abnormalities.The patient and her husband were not genetically related and had a normal general health. The family had a low socioeconomic status.**Methods:**: The patient was examined with Color Doppler and 3D system. Fetal evaluation was made by ultrasound scans for fetal growth, congenital malformations, and amniotic fluid volume. Triple test (AFP,uE3, hCG) was used too. We also collected information about family medical history. Amniotic fluid samples were taken to perform prenatal cytogenetic diagnosis.**Results:**The patients obstetrics history revealed one previous miscarriage in the second trimester (unknown causes).Her second pregnancy was evaluated late, at the 37th gestation week. Ultrasonography revealed a singleton pregnancy with growth deficiency, absence of the skull bones, development abnormalities of cerebral hemispheres, dilatation of ventricles, absent cerebellum, cardiac defects, truncus arteriosus communis, anomalies of the aortic arch, abnormal abdominal wall and skeletal defects.Lymphocytes from both parents blood were analyzed for chromosomal abnormalities and showed normal karyotype for the father (46, XY) and chromosome instability for the mother. The maternal serum tests were positive. Fetal chromosomal analysis showed a deletion of 11q and chromosome instability.**Conclusions:**acrania was correctly identified at the first scan; prenatal diagnosis was confirmed postnatally;genetic counseling and early evaluation based on both 3D ultrasonography and cytogenetic analysis should be considered for the next pregnancy.

A set of putative functional IDE and PLAU variants shows significant, replicable association with Alzheimers Disease. *M. Carrasquillo[1], S. Younkin[1], M. Kashino[1], S. Wilcox[1], T. Dincman[1], L. Younkin[1], L. Ma[1], F. Zou[1], N. Taner[1,2], M. Allen[1], R. Petersen[2], N. Graff-Radford[1], S. Younkin[1]* 1) Mayo Clinic, Jacksonville, FL; 2) Mayo Clinic, Rochester, MN.

In IDE and PLAU, which are excellent positional and functional candidate genes for late onset Alzheimers Disease (LOAD), conserved regions (>70% human vs. mouse) were screened for variants likely to be functional. Using logistic regression, conserved IDE variants were tested for association with AD in three large case/control series. Six conserved variants in non-coding regions formed 4 haplotypes with frequencies over 1%: three were protective, one was risky. In the young age group where association was strongest, these 4 haplotypes showed the same significant association with AD in the exploratory (nominal global p = 0.010) and two follow-up series (global p = 0.013 and 0.007). The three protective haplotypes (H2, H3, H4) pair to form 6 genotypes likely to be protective. These were used as a referent group and compared to the four remaining common genotypes (H1/H1, H1/H2, H1/H3, H1/H4). Three genotypes were equivalently risky, one (H1/H4) was indistinguishable from the referent group, and they showed the same significant association with AD in the exploratory (nominal global p = 0.005) and two follow up series (global p = 0.002 and 0.004). Compared to the set of 7 protective genotypes (16%), the combined set of 3 risky IDE genotypes (84%) had ORs of 4.1, 4.3, and 2.6 in the three series (p = 0.0004, 0.0005, and 0.0015). In the combined series, the OR was 3.2 (2.1-5.0) for the set of risky genotypes compared to the set of 7 protective genotypes with a p-value of 6.5x10-8 and a PAR of 65% (48% - 77%). Importantly, we observed a significant 1.5-fold elevation of IDE mRNA in the cerebella of 140 subjects with protective genotypes as compared to 22 subjects with risky genotypes (p=0.005). These results indicate that IDE is likely to be an important LOAD gene with variants that influence risk of AD by altering IDE expression. Similar analysis of variants in conserved regions of PLAU showed that multivariate PLAU haplotypes and genotypes also show significant association with LOAD.

Isolated renal transplantation in an adult with cobalamin-responsive methylmalonic acidemia. *A. Gropman[1], K. O'Brien[2], J. Sloan[3], C.P. Venditti[3]* 1) Dept Pediatrics, Georgetown Univ Medical Ctr, Washington, DC; 2) MGB, NHGRI, NIH, Bethesda, MD; 3) GDRB, NHGRI, NIH, Bethesda, MD.

Solid organ transplantation has been used as a therapeutic modality in methymalonic acidemia but the nature and indication for transplantation as well as the number of transplant recipients is unknown. We describe a 33 yr old female with vitamin B12-responsive methylmalonic acidemia who underwent kidney transplantation for renal failure. The patient presented in the first year of life with a poorly defined renal tubular acidosis syndrome and had intermittent decompensations during the first few years of life before the diagnosis of was established at age 8. Her renal function declined over the next decade and she progressed to needing hemodialysis at the age of 24. At age 31, she received a cadaveric renal allograft and has been stable from a renal perspective since that time. She had intermittent compliance with her medical regimen - which consisted of IM OH-cobalamin and carnitine - after transplantation but has maintained a low protein diet of ~0.6 g/kd/d of whole protein. She had not received cobalamin for more than one-year prior to her visit. Evaluation at our center demonstrated plasma MMA levels in the 135-223 uM range (normal= <0.4 uM) with a urinary MMA concentration of the 223 mg/g/Cr (normal=<3 mg/g/Cr). The amino acid profiles showed elevated glycine, alanine, glutamine, and proline suggestive of inadequate metabolic control. The plasma B12 level was in the normal range at 288 pg/ml ([normal range: 220-960 pg/ml]). Cobalamin-responsiveness was determined by injecting 2 mg OH-cobalamin IM twice over a two-day period, waiting one day and reassessing metabolic parameters. After injection, plasma B12 levels rose to >200,000 pg/ml. Metabolic studies revealed a 10-fold drop in the plasma (19 uM) and urinary MMA concentrations (18 mg/g/Cr). We conclude that cobalamin therapy and dietary management are still required after solid organ transplantation, even in patients who have milder MMA syndromes, and that renal transplantation by itself does not normalize metabolism in B-12 responsive patients.

Development of A Coordinated Approach to Experimental Design and Analysis. *K.P. Clancy[1], M. Duan[2], J. Yen[2], F. Liang[2]* 1) Bioinformatic Sciences, Invitrogen Corp, Frederick, MD; 2) Bioinformatic Sciences, Invitrogen Corp, Carlsbad CA.

With modern genomics, scientists now examine groups or families of genes rather single genes, and need a more coordinated approach to experimental design. Software and databases that manage the relationships between biological data, reagent applications and resultant data, and protocols facilitates experimental planning and helps scientists coordinate performancing and reporting more complex experimental methodologies. We developed a Matched Reagents database, by linking NCBI RefSeq genes and gene products to associated designed reagents. Methods to detect ongoing changes to RefSeq records that affect pre-designed reagents were incorporated to manage the transient nature of this data. Genes were associated with GeneGO data to incorporate pathway data and other public data, including dbSNP, OMIM and others were included. A web tool, iPath, was developed to browse and evaluate the pathways and genes in this database for biological context and applicable technologies. Genes and experimental reagents were then coordinated with experimental technologies using more than a quarter of the protocols from the Wiley Current Protocols series and the entire Invitrogen product protocol collection. Protocols were parsed as a series of XML documents and entities that corresponded to experimental reagents were identified, marked up, and updated in the Matched Reagents database in a vendor neutral fashion. Bioinformatic tools needed for design of reagents were identified and incorporated. iProtocol, a second web based tool, was developed to permit the browsing and identification of protocols and associated reagents. This is a generalizable approach to coordinating the activities and reagents of groups of laboratories. The database has increased our efficiency for targeting genes with appropriate ranges of technologies. This database currently coordinates 65,000 genes with > 430,000 individual Invitrogen products and > 1,500,000 competitor products. Such a database approach can form the basis of a more complex system for automated or semi automated experimental design softwares.

Chromosome Rings/Markers as a Model to Determine Rearrangement Mechanisms. *C. Fitzpatrick[1], L. Christ[2], C. Crowe[3], M. Graf[4], S. Schwartz[1]* 1) University of Chicago; 2) Case Western Reserve University; 3) Metro Heath Medical Ctr, Cleveland OH; 4) TGen, Pheonix AZ.

The underlying mechanisms of chromosome rearrangements and how they translate to disease phenotypes have recently begun to be discovered. Genomic architecture can predispose a chromosomal region to rearrangement and can be an important tool in understanding how such rearrangements occur. From a collaborative study of marker chromosomes, we have ascertained 172 marker/ring chromosomes as well as 17 constitutional ring chromosomes (i.e. the abnormal complement of one normal chromosome). The pattern of chromosomal involvement of accessory rings shows no specific chromosomal predominance. However, the pattern identified among constitutional rings shows a clear over-representation of chromosomes 9, 18, and all acrocentric chromosomes. Because few studies have been reported that examine the structure of either type of ring chromosome, we have expanded our initial analysis on a subgroup of the marker/rings to include breakpoint analysis using BACs, fosmids and real time PCR in an attempt to better understand the mechanism of formation. Analysis of three constitutional rings derived from chromosome 9 has revealed that all breakpoints involved are unique with the exception of two out of three rings having an intact subtelomeric region. Likewise, analyses of three marker rings derived from chromosome 17 also contain different breakpoints, all of which were facilitated by low copy repeats (LCRs). This study demonstrates several important findings regarding ring and marker chromosomes: (1) There are specific chromosome preferences in constitutional ring chromosomes, due either to an associated phenotype or the inherent chromosome structure (2) both types of ring chromosomes exhibit breakpoint heterogeneity and (3) the vast majority of breakpoints appear to be facilitated by at least one repetitive region of DNA: LCRs and pericentromeric repeats in the case of chromosome 17, subtelomeric regions in chromosome 9 and the acrocentric short arm regions. These results suggest that the underlying mechanism involved may be more complex than the classic model of homologous recombination.

Identification of a gene expression signature and an increased plasticity in periosteal fibroblasts from Apert syndrome patients. *R. Fanganiello[1], A.L. Sertie[1], N. Oliveira[1], E.M. Reis[2], E. Yeh[1], D. Bueno[1], N. Alonso[4], S. Cavalheiro[3], I. Kerkis[5], S. Verjovski-Almeida[2], M.R. Passos-Bueno[1]* 1) CEGH-IBUSP, Brazil; 2) IQ-USP, Brazil; 3) UNIFESP, Brazil; 4) FM-USP, Brazil; 5) Inst. Butanta, Brazil.

FGF-FGFR signaling is critical in skeletal growth and development. One of the most severe forms of craniosynostosis is Apert syndrome (AS), caused by mutations in *FGFR2*. The signaling pathways activated by FGFR2 are responsible for the processes of cell growth and differentiation at the sutural margins and alterations in these pathways can lead to the premature fusion of these margins. To better understand the signaling pathways and transcription factor networks unchained by FGFR2 activation we investigated, through oligonucleotide microarray technology (Codelink system), the gene expression of the coronal suture periosteum fibroblasts of seven AS patients (S252W mutation) and compared them to those of seven wild type cultured periosteal fibroblasts. We found changes (1.8 fold difference) in the expression profile of 271 genes with a SNR|0.4| and pvalue0.05. Among this group, 63 and 17 genes were found to be diferentially expressed in at least 50% and 100%, respectively, of the 14 groups of genes generated using a leave-one-out statistical procedure, with a SNR|0.75|. In accordance to these data, 6 genes were found differentially expressed between control and mutant cells in quantitative Real Time PCR experiments. This data were also confirmed by an independent experiment where primary cultured control periosteal fibroblasts were treated with high concentration of FGF2, used to simulate the phosphorylation status of mutant *FGFR2*. Furthermore the periosteum cells of one AS patient and one control were submitted to bone differentiation protocols, and surprisingly, only the AS cells were able to differentiate into osteoblasts. This data lead to the identification of an expression profile signature involved in the molecular mechanisms of AS. In addition, our results suggest that the *FGFR2* mutation lead to an increased cell plasticity. CEPID / FAPESP; CNPq robertofanganiello@yahoo.com.

Population screening for fragile X syndrome and sex chromosome aneuploidies by quantification of methylated FMR1 DNA. *B. Coffee[1], I. Albizua[1], M. Friez[2], R. Stevenson[2], S.T. Warren[1]* 1) Department of Human Genetics, Emory University, Atlanta, GA; 2) Greenwood Genetics Center, Greenwood, SC.

The vast majority of fragile X syndrome is due to aberrant changes in chromatin structure, as a consequence of CGG repeat expansion, that silences expression of the FMR1 gene. One of the epigenetic changes concomitant with CGG repeat expansion is DNA methylation. We have developed a rapid high-throughput quantitative methylation sensitive PCR method to assess FMR1 methylation in DNA from dried blood spots to screen for fragile X syndrome. In addition, this screen will detect sex chromosome aneuploidies when there is a deviation from the expected FMR1 DNA methylation pattern. Detection of methylated FMR1 DNA in males would be consistent with either fragile X, Klinefelter Syndrome (or its variants), or 46,XX males. In females, absence of methylated FMR1 DNA would be consistent with Turner Syndrome or 46,XY females. An increase in FMR1 DNA methylation would be consistent with either fragile X, 47,XXX and its variants. The Q-MSP method is very sensitive and easily detects low levels of methylated FMR1 DNA. The sensitivity of the assay allows for the detection of mosaic fragile X and Klinefelter Syndromes. In addition, the sensitivity allows for the pooling of male samples into large groups, permitting the simultaneous screening of up to one hundred males in a single experiment. To determine if Q-MSP can be used to screen for fragile X syndrome in females, we screened a cohort of full mutation carrier females. Greater than 80% had a FMR1 DNA methylation index greater than 2 SD above the mean normal FMR1 methylation index. In summary, the Q-MSP method can be used for population screening for fragile X syndrome and sex chromosome aneuploidies in a single assay by detection and quantification of methylated FMR1 DNA.

A Novel Multivariate Method for the Analysis of Psychometric and Genotype Data. *T.A. Greenwood[1,2], L.M. Evans[1], S.G. Liang[1], J.R. Kelsoe[1], N.J. Schork[1,2]* 1) Department of Psychiatry, University of California, San Diego, La Jolla, CA; 2) Center for Human Genetics and Genomics, University of California, San Diego, La Jolla, CA.

Novel statistical methods are needed to model the complex interaction of personality and genetics. Traditional strategies that condense psychological exam or psychometric data (e.g., translating items on a questionnaire into global scales) do not take full advantage of the data. We describe a method for testing the relationship between variation in a similarity matrix constructed from items on a psychometric exam and ancillary information, such as genotypes, collected on a sample of individuals. This procedure does not rely on data reduction strategies, such as factor or cluster analysis. This method is also the perfect companion for heat map and tree-based representations of the item data as organized by some feature or grouping factor (e.g., genotypes) meant to reveal the relationship between the items. We have applied this method to a sample of bipolar subjects that have genotypic data for 160 SNPs within a 2.8Mb region on chromosome 22. These subjects have also completed the Cloninger Temperament and Character Inventory (TCI) and Akiskals TEMPS-A. The TCI distinguishes four dimensions of temperament (harm avoidance, novelty seeking, reward dependence, and persistence) and three dimensions of character (self-directedness, cooperativeness, and self-transcendence). The TEMPS-A assesses four types of temperament, hyperthymia, dysthymia, cyclothymia, and irritability, and includes a measure of anxiety. Using this technique, we have identified a number of associations. We also compare our method with other methods.

Hierarchical Modeling in Linkage Disequilibrium Mapping. *G. Chen, E. Jorgenson, J. Witte* Institute for Human Genetics, UCSF, San Francisco, CA.

The completion of the HapMap project and advances in high-throughput genotyping methods has made feasible genome-wide association (GWA) studies. Such studies generally evaluate the relationship between hundreds of thousands of single nucleotide polymorphisms (SNPs) and one or more phenotypes. A critical unresolved issue in GWAs is how to analyze the enormous amount of information generated in a manner that is most likely to detect causal variants. The conventional analysis approach entails estimating the association between each SNP (or multiple SNPs) and a phenotype, and then using the corresponding p-values to prioritize the results. This approach, however, ignores existing information about the SNPs, can lead to spurious results as well as suffer from low power. To address these issues, we propose here a hierarchical model: adding a prior model that incorporates known information about the SNPs into a conventional analysis. In particular, we develop a hierarchical model that uses the following information on the SNPs: 1) potential functionality; 2) whether non-synonymous; 3) evolutionary conservation; 4) previously associated or in linkage region; 5) LD with neighboring SNPs. We show empirically how integrating this information in a hierarchical model may improve the ability of GWAs to determine the location of causal variants.

Translocation of neo(20qter->q13.2) to 22qter detected in prenatal diagnosis samples. *R.E. Falk[1], D.L. Van Dyke[2], R.G. Meyer[2], R.A. Mota[2], D. Krakow[1], R.R. Schreck[1]* 1) Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA; 2) Cytogenetics Laboratory, Mayo Clinic, Rochester, MN.

A healthy 40-year old G2P1 woman presented for CVS at 11+4 weeks of gestation. Cytogenetic analysis of cultured villi showed mosaicism with additional material at distal 22q [specifically, add(22)(q13)] in seven of 22 cells. FISH with a probe set for the TUPLE1 and ARSA sequences showed normal number and orientation of the signals. A whole chromosome 22 painting probe, which was used to investigate the possibility of an inverted duplication of chromosome 22 versus origin from of the extra material from a different chromosome, failed to hybridize to the add(22)(q13) segment. The couple was counseled regarding the high likelihood of confined placental mosaicism. However, evaluation of amniocytes confirmed mosaicism with the add(22)(q13) in 15 of 18 colonies. Multicolor-FISH (M-FISH) showed hybridization of the wcp20 to the add(22)(q13) region. Confirmation studies with subtelomeric FISH probes for chromosomes 20 and 22 showed a normal signal pattern on each chromosome 20 and on the normal chromosome 22. The add(22) showed a 22qter signal proximal to the added segment, a 20qter signal immediately distal to the 22q subtelomeric probe signal and a second 20qter signal at the terminal end of the add(22) segment. Correlation of the FISH and G-banding patterns suggested that the additional material on chromosome 22 represented an inverted duplication of the region 20q13.2 to 20qter. Hence, the abnormal cells had four copies (partial tetrasomy) of distal 20q. Fetal ultrasound assessment showed a flat midface, absent nasal bone, abnormal ear shape/position, possible mild tricuspid regurgitation, echogenic bowel, generally decreased muscle tone, clenched hands and mild unilateral equinovarus foot position. Limited fetal evaluation after pregnancy termination confirmed strikingly dysmorphic facies and abnormal foot position. Tetrasomy of distal 20q is extremely rare. One possible etiologic mechanism of this abnormality is formation of a neochromosome 20q that subsequently attached to distal 22q.

Comprehensive linkage disequilibrium mapping of schizophrenia candidate genes in a large European-ancestry sample. *J. Duan[1], M. Martinez[2], A.R. Sanders[1], G. Burrell[1], C. Hou[1], D. He[1], D. Schwartz[1], N.G. Buccola[3], B.J. Mowry[4], R. Freedman[5], F. Amin[6], D.W. Black[7], J.M. Silverman[8], W.F. Byerley[9], R.R. Crowe[7], C.R. Cloninger[10], D.F. Levinson[11], P.V. Gejman[1]* 1) ENH & Northwestern Univ, Evanston, IL; 2) INSERM, Toulouse, France; 3) LSU Health Sciences Center, New Orleans, LA; 4) QCSR and University of Queensland, Brisbane, Australia; 5) Univ. of Colorado Health Sciences Center , Denver, CO; 6) Atlanta VA Med Ctr & Emory Univ, Atlanta, GA; 7) Univ. of Iowa, Iowa City, IA; 8) Mt. Sinai School of Medicine, New York, NY; 9) UCSF, San Francisco, CA; 10) Washington University, St. Louis, MO; 11) Stanford Univ, Palo Alto, CA.

Linkage, association and cytogenetic data have nominated plausible schizophrenia (SZ) candidate genes, but replication studies have produced mixed results, and there is no definitive evidence for a DNA sequence with an identifiable pathogenic mechanism. We have studied 14 SZ candidate genes (AKT1, ARVCF, CHRNA7, COMT, DAOA, DISC1, DRD2, DTNBP1, HTR2A, NRG1, PPP3CC, RGS4, and the STX7-TAAR6 gene cluster) in a European-ancestry sample of 1,673 cases with final diagnoses of SZ or schizoaffective disorder and 2,146 controls (screened by self-report to exclude those with possible psychotic or bipolar disorders). We used SNPlex and Taqman methods to genotype comprehensive maps of each gene (800 SNPs in 2.2 Mb of sequence) consisting of a framework of HapMap SNPs to tag common variation and additional SNPs that had been reported as associated to SZ, or are missense or within functional elements; and 187 ancestry-informative markers (AIMs) for continental populations, analyzed in relation to self-reported ancestry. We achieved high call rates and low error rates. Analysis of AIMs demonstrated minimal case-control allele frequency differences. We have analyzed 118 SNPs in ARVCF, COMT, DRD2 (Ser311Cys), HTR2A, NRG1 (core SNP haplotype), and TAAR6. We observed nominal p-values <0.05 for SNPs located in HTR2A, NRG1 and TAAR6. Interpretation of the findings will require completion of analyses of the remaining genes and of empirical gene- and experiment-wise p-values, as well as further analyses of possible population substructure.

GALNT3 mutations cause familial tumoral calcinosis by decreasing intact FGF23. *H.J. Garringer[1], H. Boztepe[3], R. Tankakol[3], S.M.J. Mortazavi[2], F. Esteghamat[2], M. Malekpour[2], K.E. White[1]* 1) Med & Mol Gen, IUSM, Indianapolis, IN; 2) Imam Khomeini Univ Hosp, Tehran, Iran; 3) Istanbul Univ, Istanbul, Turkey.

Phosphate homeostasis, required for normal bone development and maintenance, is dependent upon complex endocrine interactions. Disruption of these regulatory pathways disturbs serum phosphate levels, and leads to disorders such as familial tumoral calcinosis (TC). TC is characterized by high serum phosphate concentrations, severe ectopic calcifications, and results from mutations in either fibroblast growth factor-23 (FGF23) or GalNac transferase-3 (GALNT3). FGF23 is critical in the regulation of renal reabsorption of phosphate, however the molecular pathogenesis of TC is unknown. TC patients with FGF23 mutations have low/low-normal serum levels of biologically active FGF23 when analyzed with an ELISA specific for full length FGF23 protein. In contrast, these patients have markedly elevated serum levels when assessed with an ELISA that recognizes intact FGF23 as well as C-terminal fragments. GALNT3 initiates O-glycosylation of proteins within the Golgi. Mature FGF23 requires O-glycosylation, thus, we hypothesized that mutations in GALNT3 cause TC via decreasing serum levels of active FGF23 protein. Herein we identified novel TC mutations in GALNT3: a nonsense mutation (W487X), and a frameshift mutation (S368fsX375) that result in severely truncated GALNT3 protein products. To determine the molecular etiology of TC in these GALNT3-null patients, we analyzed serum levels of FGF23. In this regard, mean serum intact levels of FGF23 were low-normal whereas the C-terminal FGF23 concentrations were 20 fold the normal mean, thus the serum FGF23 profile of these patients with GALNT3 mutations is similar to the serum profile of TC patients with FGF23 mutations. In summary, our findings demonstrate that decreased serum levels of intact, active FGF23 protein lead to the TC phenotype, and the inability to produce sufficient amounts of biologically active FGF23 is a result of loss of function mutations in either FGF23 or in GALNT3. Thus, due to improper processing of FGF23 in GALNT3-TC patients, we conclude that GALNT3 has a critical regulatory role on FGF23 glycosylation and production.

A Parkinson's Disease Mutation genotyping Array for the parkin Gene. *L. Clark[1], E. Haamer[2], H. Mejia-Santana[3], J. Harris[4], S. Lesage[5], A. Durr[5], S. Janin[5], K. Hedrich[6], E.D. Louis[3,4], L.J. Cote[3,4], H. Andrews[4], C. Waters[4], B. Ford[4], S. Frucht[4], W. Scott[7], C. Klein[7], A. Brice[5,8], H. Roomere[2], R. Ottman[9], K. Marder[3,4]* 1) Dept Pathology & Taub Inst, Columbia Univ, New York, NY; 2) Asper Biotech, Tartu, Estonia; 3) Gertrude H. Sergievsky Center, Columbia Univ, New York, NY; 4) College of Physicians and Surgeons, Columbia Univ, New York, NY; 5) INSERM U679, Neurology and Experimental Therapeutics; 6) Department of Neurology, University of Lübeck, Lübeck, Germany; 7) Duke University Medical Center, Durham, NC; 8) Hôpital de la Pitié-Salpêtrière, AP-HP, Paris; 9) NYS Psychiatric Institute, Columbia Univ, New York, NY.

Parkin mutations account for the majority of familial and sporadic early-onset Parkinson's Disease (EOPD) cases with a known genetic association. In both cases, >100 mutations have been described in the parkin gene, many of which are recurrent and have been observed in different ethnic groups. We have designed a parkin genotyping array (PGA) that includes published parkin sequence variants and allows their simultaneous detection. The PGA (gene chip) was constructed by arrayed primer extension technology (APEX). A total of 132 DNA samples, 85 PD cases and 47 controls, previously screened for parkin mutations were used to validate the parkin array. We tested the sensitivity and specificity of the parkin genotyping array as a mutation screening method for the detection of 65 different mutations. Investigators at AsperBiotech performed analysis blind to the mutation status of subjects. Excluding whole exon deletions and duplications, the array detected all disease-associated alleles in PD cases and all parkin SNPs that were included on the chip. We envision several potential uses for the chip. 1) In mutation screening of the parkin gene when used in combination with DHPLC and semi-quantitative PCR to ensure detection of all mutations and 2) As a mutation screening tool for recurrent mutations and risk alleles in epidemiological studies. In summary, the genotyping array is an efficient screening tool for detection of known mutations and may be developed to simultaneously capture genetic information in several genes at once.

Acute in vivo exposition to curcumin and copper by means of comet assay. *A. Corona-Rivera[1,4], P. Urbina-Cano[1], L. Bobadilla-Morales[1], M.A. Ramírez-Herrera[2], M.L. Mendoza-Magaña[2], P. Díaz-Ezquivel[3], R. Troyo-Sanromán[1], J.R. Corona-Rivera[1]* 1) Laboratorio de Citogenética y Genotoxicidad, Instituto de genética Humana Dr. ECR, Dep. Biol. Mol. and Genomics, CUCS, University of Guadalajara. Guadalajara, Jalisco, Mexico; 2) Laboratorio de Neurofisiología, Dep.de Fisiología, CUCS, University of Guadalajara. Guadalajara, Jalisco, Mexico; 3) Bioterio, Dep.de Fisiología, CUCS, University of Guadalajara. Guadalajara, Jalisco, Mexico; 4) Unidad de Citogenética, OPD, Hospital Civil de Guadalajara.

Curcumin is a phytochemical extracted from Curcuma longa L, with anti-inflammatory, antioxidant and anti-carcinogenic properties. In vivo studies, indicate that curcumin is not genotoxic and even genoprotective, mainly due to its antioxidant activity. Curcumin had also showed a pro-oxidant effect in the presence of copper causing DNA breaks. The aim of the present study was to study the effect of in vivo exposition to curcumin in the presence of increasing concentrations of copper on DNA by means of comet assay. Eight groups with six BALB/c mice randomly distributed were set under the following administration scheme: control no-treated group, copper 65ppm, copper 130ppm, copper 390ppm, curcumin 0.2%, curcumin 0.2%+copper 65ppm, curcumin 0.2% +130ppm, curcumin + 390ppm. Blood samples were obtained after 48hr and processed to perform the comet assay. We used whole blood on two layers of agarose per microgel and dehydration to be storaged until observation and propidium iodide stained. Tail length was measured in m from fifty nucleoids in triplicate. The in vivo exposition to curcumin for 48 hr did not cause genomic damage. Copper 390ppm was genotoxic. DNA damage increased (163%) for copper 390ppm exposition. While curcumin in the presence of copper 390ppm showed a decrease of DNA damage. It was also evident that curcumin did not induced DNA damage alone or in combination with copper. We agreed with other studies that the differences observed for in vivo exposition is related with complexes interactions, that decreases the blood and tissue concentration until excretion. Curcumin protected against DNA damage induced by copper 390ppm, showing a genoprotector effect.

Inhibition of proteasome and SGG/GSK3 kinase prevents clearance of phosphorylated tau in *Drosophila*. *O. Blard, T. Frebourg, D. Campion, M. Lecourtois* Inserm U614, Faculty of Medicine, Rouen, France.

Tauopathies, including Alzheimers disease (AD), are a group of neurodegenerative disorders characterized by the presence of intraneuronal filamentous inclusions of abnormally phosphorylated tau protein. In AD brains, it has been shown that the level of abnormally phosphorylated tau is higher than in age-matched control brains, suggesting that abnormally phosphorylated tau is resistant to degradation. Using a *Drosophila* model of tauopathy, we studied the relationship between tau phosphorylation and degradation. We showed that *in vivo* reduction of proteasome activity results in an accumulation of high molecular weight forms of hyperphosphorylated tau. We also found that GSK3-mediated hyperphosphorylated forms of tau are degradable by the proteasomal machinery. Unexpectedly, GSK3 inactivation resulted into a massive accumulation of high molecular weight species consisting of hyperphosphorylated tau. This results suggests that, depending on the kinase(s) involved, tau phosphorylation state affects its degradation differently. We thus propose a model for tauopathies whereby, depending on toxic challenges (e.g. oxidative stress, exposure to amyloid peptide, etc.), abnormal phosphorylation of tau by kinases distinct from GSK3 leads to progressive accumulation of hyperphosphorylated tau oligomers that are resistant to degradation.

Investigating *HOX* genes as autism susceptibility loci using an integrative approach of comparative genomics and genetic analysis. *N. Gharani, R.A. Zimmerman, B.J. Smith, S. Murray, J. Raincrow, C-H. Chiu* Dept Genetics, Rutgers Univ, Piscataway, NJ.

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder with a complex genetic basis. Homeodomain proteins are key regulators of early embryonic development and some members play a critical role in the specification of discrete regions of the central nervous system. We previously demonstrated that the homeodomain transcription factor engrailed 2 (*EN2*) is associated with ASD. The aim of this study was to investigate members of the *HOX* homeodomain gene family, encoded within the four *HOX* gene clusters (*HOXA-HOXD*), as ASD susceptibility loci using association methods and 167 families (n=753) from the autism genetic resource exchange (AGRE). Each of the 4 *HOX* gene clusters, including 5 and 3 flanking sequences cover >300kb of DNA and contain many hundreds of single nucleotide polymorphisms (SNPs). We have taken an integrative approach that combines comparative genomics with linkage disequilibrium (LD) map data to select and prioritize a subset of informative tagSNPs for further analysis. Here we illustrate this approach and present data for the analysis of the *HOXA* cluster on chromosome 7p15. Comparative genomics and the criterion of evolutionary conservation were previously used to identify Phylogenic Footprint Clusters (PFCs) inferred to be putative *HOXA* cis-regulatory sequences (Chiu et al 2002 PNAS 99:5492-5497). Here we used the dbSNP resource to identified 17 potentially functional SNPs within PFCs, intronic, or protein coding sequences. Next we used empirical HapMap SNP data together with the Tagger program to select a subset of 42 informative tagSNPs based on pairwise SNP LD data (r20.8) and inclusive of the 17 potential functional SNPs. 20 SNPs have thus far been genotyped in the AGRE families and analyzed for evidence of association with ASD using the pedigree association program PDTPHASE 2.404. Nominal significant association has been obtained for 2 SNPs both individually and as a haplotype (*rs1801085* P=0.041; *rs10486494* P=0.013; haplotype P=0.0045). These preliminary data suggest that variation in specific *HOXA* genes may play a role in the etiology of ASD.

Fragile X prenatal analyses show full mutation females at increased risk for mosaic Turner syndrome: Loss of maternal X? *C. Dobkin, G. Radu, X. Ding, W.T. Brown, S.L. Nolin* Dept Human Genetics, NYS Inst Basic Research, Staten Island, NY.

Analysis of 475 prenatal samples for fragile X revealed 5 with 45,X/46,XX mosaicism for Turner syndrome among 86 females that carried the full mutation (>200 CGG repeats). In two cases 50% of the cells were 45,X and in the other three 14%. None of the other 160 female fetuses was found to be mosaic for the X chromosome. This highly significant association of Turner mosaicism and the fragile X full mutation is much more common than expected. Isolated cases of mosaic Turner syndrome in full mutation females have been reported previously but an increased prevalence was not apparent from these reports (Shapiro et al., AJMG 51:507, 1994; Tejada et al., J Med Genet 31:76, 1994; Wilkin et al., Prenat Diagn 20:851, 2000).

We were interested to determine whether the maternal chromosome with the full mutation or the paternal chromosome was lost. Analysis of polymorphisms identified the parental origin of the lost chromosome in 3 of the 5 cases. Interestingly analysis of the two cases with 50% 45,X showed that the maternal X had been lost. Since approximately 75% of Turner syndrome cases show a loss of the paternal X, our results suggest that the presence of the fragile X mutation on the maternal chromosome may result in its loss.

A new genetic isolate with a unique phenotype of syndromic oculocutaneous albinism: clinical, molecular and cellular characteristics. *A. Blumenfeld[1], N. Schreyer-Shafir[1], M. Huizing[2], Y. Anikster[3], Z. Nusinker[1], I. Bejarano-Achache[1], G. Maftzir[1], L. Resnik[1], A. Helip-Wooley[2], W. Westbroek[2], L. Gradstein[4], A. Rosenmann[1]* 1) Ophthalmology Dept., Hadassah - Hebrew Univ. Hospital, Jerusalem, Israel; 2) National Human Genome Research Institute, NIH, Bethesda, MD; 3) Metabolic Disease Unit, Safra Children Hospital, Sheba Medical Center, Tel Hashomer, Israel; 4) Ophthalmology Dept., Soroka Medical Center, Beer-Sheva, Israel.

An extended, highly consanguineous Israeli Bedouin family with at least 20 individuals exhibiting a unique phenotype of oculocutaneous albinism (OCA) was identified. All known OCA genes were excluded in this family. Electron microscopic analysis of platelets revealed absence of dense bodies, suggesting a diagnosis of Hermansky-Pudlak syndrome (HPS). HPS is a rare autosomal recessive disorder of lysosome-related organelle biogenesis, clinically characterized by OCA and platelet dysfunction, sometimes accompanied by other systemic pathologies. All human HPS genes (*HPS1-8*) and 5 genes corresponding to murine HPS models were evaluated. Haplotype analysis and homozygosity mapping of the *HPS* loci revealed linkage to chromosome 10 in the studied family. Subsequently, a novel insertion mutation, c.1066-1067insG was identified in *HPS6*. Most frameshift mutations generating premature termination codon cause mRNA nonsense mediated decay (NMD), while intronless genes like *HPS6* are usually not monitored by NMD. Expression analysis revealed no mRNA decay in patient's fibroblasts, hence truncated protein is most probably produced. Confocal microscopy revealed abnormal distribution of LAMP-3 (lysosomal associated membrane protein-3) in fibroblasts from the patients, indicating abnormal trafficking of lysosomal lineage organelles. So far, a single HPS-6 patient phenotypically similar to HPS-3 and HPS-5 has been identified. The HPS-6 phenotype in the studied family is unique since it resembles OCA and not HPS. Therefore, our finding broadens the phenotypic definition of HPS. Two major genetic isolates of HPS-1 and HPS-3 patients were previously diagnosed in Puerto-Rico. The extended Bedouin family is the largest isolate of non-Puerto-Rican HPS patients.

Skewed X-inactivation and X-linked recessive lethal infantile spinal muscular atrophy (*XL-SMA*) as co-inherited traits in a Spanish pedigree: possible clues to disease gene identification. *M.E. Ahearn[1], F. Martinez[2], J. Tarleton[3], J. Laursen[3], K.O. Yariz[1], Y.S. Fan[1], H. Zhu[1], J. Ramser[4], A. Meindl[4], L. Baumbach[1]* 1) Miller Sch Med, Univ. Miami, Miami, FL; 2) Instituto se Biomedicina, Valencia, Spain; 3) Fullerton Genetics Ctr. Lab, Asheville, NC; 4) Gynaekologische Tumorgenetik Ismaningerstr, Munchen, Germany.

Our group has previously described *XL-SMA* syndrome (MIM 30021) and our efforts in disease gene identification. Consistent with other X-linked recessive disorders, only *XL-SMA* males families are affected; obligate carrier females are asymptomatic. Recent cytogenetic and molecular analyses of one *XL-SMA* Family 3 (from Spain) have provided possible new clues. Clinical features of Family 3 *XL-SMA* males include those common to all affected males (AMs); severe congenital hypotonia, congenital contractures of all extremities, muscle biopsy with neurogenic atrophy, and neonatal/infant death from respiratory insufficiency. Family 3 AMs display dysmorphic facies seen in other *XL-SMA* males, but evidence additional dysmorphic findings and clinical features not described in other *XL-SMA* families. Their disease course is more severe with death within the first 24-72 hrs postpartum and represents the most severe disease phenotype. Linkage studies using microsatellites and SNPs have demonstrated that the family links to the current XL-SMA diseasse region (Xp11.3-q11.2). Due to severity of disease presentation, X-inactivation patterns were analyzed in Family 3 using standard androgen receptor methylation assays. These studies were independently performed in two laboratories using identical patient materials. Carrier females and selected non-affected males were tested. Each carrier female showed skewed X-inactivation, with ratios ranging from 86:14 to 90:10. Based on constructed haplotypes, skewed inactivation occurs of the at-risk X chromosome. In hopes of detecting a microdeletion, we are analyzing this family with Array CGH and high density SNP mapping. Skewed X-inactivation has been observed in selected other X-linked disorders, however, families with stably transmitted X-inactivation are rare, and may provide important clues to gene discovery.

Human Cholestryl Ester Transfer Protein (TaqIB) Polymorphism among Filipinos with Cardiovascular Risk Factors. *E.C. Cutiongco[1], R.S. Santos[2], F.R. Punzalan[2], F.B. Geronimo[2], R.V. Tangco[2], R.G. Sy[2]* 1) Institute of Human Genetics, National Institutes of Health Manila, Philippines; 2) Lipid Research Unit, University of the Philippines-Philippine General Hospital.

BACKGROUND:HDL-C has emerged as an important independent predictor of cardiovascular disease. The FNRI and NNHes Study Group in the Philippines reports a high prevalence of low HDL among Filipinos. Most cases of low HDL-C are associated with secondary causes like Metabolic Syndrome. A primary cause of reduced HDL-C such as increased Cholesteryl Ester Transfer Protein activity has been identified.. OBJECTIVES: 1. To determine the phenotype and frequency of Cholesteryl Ester Transfer Protein (TaqIB) polymorphism among Filipinos with cardiovascular risk factors. 2. To determine the association of TaqIB polymorphism with HDL-C levels among Filipinos with cardiovascular risk factors. DESIGN: Cross-sectional Study METHODOLOGY: Fifty patients were included in this pilot study and were examined with respect to genotype, lipid profiles, blood sugar and other cardiovascular risk factors. Polymerase Chain Reaction (PCR), Restriction Fragment Length Polymorphism (RFLP) and Agarose Gel Electrophoresis techniques were used to determine the CETP TaqIB Polymorphism. Analysis include descriptive statistics, Chi square test and Fishers correlation test using Stata version 6. RESULTS: Out of 50 patients, 66% were females and 34% were males with a mean age of 55 y/o and a BMI of 27 kg/m2. The following risk factors were identified: hypertension (92%), dyslipidemia (88%), obesity (68%), smoking (50%), diabetes mellitus type 2 (18%) and family history of premature CAD (14%). The genotype frequencies of B1B1; B1B2; B2B2 were 40%, 50% and 10% respectively. The B1B1 homozygote was associated with lower HDL-C levels (45.358.82 mg/dl) compared to B1B2 (48.9610.10mg/dl) and B2B2 (48.9910.13 mg/dl)). CONCLUSIONS CETP-TaqIB Polymorphisms exist among Filipinos with cardiovascular risk factors. The frequency of TaqIB polymorphism among Filipinos with cardiovascular risk factors were B1B1 (40%), B1B2 (50%) and B2B2 (10%). B1B1 polymorphism is more common than B2B2 and associated with low HDL-C.

Association of *PON2* variants with PON activity in systemic lupus erythematosus (SLE). *S. Dasgupta[1], F.Y. Demirci[1], R.L. Minster[1], M. Kenney[1], P. Shaw[2], A. Kao[2], C. Kammerer[1], F. Bontempo[3], S. Manzi[2], M.I. Kamboh[1]* 1) Dept Human Genetics; 2) Lupus Center of Excellence; 3) Dept Medicine, Univ Pittsburgh, Pittsburgh, PA.

Several studies have implicated the association of low paraoxonase (PON) activity with coronary artery disease (CAD). SLE is a chronic autoimmune disease and patients with SLE are known to have increased morbidity and mortality from CAD. Two SNPs in the PON1 gene (codon 55 and codon 192) are known to be major regulators of serum PON activity, though the extent of contribution from subsequently characterized PON2 and PON3 genes remain to be determined. The purpose of this study was to test the impact of *PON2* SNPs on SLE risk and PON activity. For this purpose, 345 Caucasian SLE patients and 450 healthy control women were genotyped for five *PON2* tagSNPs using either Pyrosequencing or restriction analysis. None of the SNPs were associated with SLE risk. However, in single-site analyses, two SNPs (codon 311 and rs11982486 SNPs) showed significant gene-dosage effects on serum PON activity (p=5.24E-09 and p=0.0096, respectively). When all five *PON2* SNPs and two *PON1* SNPs (codon 55 and codon 192) were included in a multiple linear regression model, the significant effect of *PON2* SNP rs11982486 was lost (p=0.98) but it remained significant for *PON2*/codon 311 SNP (p<0.0001) and for two *PON1* SNPs (p<0.0001). On the other hand, the association with an additional *PON2* SNP (rs17876193), which was not significant in the single-site analysis, became significant using the multiple regression (p=0.0028). The contribution of the *PON1*/codon 192, *PON1*/codon 55, *PON2*/codon311, and *PON2*/rs17876193 SNPs on PON activity was 28.6%, 4.8%, 2.8% and <1%, respectively. These data indicate that in addition to the known effect of *PON1* on PON activity, genetic variation in *PON2* also contributes towards PON activity. Since the PON1 and PON2 genes are clustered in a segment of chromosome 7, their combined genetic effects appear to explain the bulk of variation in PON activity. Further analysis of additional *PON2* polymorphisms will reveal the exact roles of *PON2* variants in both the SLE risk and serum PON activity.

A genome-wide portrait of gene copy number variation spanning over 60 million years of human and primate evolution. *L. Dumas[1], Y. Kim[2], A. Karimpour-Fard[1], M. Cox[1], J. Hopkins[1], J. Pollack[2], J. Sikela[1]* 1) Human Medical Genetics, University of Colorado Health Sciences Center, Aurora, CO; 2) Department of Pathology, Stanford University, Stanford, CA.

Gene duplication and loss are key evolutionary forces that can underlie the emergence of important species-specific traits. A previous genome-wide cDNA array-based comparative genomic hybridization (aCGH) analysis we reported, involving pair-wise comparisons between human and four great ape species, identified over 1,000 genes that showed lineage-specific gene copy number variations (gCNVs) in these species. As a further step toward development of an evolutionary history of primate gene duplication and loss, we have now applied cDNA aCGH to more distant primate species that span over 60 million years of primate evolution. Using human as the reference sample in all experiments and using human cDNA arrays (>41,000 cDNAs) covering 24,473 genes, we identified 1,960 lineage-specific aCGH-predicted gCNVs for these 10 species: 67 in human, 54 in bonobo and chimp, 65 in gorilla, 67 in orangutan, 334 in gibbon, 243 in Old World monkeys (macaque and baboon), 367 in marmoset and 763 (increases) in lemur. For each species except lemur, gene copy number increases consistently outnumbered decreases (861/336), suggesting that sequence divergence did not significantly interfere with copy number predictions. Q-PCR was used to confirm aCGH-predicted changes for several genes, including a number that are potentially relevant to important species-specific traits. Interestingly, aCGH-predicted gene copy number increases in distant primates must reflect expansions that were sufficiently great enough to overcome any reduction in hybridization that was due to sequence divergence. Such gene increases represent excellent candidates to be important to biological traits unique to these distant species. Finally, because the reference DNA is kept constant for all comparisons, cDNA aCGH datasets from a wide range of species can all be interrelated to one another and an evolutionary portrait of gene copy number gain and loss for over 24,000 human genes can be assembled that spans much of human and primate evolutionary history.

Towards the characterization of modifier genes for glaucoma in a huge French-Canadian pedigree carrying the K423E myocilin mutation: identification of clusters for age at onset of the disorder. *P. Belleau[1], K. Lebel[1], R. Arseneault[1], J.L. Anctil[2], A. Duchesne[1], G. Côté[2], M.A. Rodrigue[1], V. Raymond[1]* 1) Ocular Genetics & Genomics, CREMO, Laval University Hospital (CHUL) Res Ctr, Québec City, PQ, Canada; 2) Ophthalmology Dept, St-Sacrement Hospital, Québec City, PQ.

Glaucoma is the 2nd leading cause of blindness worldwide. 11 loci and 3 genes have been characterized for its most common form, primary open-angle glaucoma (POAG). We reported a huge autosomal dominant POAG pedigree in which carriers of the K423E MYOC mutation displayed wide phenotypic variability for age at onset (AAO). As a 1st step towards characterization of modifier gene(s) responsible for this variability, we analyzed if sub-phenotypes for the disorder clustered in particular regions of the pedigree. Of the 709 individuals studied, 145 were mutant heterozygotes. Complete ophthalmologic records were obtained for 103 of the carriers. 86 were diagnosed POAG while the 17 other heterozygotes displayed intraocular hypertension (OHT), a symptom always preceding glaucoma in the family. AAO of the disorder (either POAG or OHT) ranged from 8 to 63 years old. Mean AAO was 27 years old (men: 25, women: 29). Penetrance was 73% in carriers aged 35. We defined the neighborhood of each carrier as a set of pedigree members where the kingship coefficient between the carrier and other members was 0.0625. For each heterozygote, we next calculated the median of age at onset for this carriers neighborhood. Each heterozygote was classified into 3 categories according to this median (25, 26 to 34 or 35). We observed 5 distinct clusters relative to AAO. In clusters 1, 2 and 3 (43 carriers), the majority of heterozygotes showed a median for AAO relative to their neighborhood 35. In clusters 4 and 5, this median was 25. In each cluster, descendants originated from 1 distinct ancestor. Our study confirmed that age at onset displayed wide phenotypic variability in this kindred. Interestingly, heterozygotic carriers clustered into large extreme groups relative to age at onset. The observation of age-related clusters strongly suggests the presence of at least 1 modifier gene for MYOC-associated glaucoma in this pedigree.

Molecular and functional analysis of paraplegin gene (*SPG7*) mutations in patients with spastic paraplegia. *D. DiBella[1], F. Lazzaro[2], M. Plumari[1], C. Gellera[1], C. Mariotti[1], M. Muzi-Falconi[2], S. Baratta[1], F. Taroni[1]* 1) Division of Biochemistry and Genetics, IRCCS Istituto Neurologico Carlo Besta, Milan, Italy; 2) Department of Biomolecular Sciences and Biotechnologies, University of Milan, Italy.

Hereditary spastic paraplegias (HSP) are a clinically and genetically heterogeneous group of neurodegenerative disorders. Mutations in the *SPG7* gene are responsible for autosomal recessive HSP with both pure and complex phenotypes. This gene encodes paraplegin, a major component of the hetero-oligomeric ATP-dependent m-AAA metalloprotease located in mitochondria and involved in proteolytic and chaperone-like functions. We have sequenced the paraplegin gene in 57 unrelated index cases, including 41 isolated cases and 16 cases compatible with an autosomal recessive pattern of inheritance. The majority of patients exhibited a complex phenotype characterized by spastic gait and signs of cerebellar involvement. Pathogenic mutations (3 frameshift, 4 nonsense, and 3 missense) were found in 8/41 (19.5%) sporadic patients but in none of the familial cases. The mutations were not found in a large control population. Four patients (4/8, 50%; 4/41, 9.8%) had mutations on both alleles, a proportion higher than that in previously reported series. Of the remaining 4 patients, 3 carried a single heterozygous missense mutation, suggesting the possibility of a dominant effect of the mutated protein. We have modelled the identified *SPG7* missense mutations in a yeast system in which the human m-AAA is functionally reconstituted by coexpressing its two components AFG3L2 and paraplegin. Notably, functional analysis clearly indicated that the mutation Ala510Val, previously described as a polymorphism (McDermott 2001; Wilkinson 2004) and more recently identified in numerous HSP patients (Elleuch 2006), produces a respiratory-deficent phenotype in yeast cells expressing the mutant protein. Investigation on the dominant effect of the Ala510Val substitution and functional analysis of the remaining missense mutations is in progress. [Partly supported by grants FIRB RBNE014HJ3010, Telethon-UILDM GUP04009, and Fondazione Mariani R0544 to FT].

High-throughput gene expression analysis of skin from psoriatic patients and normal controls. *J. Ding[1], J.E. Gudjonsson[2], R.P. Nair[2], P.E. Stuart[2], D. Ghosh[1], J.J. Voorhees[2], G.R. Abecasis[1], J.T. Elder[2]* 1) Dept Biostatistics, Univ Michigan, Ann Arbor, MI; 2) Dept Dermatology, Univ Michigan, Ann Arbor, MI.

Gene expression has been proposed as an intermediate phenotype that can increase power in complex trait gene-mapping studies. Psoriasis, an immune-mediated, inflammatory and hyperproliferative disease of the skin and joints, provides an ideal model system to evaluate this paradigm since the disease tissue is readily accessible. Susceptibility to psoriasis is influenced by multiple genetic and environmental factors, notably including HLA-Cw6.

To characterize global expression in psoriatic patients, we collected involved and uninvolved skin from psoriatic patients and normal skin from controls (40 patients and 40 controls in total, corresponding to 120 skin samples) and performed microarray experiments using Affymetrix U133 Plus 2.0 arrays. Principal Component Analysis (PCA) on all samples showed that involved psoriatic skin could be perfectly separated from uninvolved psoriatic skin and normal skin while the latter two groups were mixed together. Genes involved in immune response, epidermal cell differentiation and cell proliferation regulation were significantly enriched in a list of 666 up-regulated transcripts. When PCA was applied only to uninvolved skin and normal skin, the two groups could be separated with a small fraction of misclassified samples, indicative of distinguishable but not dramatic differences in gene expression between the two groups. In this comparison, we identified only 2 consistently up-regulated transcripts and 7 consistently down-regulated transcripts. The genes encoding these transcripts present excellent candidates and may be involved in modulating susceptibility to psoriasis.

HLA-Cw6 has been shown to be the major genetic risk factor that confers susceptibility to early-onset psoriasis. HLA-Cw6 carriers have a 5 to 20-fold increase in susceptibility. We contrast gene expression differences between HLA-Cw6 positive and negative psoriatics. Our results illustrate the potential use of gene expression as an intermediate phenotype in gene-mapping studies and further the genetic dissection of psoriasis.

Penoscrotal transposition and other anomalies in patients with distal 13q deletion syndrome. *J.R. Corona-Rivera[1,2], J. Acosta-León[2], V.A. Gutiérrez-García[1], E. López-Marure[2], A. Corona-Rivera[1], L. Bobadilla-Morales[1]* 1) Instituto de Genética Humana, CUCS, Universidad de Guadalajara, Guadalajara, México; 2) División de Pediatría, Hospital Civil de Guadalajara "Dr. Juan I. Menchaca", Guadalajara, México.

Complete or partial penoscrotal transposition (PST) is a rare abnormality, frequently associated with other malformations involving the genitourinary, cardiovascular or skeletal systems. Deletions of different portions of chromosome 13 are related with variable features of 13q deletion syndrome as well as with complete or partial PST. We report a patient with del(13)(q32) and partial PST and review the 13q breakpoints previously related to PST. The propositus was born to 20-years-old G2, P2 mother and 37-years-old healthy non-consanguineous father. He was born at term after uncomplicated pregnancy and delivery. Birth weight was 1,800 g and length 44 cm (both below 3rd centile). He presented delayed development of motor skills. Physical examination showed weight 2200 g (-4.7 SD), height 47.5 cm (-3.4 SD), OFC 31.2 cm (-4.8 SD). He had a 1.3 cm cystic mass just below the posterior fontanelle, broad nasal bridge, upward obliquity of the palpebral fissures, small receding chin, short neck, hearth murmur, single palmar crease on both hands, extra digital creases on some digits and hiperconvex nails. He also had partial PST with bifid but well developed scrotum and testis, hypospadias and a perineal sinus. Ultrasonographic evaluation of cranial cystic mass suggested a dermoid cyst. An echocardiogram showed patent foramen ovale. Cystography and abdominal ultrasound did not show other renal or genitourinary anomalies. Cranial CT scan showed mild atrophy. Karyotype was 46,XY,del(13)(q32). The overall phenotype in our patient was strikingly similar to those previously reported in patients with different deletion of chromosome regions located betweeen 13q22-13q34. Since some previous reports of PST had a similar pattern of defects of the facial, genital, cardiovascular and limb regions, but with apparent normal karyotype, we speculate that cryptic deletions of the PST critical region may be related to this cases.

Could Glucocerebrosidase mutations affect alpha-synuclein aggregation by toxic gain-of function? *O. Goker-Alpan[1], D. Urban[1], B. Stubblefield[1], M. Cookson[2], B. Giasson[3], E. Sidransky[1]* 1) MGB/NHGRI, NIH, Bethesda, MD; 2) NIA, NIH, Bethesda, MD; 3) UPenn, Philadelphia, PA.

Accumulation of protein aggregates are linked to many diseases, including the neurodegeneration associated with Parkinson disease. Recent clinical studies implicate an association between mutations in glucocerebrosidase (GBA), the lysosomal enzyme deficient in Gaucher disease, and the development of parkinsonism. GBA mutations are identified at an increased frequency in subjects with different synucleinopathies. Certain GBA mutations exhibit temperature-sensitive activity changes that indicate protein misfolding. In fibroblasts from patients with Gaucher disease, these GBA mutations resulted in improper trafficking. Growth at 30C corrected the trafficking to the lysosomes, restoring enzyme activity and increasing steady-state protein levels. To explore whether misfolded glucocerebrosidase (GC) has an effect on alpha-synuclein aggregation, brain samples from five subjects with parkinsonism who carried GBA mutations were examined. Mutant glucocerebrosidase was present in alpha-synuclein positive inclusions in both GBA hetero- and homozygotes with parkinsonism. Both ubiquitinated and non-ubiquitinated aggregates showed immunoreactivity for GC, and GC positive aggregates were localized with lysosomal markers. Next, the functional relationship between these proteins was examined by co-expression of A53T-synuclein and wild-type or mutant GBA in COS7 cells. Overexpresion of mutant GBA induced the formation of juxta-nuclear LB-like inclusions. These synuclein- and GC-positive inclusions stained positive with aggresome markers and altered the distribution of intermediate filaments in the cell. Deposition of misfolded proteins into aggresomes is a common feature of neurodegenerative diseases associated with a toxic gain-of-function mechanism. These results suggest that mutations in GBA may likewise enhance synuclein aggregation by this mechanism.

Use of C8-DCH--glucopyranoside for high-throughput screening of modulators of lysosomal -glucosidase in intact cells. *E. Goldin[1], D. Urban[1], K. Hruska[1], S. Ziegler[1], E. Sidransky[1], C.R. Kaneski[2]* 1) MGB/NHGRI, NIH, Bethesda, MD; 2) DMNB/NINDS, NIH, Bethesda, MD.

Recently, a new substrate for lysosomal -glucosidase (GC) based on 2,3 dicyanohydroquinone (DCH) has been reported. This substrate, 4-octyl-2,3-dicyano-1,4-hydroquinonyl--D-glucopyranoside (C8-DCH--glu), is hydrolyzed by living cells to a highly fluorescent product (C8-DCH) that can be detected using a standard microplate reader (excitation: 380 nm, emission: 445 nm). A Flp-in-CHO system expressing wild-type or mutant constructs of human glucocerebrosidase (GBA) cDNA with a V5 tag was used to test the use of this substrate for high-throughput screening. CHO cell lines carrying wild-type GBA or one of two common Gaucher mutations, N370S and L444P, were generated. Expression of the mutant and wild-type GC constructs was similar when measured by Western blot with anti-V5 antibody. When incubated with C8-DCH--glu, intact cells expressing the mutant constructs had negligible activity above the baseline found in vector-transfected cells, but the wild-type construct demonstrated a time-dependent increase in fluorescence. Glucocerebrosidase activity in intact cells exposed to the C8-DCH--glu substrate corresponded well to the levels measured in cell homogenates using a standard 4MU method. Hydrolysis of C8-DCH--glu was completely suppressed by the known GC inhibitor, CBE. The difference in activity observed between cells expressing wild-type and mutant GC suggests that this system will enable the determination of changes in GC activity in its native environment and will be useful in monitoring the response to treatments *in vivo*.

Radiation induced genomic damage in the presence of curcumin by comet assay in a murine model. *L. Bobadilla-Morales[1], R. Silva-Cruz[1], K. Contreras-Venegas[1], C. Ortega-de la Torre[1], L. Ruvalcaba-Ortega[1], J.J. Vargas-Lares[1], M.A. Ramírez-Herrera[2], M.L. Mendoza-Magaña[2], J.R. Corona-Rivera[1], A. Corona-Rivera[1,3]* 1) Laboratorio de Citogenética y Genotoxicidad, Instituto de genética Humana Dr. ECR, Dep. Biol. Mol. and Genomics, CUCS, University of Guadalajara. Guadalajara, Jalisco, Mexico; 2) Laboratorio de Neurofisiología, Dep.de Fisiología, CUCS, University of Guadalajara. Guadalajara, Jalisco, Mexico; 3) Unidad de Citogenética, OPD, Hospital Civil de Guadalajara.

Introduction: ionizing radiation is mutagenic to DNA. Curcumin behave anti-oxidant and anti- inflammatory proprieties. Previous studies suggest that curcumin may decrease genetic damage due to ionizing radiation. The goal of this work is to test the radioprotective effect on genetic material of gradual doses of curcumin by comet assay. Methods. We established 6 groups (n=36) of 5 weeks male Balb-C mice, as follow: (1) negative control, (2) exposed to 6 Gy of gamma radiation, (3) curcumin 0.1%, (4) 6 Gy of gamma radiation + curcumin 0.01%, (5) 6 Gy of gamma radiation + curcumin 0.1%, (6) 6 Gy of gamma radiation + curcumin 0.5%. After 24 hs. Comet assay was performed measuring tail length, total length and qualitatively (categories 0 to 4). Results. The observed frequency of damage was similar between control and curcumin 0.01%. Statistical increase was observed in radiated mice. Increased values of damage were observed in radiation + curcumin 0.1% and 0.5% versus control and similar to radiation condition. Conclusions. Genomic damage due to ionizing radiation using comet assay was reduced in the presence of curcumin at doses of 0.1% and 0.5%. This indicate that curcumin was radioprotective of genetic damage.

ChIP on CHIP analysis of neocentromere inner kinetochore chromatin domain structure. *A.L. Alonso[1], F. Cheung[1], D. Hasson[1], B. Fritz[2], A. Ladurner[2], P.E. Warburton[1]* 1) Mount Sinai Sch Medicine, New York; 2) EMBL, Heidelberg.

Human neocentromeres are fully functional variant centromeres that have arisen epigenetically in ectopic chromosomal locations and are devoid of alpha satellite DNA. Neocentromeres allow detailed studies of centromeric DNA and chromatin organization not possible at endogenous centromeres. We have used chromatin immunoprecipitation (ChIP) and genomic microarray (CHIP) analyses with antibodies to three Centromere Proteins CENP-A, -C and -H to define the inner kinetochore chromatin domains at different resolutions on chromosome 13-derived neocentromeres. Using a contiguous 126 BAC genomic microarray spanning 14Mbp from 13q31.3 to 13q33.1, we have mapped the centromere-specific histone H3 variant CENP-A to distinct chromatin domains of 130kb, 215kb and 275kb in three independent 13q32 neocentromeres, suggesting little role for primary DNA sequence in neocentromere formation. Further analysis of two neocentromeres on the BAC array showed that both CENP-C and -H colocalize to the same chromatin domains as CENP-A, defining a distinct inner kinetochore chromatin structure. We then generated a high resolution microarray containing 257 unique PCR fragments that cover ~350kb including the 130kb CENP-A domain, with lower density coverage extending about 1 Mbp on either side. ChIP CHIP analysis on this PCR array showed that the 130kb CENP-A domain defined by the BAC array consisted of two distinct domains of ~102kb and ~7.8kb, both of which showed precise colocalization with CENP-A, -C and -H. These two domains were separated by an ~149kb devoid of these CENPs, which fits with current higher-order chromatin looping models. Finally, to investigate the CENP-A chromatin structure at a nucleosome level, we designed an oligo array that contains overlapping 70mers to two unique sequences of 1540 bp, within the ~102kb domain. Hybridization of CENP-A ChIP DNA enhanced for mononucleosomes showed one CENP-A nucleosome in one region versus three CenpA nucleosomes in the other. These data show that CENP-A chromatin is not contiguous within the kinetochore domain. Instead, CENP-A is interspersed throughout kinetochore chromatin with different CENP-A nuclesome density.

False interphase FISH result due to chromosomal heteromorphism. *L. Dong[1], A. Hajianpour[1], R. Habibian[1], D. Burkhardt[1], S. Kou[1], B. Diaz[1], Q. Huang[1], J. Chang[1], B. Huang[1,2]* 1) Genzyme Genetics, Monrovia, CA; 2) Genzyme Genetics, Orange, CA.

FISH using chromosome-specific DNA probes on uncultured specimens is an efficient method for expanding the capabilities of prenatal cytogenetics & PGD. The clear advantage of interphase FISH is the rapid availability of results; however, chromosomal heteromorphisms can give rise to a result that is discordant with the cytogenetic analysis. We present two cases. Case 1: Transabdominal CVS was performed on a 36 year old at 11 weeks gestation. FISH studies were performed using the AneuVysion assay kit that includes -satellite probes for chromosomes X, Y, & 18 centromeric regions, as well as locus specific probes for chromosomes 13 & 21. Of 82 interphase cells evaluated, the majority showed one signal for chromosome 18 but in ~30% of the cells there was a questionable tiny signal in addition to an intense larger signal. The conventional cytogenetic result was normal. Further investigation using the same probe on metaphase cells revealed that one chromosome 18 had a consistently diminished signal. The false positive monosomy 18 interphase FISH result was therefore attributed to polymorphism of one of the individuals chromosome 18 centromeres. Similar cases have been reported in the literature with findings of false positive monosomy 18 & false negative trisomy 18. Case 2: Pre-PGD FISH studies were performed on a 29 year old female due to a balanced translocation involving chromosome 15 found in her partner. One of the probes initially tested was -satellite DNA probe D15Z4 at 15p11.1-q11.1 (Vysis, Inc.); 50 interphase & 5 metaphase cells were analyzed. One chromosome 15 showed diminished signal in metaphase cells, while only one signal was detectable in most interphase cells. Consequently, a probe for chromosome 15 which does not include the polymorphic region was selected for evaluation. These cases show that careful interpretation of interphase FISH analysis is necessary & that conventional cytogenetic analysis should be performed whenever possible. For PGD cases, the best approach is to perform pre-PGD FISH studies on both parents in order to prevent false PGD results.

Genome assembly comparison to identify structural variation in the human genome. *L. Feuk[1], R. Khaja[1], J. Zhang[1], J.R. MacDonald[1], Y. He[1], A.M. Joseph-George[1], J. Wei[1], M.A. Rafiq[1], L. Armengol[2], X. Estivill[2], C. Lee[3], S.W. Scherer[1]* 1) Dept Genetics & Genomic Biol, Hosp Sick Children, Toronto, ON, Canada; 2) Genes and Disease Program, Center for Genomic Regulation, Barcelona, Catalonia, Spain; 3) Department of Pathology, Brigham and Womens Hospital, Boston, MA, USA.

The most sensitive method for identifying all variation existing between two DNA donors is through direct comparison of accurately completed sequence assemblies of the genomes under study. We developed a new algorithm and applied it for comparison of two existing human genome assemblies, Celera's R27c compilation and the human Build 35 reference sequence. The differences identified range from single nucleotide changes to large structural changes. These include variants where sequence is gained or lost in one assembly compared to the other, as well as inversions. Fluorescence in situ hybridization and PCR based assays were used for experimental validation of assembly differences. Using a combination of literature, database and experimental analyses, we validated 100 structural variants and more than 1.5 million SNPs. In some cases the differences were simple insertion and deletions, but when copy number variants, segmental duplications, or repetitive DNA were involved the relationship was more complex. The results also show that 3Mb of sequence from the R27c assembly can be used to partially fill euchromatic gaps in the reference sequence. Our results highlight the need for comprehensive annotation strategies in order to fully interpret data from CNV-scanning and personalized sequencing projects.

The HR-Amp: A closed-tube mutation scanning and genotyping platform for genetic analysis. *D.A. David[1], C.T. Wittwer[2], J.T. Mckinney[1], S.F. Dobrowolski[1], V.E. Dujols[1], L.M. Nay[1], R.J. Pryor[2], D.D. Hawks[1], S.K. Marland[1]* 1) Idaho Technology Inc., Salt Lake City, UT; 2) University of Utah, Salt Lake City, UT.

HR-Amp is a new instrument that combines rapid-cycle PCR and high-resolution melting analysis (Hi-Res Melting). Genotyping and mutation scanning are enabled without labeled probes or separations by the saturating DNA binding dye (LCGreen PLUS). Rapid cycle PCR is performed within LightCycler glass capillary tubes for optimal speed, optical clarity and temperature homogeneity. The HR-Amp amplifies 32 samples at the same time within 15-30 min. High-resolution melting is performed immediately after PCR in the same instrument without any separation or reagent addition steps. Each sample is automatically translated into a precision thermal block and high-resolution melting performed and analyzed similar to our single sample platform, HR-1. Major applications include mutation scanning and genotyping. Heterozygous sequence alterations of amplified DNA are detected by melting curve shape, providing a homogeneous method for mutation scanning based on heteroduplex formation. Genotyping is enabled by either small amplicon melting or unlabeled probe analysis. The melting signature of both the amplicon and the probe are obtained during the same melting acquisition, taking only 1-2 minutes per sample. Single base changes, as well as small insertions or deletions are easily analyzed. Sensitivity and specificity for single base changes are 100% for amplicons up to 400 bases with a sensitivity of >96% and a specificity of >98% for 400-1,000 bp amplicons. No allele-specific amplification or real-time PCR monitoring are necessary. Several alleles can be genotyped from one melting analysis. Advantages of HR-Amp as a genotyping platform include speed (PCR in 20 min, analysis in 1-2 min/sample), solution-based closed-tube analysis, no need for sample transfers or reagent additions, both scanning and genotyping capability, and nondestructive analysis with the sample immediately available for sequencing in the rare case that it is necessary. Indeed, by combining scanning and genotyping capabilities, we estimate that 99% of the need for sequencing will be eliminated.

DNA repair capacity as a lung cancer risk predictor in never smoker probands and their first degree relatives. *O. Gorlova, S.-F. Weng, Y. Zhang, C. Amos, M. Spitz, Q. Wei* Dept Epidemiology, Univ Texas MD Anderson CA Ctr, Houston, TX.

We present estimates of risk of lung cancer associated with suboptimal DNA repair capacity (DRC) in lifetime never smokers, and estimates of risk of lung and other cancers in first degree relatives of never smoking individuals (lung cancer cases and matched controls) associated with probands DRC status. We use the data from one of the largest lung cancer case-control studies that included 2701 first-degree relatives of 348 never smokers (163 lung cancer cases and 185 controls) for whom the data on DRC was available. To evaluate whether there was an excess risk of cancer among first-degree relatives of individuals with suboptimal DNA repair capacity (below the control median or the first quartile) measured by host-cell reactivation assay, we performed unconditional logistic regression using generalized estimating equations, accounting for relatedness within families, adjusting for age and gender of proband and of relative, ethnicity of proband, type of relationship to proband, smoking status of relative, birth cohort of relative, and case-control status of the relative. Suboptimal DRC level (below the control median) conferred a significantly increased lung cancer risk in never smokers (OR=1.81, 95%CI [1.17-2.81], p=0.007). There was a 3.75-fold risk for individuals in the first (lowest) quartile of DRC (95%CI [1.97-7.14]) compared to individuals in the highest quartile. Relatives of probands that had lowest DRC (first quartile) were significantly more likely to be diagnosed with lung cancer (OR=5.05, 95% CI [1.51-16.9]), but not with snoking-related or all cancers compared to relatives of probands with highest DRC (fourth quartile). The case-control status of the proband turned out not to be significant when probands DRC status was included in the model. Relatives of probands with DRC below the control median had a significantly earlier age at diagnosis with lung cancer and a smoking related cancer than relatives of probands with DRC above the control median (p=0.019 and 0.026, respectively). No effect of probands DRC was found on age at diagnosis with all cancers.

Clinical relevance of *KRAS* mutation detection in metastatic colorectal cancer treated by Cetuximab plus chemotherapy. *F. Di Fiore[1,2], F. Blanchard[3], F. Charbonnier[2], F. Le Pessot[2,3], M-P. Gallais[4], L. Bastit[5], A. Killian[2], R. Sesboüé[2], J-J. Tuesch[2,6], A-M. Queuniet[7], B. Paillot[1], J-C. Sabourin[2,3], F. Michot[2,6], T. Frebourg[2]* 1) Department of Gastroenterology, University Hospital, Rouen, Northwest Canceropole, France; 2) Inserm U614, Faculty of Medicine, Rouen, Northwest Canceropole, France; 3) Department of Pathology, Rouen University Hospital, Northwest Canceropole, France; 4) Department of Hepato-Gastroenterology, Caen University Hospital and Francois Baclesse Centre, Caen, Northwest Canceropole, France; 5) Oncology Unit, St Hilaire Medical Centre, Rouen, France; 6) Department of Surgery, Rouen University Hospital, Northwest Canceropole, France; 7) Department of Hepato-Gastroenterology, Elbeuf Hospital, France.

The variability of the metastatic colorectal cancer (MCRC) clinical response to anti-EGFR agents, such as cetuximab, has highlighted the urgent need to identify reliable predictive markers. We therefore evaluated the recently suggested predictive value of *KRAS* mutation in MCRC patients treated with cetuximab plus chemotherapy. Fifty nine patients with a chemotherapy-refractory MCRC treated with cetuximab plus chemotherapy were included. *KRAS* exon 2 and *TP53* exons 5-8 were directly sequenced from tumor DNA and the presence of *KRAS* mutation in a small fraction of tumor cells was assessed by the PCR-LCR method. A *KRAS* mutation was detected in 18/59 of the tumors and, in 2 cases, was missed by sequencing analysis. Among the patients with a *KRAS* mutation, 15 presented a progressive disease. No *KRAS* mutation was found in the 12 patients with clinical response. *KRAS* mutation was therefore associated with disease progression ($p = 0.0005$). Among patients without detectable *KRAS* mutations, *TP53* mutation was associated to a clinical response ($p=0.01$). This study confirms that mutation of *KRAS*, downstream of the EGFR pathway, is highly predictive of a non-response to cetuximab plus chemotherapy in MCRC, highlights the need to use sensitive molecular methods to detect somatic mutations conferring resistance and suggests that sequential detection of *KRAS* and *TP53* mutations may have a potential use in clinical practice.

Compromised mRNA processing and epilepsy in Brunol4 mutant mice. *W. Frankel, Y. Yang, C. Mahaffey, T. Maddatu, G. Cox, J. Graber* The Jackson Laboratory, Bar Harbor, ME.

Idiopathic epilepsy is a common disorder with a strong genetic component, usually exhibiting complex inheritance. While both complex and monogenic seizure-prone animals have been reported, multigenic models with well-defined genetic lesions have yet to be developed. Here we describe frequent-flyer mice, in which a transgene insertion disrupts expression of the Bruno-like 4 gene. *Brunol4* haploinsufficiency results in dominant, recurrent limbic and tonic-clonic seizures in older, relatively healthy mice. Null homozygous mutants, which are small and do not thrive as well as littermates, have a more severe phenotype including both convulsions and absence seizures with frequent spike-wave discharges. Both genotypes show low seizure threshold before overt seizure onset. All mutant phenotypes are affected by strain background. *Brunol4* encodes a brain-specific RNA binding protein. Its expression is concentrated in neurons of brain regions controlling synchronization and oscillation (e.g. cerebral cortex, hippocampus, thalamus), suggesting it is involved in the maintenance of normal neuronal rhythmic activities. Using microarrays we identified multiple transcripts with altered expression in mutant brain, some of which were implicated in epilepsy by prior studies and others which are novel. At least four molecules critical to neuronal excitation (HTR2C, SYN2, SNCA and NSF) are selectively reduced at transcript and protein level. Analysis of the 3 UTR of these genes identified a highly conserved U/G rich motif. RNA-protein immunoprecipitation in neurons suggests direct interaction between BRUNOL4 and this sequence, the consequences of which on the target RNA are being investigated in primary culture using reporter and mRNA decay studies. We suggest that BRUNOL4 modulates neuronal excitability at the RNA level through fine-tuning the stability of transcripts encoding proteins critical to neuronal excitation. *Brunol4* mutant mice represent the first animal model where compromised mRNA processing leads to epilepsy. They also demonstrate how a single gene disruption can lead to multiple, more incremental molecular disturbances, mimicking a genetically complex disorder.

A genome-wide association study of putative functional SNPs leads to the identification of two psoriasis loci - IL12B and IL23R - in 3 independent white North American sample sets. *A.B. Begovich[1], S.J. Schrodi[1], M. Leppert[2], G. Krueger[2], M. Cargill[1]* 1) Celera, Alameda, CA; 2) University of Utah, Salt Lake City, UT.

To identify psoriasis-susceptibility loci, we carried out a multi-tiered, case-control association study focusing on functional SNPs. We screened 466 cases and 500 controls (Discovery) in disease phenotype-based pools with 26,644 gene-centric SNPs, ~70% of which were missense. SNPs associated with disease (P<0.05) were evaluated in a replication study (498 cases/498 controls) using a similar strategy. These studies identified a disease-associated IL12B 3UTR SNP which was confirmed by individual genotyping (P = 1.89E-04, OR 1.59 and P = 7.59E-07, OR 1.81, respectively). A Monte Carlo simulation addressing multiple testing showed this result was unlikely to be a type I error. IL12B was resequenced in 96 cases. A combination of 30 functional and tagging SNPs for the IL12B region were genotyped in both studies identifying a second, independent risk allele located ~60kb upstream of the IL12B transcription start site. Together these 2 SNPs mark a common psoriasis-risk haplotype (Pcomb = 8.94E-08, ORcommon = 1.46) as well as a less frequent protective haplotype (Pcomb = 4.19E-11, ORcommon = 0.53); we saw no evidence that the other two haplotypes influenced disease risk. Because IL12B encodes the beta subunit of both IL12 and IL23, we individually genotyped 17 markers in genes encoding the alpha chains of these cytokines (IL12A, IL23A) as well as their receptors (IL12RB1, IL12RB2, IL23R). Single-marker and haplotype analyses identified two IL23R missense SNPs that marked a common disease-associated haplotype in both studies (Pcomb = 7.56E-05, ORcommon = 1.47). Individuals homozygous for both the IL12B and IL23R predisposing haplotypes appear to be at substantially increased risk for psoriasis (Pcomb = 3.8E-08, ORcommon = 1.78). Both the IL12B and IL23R risk haplotypes have now been replicated in a third independent study (481 cases/424 controls; P=0.006 and P=0.003, respectively) suggesting that these genes play a fundamental role in psoriasis and implicating the IL23 pathway in psoriasis risk.

Detection of muscular dystrophy genotypes via universal condition direct sequencing (UCDS). *R.R. Bennett[1], H.E. Schneider[1], C. Feener[1], A. Lakdawalla[2], P.S. Lai[3], C.E. Barrett[1], V. Lip[1], B.L. Wu[1], B.T. Darras[1], A. Beggs[1], L.M. Kunkel[1]* 1) Dept Genetics, Children's Hosp, Boston, MA; 2) Applied Biosystems, Foster City, CA; 3) Department of Pediatrics, National University of Singapore, Singapore.

In order to provide a high throughput, low cost molecular diagnostic technique for the detection of mutations in any type of muscular dystrophy, we have developed UCDS (Universal Conditions Direct Sequencing). In collaboration with Applied Biosystems, Foster City Ca. and its VariantSEQr resequencing team, we have designed and tested primers to amplify all regions of interest (coding and non-coding exons, promoters, splice variations and UTRs) for the following list of genes: DMD, CAV3, TRIM32, CAPN3, FKRP, FCMD, DYSF, SGCA, SGCB, SGCD, SGCG, LMNA, TCAP, TTID. These assays have been designed and tested to be sure that they all amplify at a single set of PCR conditions so that a 384 well plate containing any combination of them can be assembled by a robot and placed in a thermal cycler for amplification. By using M13F and M13R tails on the primers, a universal sequencing plate can be utilized for sequencing these assays. More than 90% of the assays for all these genes have been tested for good amplification and for good sequencing. In the last year many assays have been redesigned to avoid SNPs and for better amplification and double stranded sequence coverage. We are in the final stages of automation decisions and have tested several robots to determine the best robot for this process. Robot script flow diagrams have been created and are currently being tested. Detection of mutations is facilitated rapidly using SeqScape v2.1 software (Applied Biosystems, Foster City Ca.). This software incorporates a consensus sequence based on the NCBI database, complete gene annotation of all NCBI and Celera transcripts for the gene of interest and a tab process for tabing through all variations between patients and consensus. It also features rapid identification of known polymorphisms versus unknown variants as well as insertions and deletions. Several patient mutatons have been found.

Performance of Whole Genome Amplified DNA on the Affymetrix GeneChip Human Mapping 500K Array Set.

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In recent years, several technologies for whole genome association studies have been validated. Critical to the success of these studies and an often underrated factor is the quality and quantity of input DNA. Whole genome amplification (WGA) is a robust method that utilizes 29 DNA polymerase and multiple strand displacement to generate high-fidelity copies of the genome when genomic DNA stocks are limited (Dean et al, 2002). While the performance of wgaDNA on the Affymetrix 10K GeneChip has been tested (Paez et al, 2004), the more recent 500K Array Set has not been formally investigated. We evaluated the success of WGA product on the Affymetrix GeneChip Human Mapping 250K Sty Array by assessing call rate and concordance. Ten samples (isolated from whole blood) were genotyped in tandem for both genomic DNA (gDNA) and amplified DNA (wgaDNA) on 238K SNPs. We also genotyped a reference Promega DNA in duplicate, to establish a baseline average for call rate and concordance (97.5%, 99.6%). The average call rates for the gDNA and wgaDNA were 96.1% and 93.82% respectively. The genotype accuracy between gDNA and wgaDNA samples was 98.71%, compared to the observed concordance rate of 99.8% in gDNA. To further investigate the cause of this decrease in genotyping success, we also evaluated call rates and accuracy for input wgaDNA under varied conditions: increasing concentration, mixing two independent WGA products of the same DNA, and purifying the wgaDNA. Lastly, we will present data that may suggest chromosomal regions are susceptible to poor genotype performance as a result of the WGA process.

A non-glycine sequence variant (P435T) of the COL3A1 gene associated with an autosomal dominant syndrome of joint hyperlaxity, easy bruising, pelvic organs prolapses, premature rupture of the membranes and rectal bleeding. *D.P. Germain* Department of Genetics, HEGP, Paris, France.

Vascular Ehlers-Danlos syndrome (OMIM 130050) is a life-threatening inherited disorder of connective tissue causing severe arterial and intestinal fragility and rupture. Mutations in the COL3A1 gene have been shown to underlie the phenotype. In addition to mutations resulting in haploinsufficiency, missense mutations affecting the glycine residue of the Gly-X-Y triplet of the alpha1(III) procollagen chain result in the incorporation of structurally altered molecules into fibrils and account for 2/3 of the pathogenic mutations. In contrast, no mutations have been found in the X or the Y position of the Gly-X-Y triplet (http://archive.uwcm.ac.uk/uwcm/mg/hgmd0.html). Here, we report a 14-year-old female proband presenting with a syndrome of extensive easy bruising, stretch marks, translucent skin with visible veins, and rectal bleeding. Of note, her 20-year-old sister and her 44-year-old mother both had joint laxity, distinctive facial features with thin lips and lobeless ears, and multiple pelvic organs prolapses (rectal, uterine and vaginal). The mother had premature rupture of membranes on two pregnancies. There was no history of arterial or intestinal complication. A heterozygous c.1906 C>A change was found during cDNA analysis of the probands COL3A1 gene, changing the codon CCT for proline to a codon ACT for threonine at amino acid position 435 of the alpha1(III) chain and confirmed by genomic DNA analysis (exon 25). Whether the identified sequence change located at a Y position of the Gly-X-Y repeat of collagen III triple-helical domain is a pathogenic mutation or a rare variant is unclear since 6 of the 14 threonines normally found in the type III collagen triple-helical domain occupy the Y position. However, the mutation segregated with the phenotype within the family and was absent in the probands unaffected brother. Additional molecular and clinical investigations are ongoing to establish the exact functional importance of the P435T variant. If pathogenic, whether the mutation may lead to the occurrence of life-threatening complications of vascular EDS is unknown.

Global profiling of aberrant splicing in myotonic dystrophy using the Affymetrix human exon array. *L. Bachinski[1], K.A. Baggerly[1], S. Tsavachidis[1], S.E. Olufemi[1], M. Sirito[1], J. Gamez[2], G. Bassez[3], B. Eymard[4], T. Ashizawa[5], J. Mendell[6], B. Udd[7], R. Krahe[1]* 1) Dept. Cancer Genetics, M. D. Anderson Cancer Ctr, Houston, TX; 2) Dept. Neurology, General Hosp. Vall d'Hebron Univ. Barcelona, Spain; 3) Dept. Pathology, Henri Mondor Univ. Hosp., Créteil, France; 4) Myology Inst. Salpêtrière Hosp., Paris, France; 5) Dept. Neurology, Univ. Texas Medical Branch, Galveston, TX; 6) Dept. Neurology, Childrens Hosp. Columbus, Ohio State Univ., Columbus, OH; 7) Dept. Neurology, Vasa Central Hosp. and Tampere Univ. Hosp., and Folkhälsan Inst. of Genetics, Univ. of Helsinki, Finland.

Myotonic dystrophy (DM) is the most common muscular dystrophy in adults. DM1 and DM2 are caused by similar unstable microsatellite repeat expansions - in DM1 a (CTG)n in DMPK in 19q13.3, in DM2 a (CCTG)n in ZNF9 in 3q21.3. The developing paradigm is that DM is a toxic RNA disease, mediated by the expansion of normally polymorphic microsatellites with a (CTG)n-like motif. Transcription of the mutant repeats into (CUG)n/(CCUG)n containing RNAs is necessary and sufficient to cause disease. Mutant RNAs accumulate in ribonuclear inclusions and interfere with RNA splicing, transcription and/or translation of a number of effector genes, resulting in the pleiotropic phenotype characteristic of this disease. To identify genes that are aberrantly spliced as the result of DM expansions, we used the Human Exon 1.0 ST Array (Affymetrix) to profile skeletal muscle biopsies of patients with DM1 and DM2, DM of unknown etiology, and normal (N) individuals. Altogether 184 genes were identified as potentially aberrantly spliced. We have validated two genes, TTN and SSBP3, using fluorescent RT-PCR followed by capillary electrophoresis. Peak heights were quantified using Genotyper 3.7, and isoform ratios were calculated. The isoform ratios for DM and N samples were significantly different for TTN (p-value=0.032396) and for SSBP3 (p-value=0.0023034). Validation of additional candidate genes is ongoing. Since many aspects of the pleiotrophic phenotype in DM are believed to be due to aberrant splicing, a detailed catalog of genes aberrantly spliced in DM will provide valuable entry points for the design of rational therapies.

Mutations in the gene encoding the 3'-5' DNA exonuclease TREX1 cause Aicardi-Goutières syndrome - a Mendelian mimic of congenital viral infection - at the AGS1 locus. *Y.J. crow[1], A.P. Jackson[2], P. Lebon[3], D.E. Barnes[4], T. Lindahl[4], The AGS Consortium* 1) Leeds Institute of Molecular Medicine, University of Leeds, St Jamess University Hospital, Leeds, LS9 7TF, UK; 2) MRC Human Genetics Unit, Edinburgh, United Kingdom; 3) Service de Virologie, Hôpital Cochin, St Vincent de Paul, 82 Avenue Denfert Rochereau, 75674, Paris, France; 4) Cancer Research UK, London Research Institute, Clare Hall Laboratories, South Mimms, Hertfordshire EN6 3LD, UK.

Aicardi-Goutières syndrome (AGS) typically presents as a severe neurological brain disease and is a genetic mimic of the sequelae of transplacentally acquired viral infection. Evidence exists for a perturbation of innate immunity as a pathogenic event in the disease phenotype. Herein, we show that TREX1, encoding the major mammalian 3'?5' DNA exonuclease, is the AGS1 gene; and AGS-causing mutations result in abrogation of TREX1 enzyme activity. Similar loss of function in the Trex1-/- mouse leads to an inflammatory phenotype. These observations suggest an unanticipated role for TREX1 in processing or clearing anomalous DNA structures, failure of which results in the triggering of an abnormal innate immune response. Taken together with our finding of mutations in the RnaseH complex in AGS patients, we are now in a position to define the phenotypic spectrum of the disease, which is considerably wider than previously recognised.

DNA pooling for whole genome association studies on the Illumina Infinium Assay arrays. *A.E. Baum[1], N. Akula[1], I. Cardona[1], W. Corona[1], A. Singleton[2], J. Hardy[2], S. Detera-Wadleigh[1], F.J. McMahon[1]* 1) Genetic Basis of Mood and Anxiety Disorders Unit, National Institute of Mental Health, National Institutes of Health, Bethesda, MD; 2) Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Bethesda, MD.

Conceptualized a decade ago, genome-wide association studies have only recently become practical in large samples. Costs are still a limiting factor. An initial screen which measures allele frequencies between pools of cases and controls, instead of between individuals, would greatly reduce costs. DNA pooling has been used successfully on several platforms, but has not yet been demonstrated on the Illumina Infinium system. We tested the accuracy of standard pooling methods on the Infinium I chip (109K SNPs) with DNA from 88 Caucasian control subjects who had already been genotyped individually. Three replicate pools of all 88 subjects were made from equimolar quantities of DNA as measured by pico-green. Each pool was concentrated and genotyped in duplicate. One pool was genotyped on 2 different BeadStations. Minor allele frequencies were estimated from raw intensity data (MAF=A/(A+B) or 1- A/(A+B) if A/(A+B)>0.5) and these frequencies were compared with those calculated from the individual data (MAF_{88}). As expected, MAF_{raw} only weakly correlated with MAF_{88} (r=0.86). Results were corrected for each SNPs true heterozygote intensities (k_{snp}=average(A:B) in known heterozygotes), so that MAF_k=A/(A+kB). k-corrected values were also normalized to the true positions of the homozygotes (MAF_{norm}). These transformations greatly improved the correlation between pooled and individual frequencies (MAF_{88} and MAF_k, r=0.98; MAF_{88} and MAF_{norm}, r=0.92). Variance between replicate pools was low (3-5.4%), as was variance between chips run on the same BeadStation (3.9%). Absolute error in allele frequency estimates was about 3-4%. We are now testing pooled Alzheimers Disease samples on the 550K chip to determine whether the well-known ApoE4 polymorphism can be detected.

Role of Notch Signaling in Bone Development. *F. Engin[1], G. Zhou[1], T. Yang[1], T. Bertin[1], M. M. Jiang[1], Y. Chen[1], Z. Yao[3], B. Boyce[3], B. Lee[1,2]* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Howard Hughes Medical Institute; 3) Department of Pathology and Lab Medicine, University of Rochester Medical Center, Rochester, NY.

Notch signaling is a central mechanism for controlling embryogenesis. Mutations of Notch signaling in human cause patterning defects of skeleton including Spondylocostal Dysostosis. However, Notchs *in vivo* function during bone development and pathology is poorly understood. In order to understand Notchs role in bone development, we generated osteoblast-specific transgenic mice in which Notch1 intracellular domain (N1ICD) expression was driven by Col1a1 promoter. In this gain of function model, transgenic mice showed severe, osteosclerosis and dwarfism. Histomorphometric analyses of these mice demonstrated increased mineralization and high bone mass. There was dramatic increase in the number of osteoblastic cells and abundant matrix characterized by an immature woven bone. Quantitative RT-PCR analyses showed upregulation of early osteoblast markers, while mature osteoblast markers such as osteocalcin were significantly decreased in transgenic mice. In these mice, Notch stimulated early osteoblastic proliferation by up regulating *Osterix* and *Cyclin D*, and *Cyclin E*. To elucidate the loss of function of Notch signaling, we also generated osteoblast-specific *Presenilin1, Presenilin2 (PS1/PS2)* double knockout mice. In contrast to our gain of function model, these mice showed significant osteoporosis. Histomorphometric and *ex vivo* culture studies showed decreased mineralization and accelerated differentiation of osteoblasts into osteocytes in double knockout mice, due in part to gain of *Runx2* function. These data highlight a novel central role of Notch signaling in the regulation of two distinct phases of bone formation, i.e., proliferation of early osteoblastic compartment vs. terminal differentiation into osteocytes. Hence, dysregulation of Notch signaling may account for low vs. high bone mass and/or proliferative phenotypes in humans.

The Machado-Joseph disease protein ataxin-3 interacts with NEDD8. *A. Ferro[1,2], A. Carvalho[3,4], A. Teixeira-Castro[5], C. Almeida[4], R. Tomé[4], J. Sequeiros[1,2], S. Macedo-Ribeiro[4], P. Maciel[5]* 1) UnIGENe-IBMC, Porto, Portugal; 2) Dpt of Population Studies-ICBAS, Porto, Portugal; 3) Dpt of Zoology, Coimbra, Portugal; 4) Centre for Neuroscience and Cell Biology, Coimbra; 5) Life and Health Sciences Research Institute, School of Health Sciences, Braga, Portugal.

Machado-Joseph disease (MJD/SCA3) is an autosomal dominant neurodegenerative disorder caused by the expansion of a CAG tract in the coding portion of the *ATXN3* gene. It is unknown how the resulting expanded polyglutamine (polyQ) tract in ataxin-3 (ATXN3), an ubiquitously expressed protein, triggers the disease, and why only some sub-populations of neurons are affected. The presence of ubiquitin-positive aggregates of the defective protein in affected neurons is a hallmark of most polyQ disorders. Accumulation of NEDD8, a ubiquitin-like protein in these inclusions in MJD brains was recently reported. The goal of our work was to determine if wild-type ATXN3 could interact with NEDD8. For this, we tested two normal isoforms of human ATXN3 that differ in their C-terminal (MJD1.1, MJD2.1), and N-terminal portions of the protein, which are common to both isoforms and exclude the polyQ tract, using the yeast two-hybrid and GST pull-down assays. The ceatxn-3 homologue was also tested using these assays. We observed interaction of ATXN3, encoded by both isoforms, and ceatxn-3 with NEDD8. The N-terminal part of ATXN3 seems to be sufficient for the interaction to occur since we saw interaction only with its Josephin domain (JD), indicating that this interaction is not dependent on the UIMs or the polyQ tract of ATXN3. *In situ* co-localization and His-tag pull-down assays confirmed this interaction in eukaryotic cells. The conservation of the interaction with NEDD8 in the nematode suggests a biological and functional relevance for this interaction. Molecular docking studies of the NEDD8 molecule to the JD suggest that NEDD8 interacts with ATXN3 in a substrate-like mode; our preliminary *in vitro* studies suggest that recombinant ATXN3, a known ubiquitin hydrolase, also displays deneddylase activity. Further studies must be carried out to characterize this function and to deepen the implications of these new observations for MJD.

A Markov Chain Approach to Estimating Kinship Coefficients. *K.L. Ayers[1], E. Sobel[2], K. Lange[1,2,3]* 1) Departments of Biomathematics; 2) Human Genetics; 3) Statistics, UCLA, Los Angeles, CA.

We propose a Markov chain method to estimate kinship coefficients between related individuals from dense SNP genotyping data. The output of the chain enables estimation of kinship coefficients, both globally and locally along the human genome. These in turn come into play in QTL mapping and association studies. The chain has 12 possible identity by descent states that mimic the condensed IBD states of Jacquard and Cotterman. Likelihoods can be efficiently computed via forward and backwards algorithms and optimized for maximum likelihood estimation of the underlying kinetic parameters. The equilibrium distribution permits computation of the theoretical (global) kinship coefficients. Posterior probabilities for each state at a particular marker permit estimation of local kinship coefficients. This abstract extends the model discussed in the abstract "Estimating Kinship Coefficients from High-Density SNP Genotypes for QTL Mapping and Association" by Day, Sobel and Lange. The current model is more computationally intensive, but may yield more precise estimates than the Day et al. model.

A Bardet-Biedl syndrome type 1 (BBS1) M390R mouse model results in ventriculomegaly, leptin resistance and a defect in regulation of neuronal cilia synthesis. *R.E. Davis[1], K. Agassandian[1], K. Rahmouni[1], R.F. Mullins[1], A.R. Philp[1,2], C.C. Searby[1,2], D.Y. Nishimura[1,2], M.P. Andrews[1,2], M. Tayeh[1], M.D. Cassell[1], B. Yang[1], E.M. Stone[1,2], V.C. Sheffield[1,2]* 1) University of Iowa, Iowa City, IA; 2) Howard Hughes Medical Institute, Iowa City, IA.

Bardet-Biedl syndrome (BBS) is a pleiotropic, heterogeneous disorder resulting in obesity, retinopathy, polydactyly, cognitive impairment, renal dysplasia and reproductive tract anomalies. Eleven BBS genes have been identified. The *BBS1* M390R mutation is the most common human BBS variant and accounts for ~25% of BBS cases. We have developed a novel knock-in mouse harboring the *BBS1* M390R mutation (*Bbs1*$^{M390R/M390R}$)and have extensively characterized this model. The *Bbs1* knock-in mouse model develops retinopathy and obesity as seen in human BBS patients. Histological analysis of the retina reveals degeneration of the outer nuclear layer (ONL) by 16 weeks of age, with severe attenuation increasing with age. Electroretinogram analysis confirms a progressive deterioration. Immunocytochemical analysis shows mislocalization and accumulation of rhodopsin in the ONL. In initial studies to understand the mechanism of obesity, we demonstrated elevated serum leptin levels in *Bbs1*$^{M390R/M390R}$mice. We show these mice have leptin resistance based on failure to respond to peripheral and direct central nervous system injection of exogenous leptin. Immunohistochemical analysis shows mislocalization of the leptin receptor in the hypothalamus of *Bbs1*$^{M390R/M390R}$ mice. Evaluation of the brain using magnetic resonance imaging demonstrates ventriculomegaly in these mice. Notably, a single M390R allele results in significant ventriculomegaly. Electron microscopic evaluation indicates abnormalities in ependymal cell motile(9+2) and primary(9+0) cilia of the hypothalamus consistent with a defect in retrograde intraflagellar transport and abnormal regulation of ciliogenesis. These data indicate additional phenotypes that should be evaluated in human BBS patients. We demonstrate the utility of the *Bbs1* knock-in mouse model for defining the pathological mechanisms of the BBS phenotypes and its importance for further analysis of BBS1 protein function.

Long term follow up of a 21 y/o female with methylcobalamin-treated methionine synthase deficiency. *D. Adams[1], B. Brooks[5], J. Sloan[1], J. Filiano[2], H. Levy[3,4], C. Venditti[1]* 1) NHGRI, NIH, Bethesda, MD; 2) Dept of Pediatrics and Medicine, Dartmouth Medical School, Hanover, NH; 3) Div. of Genetics, Children's Hospital, Boston, MA; 4) Dept of Pediatrics, Harvard Medical School, Boston, MA; 5) NEI, NIH, Bethesda, MD.

We present a 21 y/o female with methylcobalamin-treated methionine synthase deficiency (cblG). She was diagnosed at 5 m/o after a course marked by failure to thrive, irritability, and megaloblastic anemia. Visual evoked potential (VEP) testing showed impaired visual pathway conduction. Therapy with methylcobalamin was initiated. Shortly after diagnosis she developed infantile spasms with hypsarrythmia. Anti-epileptic medications (AEDs) were trialed without success. The seizures were only controllable by AEDs after a course of ACTH. VEPs gradually returned to normal, presumably due to optic nerve/tract remyelination. However, developmental delay and cognitive impairment persisted. Current therapies include methylcobalamin 500 mcg twice per week, L-methionine 25 mg twice daily, betaine 3 grams per day, folate and leukovorin. Urine organic acid analysis shows minimal excretion of 3-methylglutaconic acid and 3-methylglutaric acid. The total plasma homocysteine level is between 22 and 26 umol/L. The plasma methylmalonic acid level is normal at 0.15 umol/L. Plasma GAA and creatine are normal. Retinal exams are normal bilaterally; there is no evidence of optic atrophy. There is bilateral, asymmetric clonus at the ankles. A brain MRI shows normal brain structure. Magnetic resonance spectroscopy shows decreased NAA in some gray matter areas including the basal ganglia. The young woman described provides an example of the long term outcome of methionine synthase deficiency treated with methylcobalamin. Her overall course appeared to stabilize with the institution of therapy. Open issues include: 1) the contribution of her infantile spasms and hypsarrythmia to her present cognitive delays, 2) the progressivity of her lower extremity spasticity, 3) the metabolic basis of the 3-methylglutaconic and 3-methylglutaric acid excretion, and 4) the course of eye disease in treated cblG given the apparent reversal of visual tract damage.

Gene promoter methylation, genomic deletions and amplifications in biopsies from patients with hereditary breast cancer. *M.P. Carvallo[1], C. Alvarez[1], T. Tapia[1], M. Vallejos[1], S. Smalley[1], M.L. Solis[2], A. Corvalán[2], M. Alvarez[2], E. Rozenblum[3], D. Munroe[3]* 1) Department of Cell and Molecular Biology, Faculty of Biological Sciences, Pontificia Universidad Catolica de Chile, Santiago, Chile; 2) Faculty of Medicine, Pontificia Universidad Catolica de Chile, Santiago, Chile; 3) Laboratory of Molecular Technology, SAIC-Frederick Inc., NCI, National Institutes of Health, Frederick,MD, USA.

We have previously screened 54 families with breast/ovarian cancer for mutations at BRCA1 and BRCA2 genes. Close to 80% of these families did not present a mutation in either gene. In an attempt to search for other tumour suppressor genes involved in the progression of breast cancer, we began an analysis of genomic DNA from breast tumour biopsies of non BRCA1/BRCA2 patients. We analyzed the methylation status of BRCA1 and ATM promoters two genes known to be involved in breast cancer. The analysis of the promoter methylation of these two genes in 30 biopsies from women affected with hereditary breast cancer, revealed an 87% of methylation only for ATM. Besides, only 6 biopsies showed methylation in the BRCA1 promoter. There is evidence that most BRCA1 mutated tumours are negative for estrogen receptor (ER) and ERBB2. Consistent with this information, we found 4/6 breast tumour biopsies, ER negative and ERBB2 negative, showing BRCA1 promoter methylation, suggesting a possible role of this gene in tumour's progression. We performed an array-CGH analysis on 8 of these biopsies to analyze genomic deletions and amplifications. The most frequent deletions found involve: 1p31, 3q28, 6p12, 13q14, 13q33, 15q2, and the most frequent amplifications segments comprised are: 3p21, 2p23, 12q14, 10p12. Some interesting genes included in the deleted regions are: BAG2 (Bcl2 associated athanogen 2), RB, ITM2B (Integral membrane protein 2B), and TUSC3 (Tumour suppressor candidate 3) and in the amplified regions: jun, ALK (Anaplastic Lymphoma Kinase), HMGA2 (High mobility group AT-Hook 2) and MAP3K3 (Mitogen-Activated Protein kinase kinase kinase 3).Financed by Fondecyt 1040779.

Large-scale genetic investigation of genome-wide transcriptional profiles. *H.H.H. Göring[1], J.E. Curran[1], M.P. Johnson[1], T.D. Dyer[1], J.B.M. Jowett[2,3], M.C. Mahaney[1], J.W. MacCluer[1], G.R. Collier[3], E.K. Moses[1], J. Blangero[1,3]* 1) Southwest Foundation for Biomedical Research, San Antonio, TX; 2) International Diabetes Institute, Melbourne, Australia; 3) ChemGenex Pharmaceuticals, Geelong, Australia.

Quantitative differences in gene expression are thought to contribute substantially to phenotypic differences among individuals. While environmental stimuli influence the location, timing, and level of gene expression, genetic differences among individuals are also important. Here we sought to localize major genetic determinants of expression of individual transcripts located throughout the human genome. Using Illuminas Sentrix Human-6 gene expression bead chips, we measured the expression levels of 47,289 different transcripts in stored lymphocyte samples from 1,240 members of 40 randomly ascertained Mexican American families in the San Antonio Family Heart Study. 20,413 transcripts (43%) passed quality control. The levels of virtually all transcripts were found to be significantly heritable, validating these measures as potentially useful biological indicators. In order to localize transcript-specific regulators of gene expression, we performed genome-wide variance components-based linkage analysis on the expression levels of all transcripts. At a false discovery rate of 33%, 2,957 autosomal transcripts (15%) were found to be *cis*-regulated, i.e. a significant point-wise lod score was obtained at the genomic location of the gene. This suggests that a substantial proportion of genes harbor genetic variants that influence their own transcription levels. While *trans*-regulation was also found to be important, most of the major genetic factors influencing gene expression appear to be located in *cis*, with >95% of lod scores ?5 being located in the vicinity of the genomic location of the assessed transcript. This is the largest study todate on the genetics of human gene expression, both with regard to the number of phenotyped individuals and the number of investigated transcripts. Our results indicate that identification of *cis*-regulated genes may be of great utility for prioritization of positional candidate genes.

A genome-wide scan for variation in the polyadenylation signal and correlation to expression levels. *L.R.L. Davies[1], C.J. Cotsapas[1,2], R.R. Graham[1,2], D. Altshuler[1,2]* 1) Program in Medical and Population Genetics, Broad Institute of Harvard and the Massachusetts Institute of Technology, Cambridge, MA; 2) Center for Human Genetics Research and Molecular Biology, Massachusetts General Hospital, Boston, MA.

Gene expression levels, which are both heritable and highly variable across populations, may play an important role in interindividual variation, including risk to disease. Several genome-wide scans have concluded that many genetic determinants of expression levels are *cis*-acting variants. The causal alleles of these variants, however, remain undefined.

We are investigating variations in a near-ubiquitous gene feature, the polyadenylation (polyA) signal. This is a short, highly conserved 6 bp motif in the 3UTR that is bound by the cleavage and polyadenylation specificity factor (CPSF). CPSF is part of a complex responsible for cleavage of the mRNA 15-30 bp downstream of the polyA signal and subsequent polyadenylation of the transcript. We have hypothesized that variations in the polyA signal may disrupt the binding of CPSF, altering the expression levels of some transcripts by affecting the rate of polyadenylation and/or forcing the use of alternate signals downstream. In the latter case, the altered 3UTR length may have further effects on transcript stability, binding of 3'UTR proteins, or the length of the polyA tail.

Here we describe a scan of all 32,000 RefSeq isoform 3UTRs for common variation in the canonical form of the polyA signal (AATAAA). Within these coordinates, dbSNP revealed multiple single nucleotide polymorphisms (SNPs) that we predict may alter CPSF binding. We are currently genotyping these variants in the 270 International HapMap Project samples to determine their population frequencies. We will then experimentally validate whether these alleles do in fact alter the length of the 3UTR or mRNA expression levels using northern blots from EBV-transformed B cell lines derived from the HapMap samples. This scan will provide a new resource of functional alleles that may play a role in disease susceptibility.

Fine mapping and candidate gene screening within a major familial Ménière disease locus on human chromosome 14. *M.E.S. Bailey[1], Y. Lowe[1], C. MacKay[1], A.W. Morrison[2], G.A.J. Morrison[3]* 1) Div. of Molecular Genetics, IBLS, Univ. of Glasgow, Glasgow, U.K; 2) Dept. of Otolaryngology, Royal London Hospital, London, U.K; 3) Dept. of Otolaryngology, Guys and St Thomas Hospitals, London, U.K.

Ménière disease (MD) is a relatively common, complex disorder characterised by episodic hearing loss, tinnitus, and rotatory vertigo with nausea and vomiting, probably caused by increased pressure in the cochlea and vestibular system resulting from endolymphatic hydrops. MD has mid-life onset and leads to a significant reduction in quality of life. Approx. 7% of cases are familial, most compatible with autosomal dominant inheritance. Recently, a locus for familial MD on chr.12 has been reported. We have completed the fine mapping phase of a genome scan using a set of 18 MD families. Linkage analysis of 5cM scan data from all the families gave a maximum multipoint HLOD of 2.90 (? = 55% of families linked, 80% penetrance) indicating potential linkage to a single locus in chromosome 14q21-q22. Fine mapping in the linked families using additional markers has led to enhanced evidence for linkage (HLOD 4.19, ? = 55%, 80% penetrance). Meiotic recombination analysis has enabled us to delineate a bipartite critical region that must contain the causative gene. The minimum critical region (defined using recombinant unaffecteds) of 3 Mbp contains only 2 known genes, *MAMDC1* and *RPL10L*, and a putative expressed locus, *LOC645178*. All exons and putative promoters of these genes have been screened by SSCP and sequencing of patients from linked families. A maximum critical region (using only affected recombinants) of 5 Mbp containing 16 genes has also been delineated. Screening of the next best candidate gene in this region, *BTBD5*, has also been completed. No candidate causative variants have been discovered. Additional candidate genes are being screened and novel markers genotyped, but at present the identity of the predisposing gene at this locus remains elusive. Identification of the gene in the linked families should suggest candidates to screen in the remaining families and illuminate pathways contributing to susceptibility to the more common, sporadic cases of MD.

MOM-PAP-A detects ultra rare deletions and demonstrates that normal lung contains the EGFR microdeletions commonly found in lung cancers. *Z. Chen, J. Feng, S. Sommer* Molec Gen & Molec Diagnosis, City of Hope Natl Med Ctr, Duarte, CA.

In-frame deletions of 15 or 18 bp in the EGFR gene are common in non-small-cell lung cancers and predict response to the tyrosine kinase inhibitors. We hypothesized that these common deletions in tumors derive from rare mutations that occur during lung development and are enriched in normal histological lung tissue by positive selection. To test this Selection Hypothesis for EGFR Deletions (SHED), we developed Multiple Oligonucleotide Mismatch PAP-A (MOM-PAP-A), a sensitive and specific form of Pyrophosphorolysis-Activated Polymerization for Amplification of specific alleles (PAP-A) for detecting mutations other than single base substitutions, including deletions and complex mutations. MOM-PAP-A has the sensitivity to reproducibly detect one mutant molecule in mammalian DNA and can amplify the mutant segment about 4 trillion-fold without nesting. The selectivity of detection was >1×109 wild-type genomes when PAP oligonucleotides mismatch the wild-type at 2-5 bases. MOM-PAP-A revealed that the common 15 and 18 bp somatic EGFR deletions were enriched in normal lung far above that normally expected frequency for 15 and 18 bp common deletions. The common 15 and 18 bp deletions were found in about 6 per ten million normal human lung cells but not in blood cells. An EGFR exon 3 deletion and an EGFR2 deletion homologous to the EGFR 15 bp deletion were not detected in either lung or blood. EGFR and EGFR2 assays were developed for rat, revealing that only the homologous 15 and 18 bp EGFR mutations are present in lung at about 2 per million cells, but not in liver. Mutation frequencies increase ~50-fold from rat pups to adulthood and are similar in young and old adult rats despite a six-fold difference in age, consistent with substantial positive selection occurring before adulthood. The human cells with 15 and 18 bp EGFR deletions may be viewed as premalignant cells in that they are roughly 300,000 times more likely than average adult cells to give rise to lung cancer. PAP-based testing of EGFR mutations potentially offers an approach for rapid, low cost, early screening for early detection of a subset of lung cancer.

The BCL2 gene and aetiology of autoimmune disease. *K. Gendall, C. Guja, A. Colgan, R. Rodger, M. Merriman, P. Chapman, E. Gale, K. Gillespie, P. Gow, A. Harrison, J. Highton, P. Jones, J. ODonnell, S. Pearce, D. Smyth, J.A. Todd, T. Merriman* University of Otago, New Zealand.

Background Human chromosome 18q12-q23 has been linked to several autoimmune diseases, including type 1 diabetes (T1D), rheumatoid arthritis (RA) and Graves disease (GD). This may represent a common susceptibility gene or genes. The BCL2 gene is a candidate in the region due to its function as a negative regulator of apoptosis. Association of *BCL2* in autoimmune diseases has been found in two out of four published studies.

Aim To test the BCL2 gene for association in five independent autoimmune sample sets.

Results Ten *BCL2* SNPs were tested for association in a NZ autoimmune case-control cohort (1,187 RA, T1D and GD cases; 913 controls). Two were nominally associated with autoimmunity in a combination of all three case samples (rs1481032, OR = 0.87, $P = 0.03$; rs2849382, OR = 0.72, $P = 0.001$). These two SNPs were then typed in three UK case-control cohorts (384 T1D cases, 259 controls; 1,919 T1D cases, 1,772 controls; 465 GD cases, 376 controls) and a Caucasian cohort of ~2,000 T1D families. rs2849382 showed no evidence of association in any of the T1D samples (small case-control OR = 0.84, $P = 0.31$; large case-control OR = 1.12, $P = 0.184$; families OR = 1.11, $P = 0.12$) or in the UK GD sample (OR = 1.17, $P = 0.27$). The minor allele of rs1481032 was undertransmitted in the T1D families (%T = 46.8, OR = 0.88, $P = 0.04$), and some association with disease was found in the small T1D case-control group (OR = 0.62, $P = 0.02$). However, rs1481032 was not associated in the large T1D case-control group (OR = 1.01, $P = 0.90$) or the GD samples (OR = 0.83, $P = 0.18$). Meta-analysis of these data using Fishers method found some evidence of association (rs2849382 $P = 0.002$; rs1481032 $P = 0.008$).

Conclusion Nominal evidence of association with *BCL2* was found. However, a more thorough testing of markers in the gene is required.

CRYPTIC INVERSION X UNBALANCED RECOMBINATION IN NEW BORN MALE. *I. GADI*[1], *J. TEPPERBERG*[1], *R. SLOTNICK*[2], *V. JASWANEY*[1], *J. KESLER*[1], *T. ROYESTER*[1], *C. BULLEN*[1], *T. SAPP*[1], *P. PAPENHAUSEN*[1] 1) Cytogenetics, Laboratory Corporation of America, RTP, NC; 2) Robert Slotnick, Washoe Medical Center, 77 Pringle Way, Reno, NV 89502.

We report a newborn baby boy with effective nullisomy for the distal short arm of the X chromosome. The baby was born with multiple anomalies to a 33 year old white female gravida 1, para 1. The head circumference of the baby measured 32.5 cm (10th percentile), inter canthal distance=2.25 cm (about 50th percentile), outer canthal distance=6 cm (about 25th percentile), palpebral fissures=1.75 cm (50th percentile). The pregnancy was unremarkable until the 2nd trimester when the second trimester test showed an elevated risk of Down syndrome (1 in 177). Amniocyte and postnatal blood chromosome analysis revealed an apparently normal karyotype. Second trimester ultra sound showed short long bones (humerous, ulna, radius, femur, tibia and fibula) all measured at less than the 5th percentile for the gestation age. There was neonatal persistence of short limbs particularly of the long bones. Lower extremities and feet were normal, but the upper extremities showed posturing of both hands. The left hand showed ulnar deviation of the first finger and bilateral clinodactyly. Chest was normal in size, although the nipples were widely spaced and hypoplastic. External genitalia appeared normal except cryptorchidism and a suggestion of mild hypospadias. An opthalmology examination identified ocular albinism. Ancillary subtelomere FISH on the postnatal chromosomes showed a deletion of the X chromosome short arm subtelomere, and an extra copy of X chromosome long arm subtelomere attached to the distal Xp short arm. Maternal chromosome FISH revealed a pericentric inversion of an X chromosome suggesting unbalanced recombination as the cause of the abnormal X chromosome in the child. Additional FISH showed loss of loci for STS, Kallmann and presumably the genes associated with X-linked ocular albinism and Leri-Weil dyschondrostesis from the distal short arm of the X chromosome.

Knocking-Down KIF3A in Neural Crest Cells Results in Crainofacial Dysmorphologies. *N. Allen, S. Brugmann, J.A. Helms* Surgery, Stanford University, Palo Alto, CA.

KIF3A, a member of the kinesin-2 anterograde motor transport protein family, is involved in intracellular transport of molecules. It is required for cilia formation and maintenance, as well as successful signal transduction of the Hedgehog (Hh) pathway (Huangfu, 2005). We analyzed KIF3A expression patterns in wild-type mice during the growth and development of the craniofacial prominences. KIF3A is robustly expressed in both the cranial neural crest (CNC) derived mesenchyme and overlying surface ectoderm at the distal tip of the developing frontal nasal process (FNP). It is also expressed throughout the dorsal neuroectoderm in both the ventricular and marginal zones. Knocking-out KIF3A in CNC cells using the Wnt-1 Cre recombinase system produces a mutant with a myriad of craniofacial defects. At e12.5 the mutant has a significantly larger head compared to its wildtype littermates, which either causes or is the result of an extremely enlarged brain. In addition, the survival of neural crest is compromised, as the number of CNC cells within the FNP is significantly reduced in mutant embryos. By e16.5 numerous skeletal defects are evident. The nasal septum is severely truncated and is bifurcated along the sagittal axis. Furthermore, the width of lateral cartilages of the nasal capsule is significantly increased relative to the stage matched wild-type animal. We are currently performing molecular analyses in an attempt to elucidate how precluding normal KIF3A expression in CNC generates the observed phenotype.
Reference: Huangfu D., Anderson K.V. (2005) Cilia and Hedgehog responsiveness in the mouse. PNAS. 102(32), 11325-30.

High Frequency of Neurexin 1 Signal Peptide Structural Variants in Patients with Autism. *J. Feng[1], R. Schroer[2], J. Yan[1], W. Song[1], C. Yang[1], E. Cook, Jr.[3], C. Skinner[2], C. Schwartz[2], S. Sommer[1]* 1) Department of Molecular Genetics, City of Hope National Medical Center, Duarte, CA; 2) J.C. Self Research Institute, The Greenwood Genetic Center, SC; 3) Institute for Juvenile Research, Department of Psychiatry, University of Illinois at Chicago, IL.

Neuroligins are postsynaptic membrane cell-adhesion molecules which bind to -neurexins, a family of proteins that act as neuronal cell surface receptors. To explore the possibility that structural variants in the -neurexin genes predispose to autism, the coding regions and associated splice junctions of three -neurexin genes were scanned with DOVAM-S (Detection of Virtually All Mutations-SSCP) in 72 Caucasian patients with autism. In addition, segments of the neurexin 1 gene were sequenced in 131 additional Caucasian and 61 African American patients with autism from South Carolina and the Midwest. Two putative missense structural variants were identified in the neurexin 1 gene in four Caucasian patients with autism and not in 535 healthy Caucasian controls (4/203 vs 0/535, P=0.0056). Initial family data suggest that incomplete penetrance may occur. In addition, no structural variant was found in the neurexin 2 gene or the neurexin 3 gene. In the context of all available data, we conclude that mutations in the neurexin 1 gene may contribute to about 2% of autism cases.

MOLECULAR CHARACTERIZATION OF LOW AND HIGH-GRADE OLIGODENDROGLIOMAS. *M. Gadji[1], A.M. Tsanaclis[2], D. Fortin[3], R. Drouin[1]* 1) Service of Genetics, Dept of Pediatrics; 2) Dept of Pathology, Centre Hospitalier Universitaire de Sherbrooke (CHUS); 3) Service of neurosurgery, Dept of Surgery, Faculty of Medicine and Health Sciences, CHUS, Universite de Sherbrooke, Sherbrooke, QC, Canada.

Malignant gliomas are the most common primary brain tumors and include astrocytomas, oligodendrogliomas, and oligoastrocytomas. Oligodendrogliomas constitute about 5% to 30% of all gliomas and their pathogenesis is largely unknown. Molecular studies have identified loss of chromosomal arms 1p and 19q in 50-70% and 65-80% of oligodendrogliomas, respectively. The combined loss of 1p and 19q has proven to be a powerful predictor of a favorable chemotherapeutic response and survival in oligodendrogliomas. We have undertaken a retrospective study of oligodendroglial tumors followed at the CHUS using cytogenetic and molecular genetic techniques. The patient population was composed of 25 persons including 34.3% males and 64.7% females ranging in age from 18 to 77 years with a median age of 40.9 years. Fluorescent In Situ Hybridization using LSI 1p36/1q25 and LSI 19q13/19p13 probes was performed on 21 paraffin embedded tissues retrospectively. The results displayed 16 1p/19q-combined deletions (1p-/19q-); one patient displayed 1p- only, one patient displayed 19q- only and three cases did not show any deletion. To define the deletion length, we performed LOH (Loss Of Heterozygosity) screening with microsatellite marker mapping 1p and 19q after standard PCR-based LOH. Tumor DNA was extracted from 7 paraffin-embedded tissues and from three surgical biopsies. Constitutional DNA was extracted from peripheral blood leukocytes and from saliva in five cases. The preliminary results map the deletions between D1S2795 (1p36.31) locus and D1S2722 (1p34.2) locus and between D19S412 (19q13.32) locus and D19S422 (19q13.13) locus. Kaplan-Meier curves depicting the survival of patients between 0.3 and 262 months and bearing 1p-/19q- displayed a median survival time of 146 months and 136.5 months for oligodendrogliomas and anaplastic oligodendrogliomas, respectively. The average survival obtained in our study is longer than that from previously published studies, possibly due to our more aggressive treatment approaches.

Independent replication of autism association in several neuronal connectivity genes. *J.A. Duvall[1,2], J.L. Stone[1], R.M. Cantor[1], S.F. Nelson[1], D.H. Geschwind[1,2]* 1) Department of Human Genetics, University of California, Los Angeles; 2) Department of Neurology, University of California, Los Angeles.

Autism is a neurodevelopmental disorder which has a significant genetic component and that is characterized by language difficulties, social deficits, and repetitive, stereotyped behaviors. Recently we presented the results of a high density association analysis of 35 neuronal connectivity genes in 224 AGRE autism trios and identified nine genes in which nominal association was observed and for which follow-up analysis was warranted. Here we present the results of an independent follow-up analysis. From the nine genes of interest previously identified, GABRB3, NRXN1, NRXN3, NLGN4, EFNB2, SLIT1, NTN4, SEMA3A, and PLAUR, we genotyped 125 SNPs in an additional 343 AGRE autism trios. The Transmission Disequilibrium Test (TDT) was performed on individual SNPs to determine autism association. Four SNPs showing significant autism association (TDT p0.05) in both the original analysis and the follow-up analysis were identified in three genes: NRXN3, SLIT1, and GABRB3. These findings indicate that genes involved in neuronal connectivity pathways may contribute to autism in some individuals, but also suggest that the contribution from each of these variants is likely to be small. This study presents the first attempt to provide comphrehensive genetic evidence for disruption of biological pathways related to neuronal connectivity in autism pathogenesis. Association analysis using haplotype blocks and testing of other pathway members is ongoing.

***VR22* and *LRRTM3* genes have susceptibility alleles with replicable association in multiple Late-Onset Alzheimers Disease (LOAD) series.** *N. Ertekin-Taner[1,2], M. Allen[3], S. Younkin[3], L. Younkin[3], M. Carrasquillo[3], F. Zou[3], N. Graff-Radford[4], B. Boeve[2], R. Petersen[2], S.G. Younkin[3]* 1) Dept Neuroscience, Mayo Clinic, Rochester, MN; 2) Dept Neurology, Mayo Clinic, Rochester, MN; 3) Dept Neuroscience, Mayo Clinic, Jacksonville, FL; 4) Dept Neurology, Mayo Clinic, Jacksonville, FL.

Alpha-T catenin (*VR22*) on chromosome 10 is an excellent functional and positional candidate LOAD gene. Leucine rich transmembrane protein 3 (*LRRTM3*) resides within the seventh intron of *VR22*. We genotyped 8 SNPs within *LRRTM3* and analyzed them in 3 independent LOAD case-control series. Due to weak linkage disequilibrum (LD), the 8 *LRRTM3* variants formed 20 haplotypes with allele frequencies >1%, accounting for 86% of all *LRRTM3* haplotypes. *LRRTM3* single SNPs or haplotypes did not reveal significant association with LOAD with logistic regression controlling for age, gender and ApoE. The *LRRTM3* haplotype pairs formed 25 common multilocus genotypes (MLGs) which occurred 10 times in the combined series (ages 60-75). In the exploratory series, 6 protective MLGs were identified. Compared to this set of protective MLGs, the remaining MLGs were significantly risky in the exploratory series with an OR of 3.91 (1.61-9.48, p=0.003). Remarkably, this finding was replicated in both follow-up series where the risky MLGs had ORs of 2.99 (1.37-6.54, p=0.006) and 1.97 (0.99-3.94, p=0.055). In the combined series, the OR for the set of risky multilocus genotypes was 2.71 (1.68-4.37) with a p=0.000019. The set of protective *LRRTM3* genotypes are found in 15% and the risky set in 85% of control subjects. This yields a substantial population attributable risk of 59%. The entire *LRRTM3* gene is contained within *VR22*. Functional studies are underway to determine if the risky *LRRTM3* genotypes are associated with altered LRRTM3 and/or VR22 mRNA levels. We have shown that *VR22* has variants that account for the chromosome 10 A42 linkage signal we previously reported. These *VR22* variants and the *LRRTM3* variants are not in LD, suggesting independent effects. Our ongoing analysis suggests that *VR22* also has variants that independently influence risk for LOAD.

Eight genes associated with non-syndromic x-linked mental retardation: the whole is the sum of its parts. *H. Gao[1], K. Gonzalez[1], J. Filiano[2], J. Zheng[1], L. Chen[1], L. Moore[1], V. Kimonis[3], S. Sommer[1]* 1) Clinical Molecular Diagnostic , City of Hope Medical Center, Duarte, CA; 2) Dartmouth Hitchcock Medical Center, Lebanon, NH 03756; 3) Boston Children Hospital, Boston, MA 02115.

X-linked mental retardation (XLMR) has a prevalence of 2.6 cases per 1,000 in the general population, accounting for over 10% of all cases of mental retardation. It is estimated that 2/3 of X-linked mental retardation is non-syndromic (mental retardation without other distinguishing features). Recently, multiple genes have been implicated in non-syndromic XLMR. We have selected eight genes with solid evidence and high attributable rate for a non-syndromic XLMR panel. This panel includes ARX (Aristaless Related Homeobox), DLG3 (Discs, Large Homolog 3), FACL4 (Fatty Acid CoA Ligase 4), FTSJ1 (FTSJ Homolog 1), JARID1C (Jumonji/ARID domain-containing protein 1C), PQBP1 (Polyglutamine Binding Protein 1), TM4SF2 (Transmembrane 4 Superfamily Member 2) and ZNF41 (Zinc Finger Protein 41). Based on publications on individual genes, the estimated detection rate of this XLMR panel is 15-25% of non-syndromic XLMR patients, assuming little or no publication bias. We performed the first multi-gene survey. Since the XLMR panel was offered for clinical testing, 101 clinical samples had been analyzed for the 8 genes. Among them, 38 patients have X-linked family history of mental retardation or developmental delay (class I); 27 patients are sporadic cases (Class II); 36 patients lack clinical information or family history (class III). We identified eight putative mutations in class I and one in class III patients. The detection rate is 12-23% depend on what proportion are X-linked in class III patients. PQBP1 and JARID1c were the most frequently mutated genes. Current data suggests that these eight genes do account for 15-25% of XLMR mutations.

Population genetics and comparative genomics of FUS, a gene involved in malignant translocation. *V. Bekker[1], N. Orr[1], S. Chanock[1,2,3]* 1) Section on Genomic Variation, Pediatric Oncology Branch, Center for Cancer Research, NCI, Bethesda, MD; 2) Division of Cancer Epidemiology and Genetics, NCI, Bethesda, MD; 3) Core Genotyping Facility, NCI, Gaithersburg, MD.

The somatic t(11;22) translocation resulting in the fusion of *EWS* and *FLI1* is present in over 85% of cases of Ewings Sarcoma (ES). The remainder of cases arises when *EWS* fuses to one of five members of the ETS gene family (of which *FLI1* is also included). *FUS*, an oncogene found as a fusion partner in liposarcoma and acute myeloid leukemia, has been reported in rare instances to replace *EWS* in ES. Comparison of the genomic structures of *EWS* and *FUS* has revealed highly similar exons. We have characterized population genetic variation in *FUS* to gain further insight into the similarities between these two genes.

Having identified a region of striking conservation in the *EWS* breakpoint region, we conducted a similar analysis for *FUS*. We found a region of 3.2 kb which had a conservation score of 0.712 using the phastCons17way algorithm. Of the small number of cases of ES involving *FUS* reported, all breakpoints occurred in this region, of which we have resequenced 1.6 kb in the SNP500 Cancer population (32 Caucasians, 24 African Americans, 23 admixed/Hispanic and 23 Pacific Rim individuals). Six SNPs were detected and one major haplotype accounted for 93% of the total. Nucleotide diversity, pi, was $9x10^{-5}$ and population mutation frequency, theta, was $51x10^{-5}$. We detected no signatures of recent selection. We compared the linkage disequilibrium and haplotype block structure of *FUS* using HapMap phase II data. LD patterns in both the Caucasian and combined Chinese and Japanese populations were similar, with *FUS* lying at the 5-prime end of blocks of 106 kb and 87 kb respectively. There is evidence for recombination in the Yoruba population in which *FUS* resides in a 47 kb block. Overall haplotype diversity was low (Hd =0.131) and was, as expected, highest in the Yoruba and relatively low in the other populations. These data suggest no sign of recent selection and similar to *EWS* limited haplotype heterozygosity. Further studies are warranted to investigate the population genetic of *FUS*.

Clinical Abnormalities in a Woman with Interstitial Deletion of the Short Arm of Chromosome 11. *D.S. Bonner[1], B.A. Pletcher[1], G.A. Toruner[2], D.L. Streck[2], F. Alcid[3], F. Desposito[1]* 1) Dept Pediatrics, Cytogenetics, UMDNJ/NJ Medical Schol, Newark, NJ; 2) Dept Microbiology and Molecular Genetics, UNDNJ/NJ Medical School, Newark, NJ; 3) North Jersey Developmental Center, Totowa, NJ.

This report describes a 46 year-old female with dysmorphic features and severe mental retardation (MR) who has a de novo interstitial deletion of the short arm of chromosome 11. In addition, she has peripheral edema, significant kyphoscoliosis, and bipolar disorder. MRI of the brain showed arachnoid cysts in the frontal regions, enlargement of ventricles and cortical sulci, as well as a thin rim of hyperintense T2 signal in the periventricular white matter. She has a history of hypothyroidism, chronic sinusitis and chronic bronchitis. Quantitative immunoglobulins are normal, but IgG subclass studies are still pending. These tests may help to explain her recurrent respiratory infections. The patient has three other siblings who are well and without developmental problems. The cytogenetic analysis revealed an interstitial deletion in the short arm on one chromosome 11, with a karyotype designated 46,XX,del(11)(p11.2p13). Although the patient does not appear to have parietal foramina or exostoses generally found in Potocki-Shaffer syndrome (PSS), high density comparative genomic hybridization (CGH) demonstrated a 14 Mb deletion including the EXT2 and ALX4 loci. Other genes contained in this deletion are GYLTL1B, ACP2, MDK, and SLC35C1, which are associated with many of the clinical features in this patient, including MR, growth failure, kyphoscoliosis, and immunodeficiency. A review of the literature revealed a few similar cases with identical breakpoints, but mostly in newborns or young children. This patient appears to be the first reported case of an older individual with this deletion.

An Inexpensive Bead-Based Oligonucleotide Ligation Assay for SNP Genotyping. *S.E. Bruse[1], N. McGregor[1], M.A. Azaro[1], B. Xu[1], L.M. Brzustowicz[1,2]* 1) Dept Genetics, Rutgers Univ, Piscataway, NJ; 2) New Jersey Medical School, UMDNJ Newark, NJ.

Candidate gene studies typically involve genotyping of dozens of SNPs within a relatively small region. This requires a flexible technology that is inexpensive in the medium throughput range. One such technology is a bead-based assay combining the oligonucleotide ligation assay (OLA) with the Luminex flow cytometry platform. We have introduced some modifications to the standard OLA/Luminex protocol that reduce the cost per genotype, including: (1) use of far fewer beads than previously published reports (2) use of a universal biotinylated oligonucleotide for capture of the reporter molecule (3) use of FlexMAP (Luminex Corporation) microspheres and (4) incorporation of a highly multiplexed PCR. This system is capable of scoring 50 SNPs simultaneously, processing a 96 well plate in about an hour. The cost is less than $0.10 per genotype for a set of 1000 subjects. In addition to the low assay cost, the initial cost of the platform is ~$50,000, versus other platforms whose cost typically exceeds $250,000. We will present data assessing the validity and reliability of the technique, by comparison of the OLA/Luminex method with both direct sequencing and Pyrosequencing. We will present data focusing on issues of intra-well, inter-well, and inter-plate variability and the negligible impact that using fewer beads has on the accuracy of genotype calling. Finally, we will assess assay conversion rates and assay call rates by presenting data from dozens of multiplexed assays that have been used in candidate gene studies of neuropsychiatric disorders.

Genome-wide association scans identify SNPs associated with Metabolic Syndrome risk factors. *K.A. Frazer[1], J.S. Kooner[2], C.A. Aguilar-Salinas[3], D.A. Hinds[1], C.L. Hyde[4], J.C. Chambers[2], G.R. Warens[4], J. Scott[2], D.S. Lee[4], P.M. Milos[4], D.R. Cox[1], J.F. Thompson[4]* 1) Perlegen Sciences, Mountain View CA; 2) Imperial College of Medicine, London; 3) National Institute of Medicine, Mexico City; 4) Pfizer, Groton CT.

Metabolic syndrome is characterized by a cluster of health risks, increased blood pressure, insulin resistance, abdominal obesity, low HDL cholesterol, and increased triglycerides, in one individual resulting in increased risk for heart disease, stroke and diabetes. Although each of these health risk factors has genetic components, it is unclear whether metabolic syndrome is a distinct heritable medical condition. To identify regions of the genome associated with Metabolic syndrome and its subphenotypes, we performed two whole-genome association studies, individually genotyping DNA from 500 individuals with Metabolic syndrome and 500 matched controls for over 200,000 SNPs in each study. Both studies used individuals living in West London, UK, one cohort was composed of males of South Asia ancestry and the other cohort was composed of males of Northern European ancestry. For the replication phase a set of 5000 SNPs associated with Metabolic syndrome or a subphenotype in the initial two whole-genome studies was genotyped in 4000 additional individuals. Four cohorts were included in the replication phase: South Asia females, Northern European females, Mexican females, and Mexican males. Stratification into the subphenotypes confirmed significant associations of many SNPs, including some located in genes previously shown to be associated with HDL cholesterol and triglycerides levels. Several novel associations of SNPs in genomic intervals not previously identified as associated with the various subphenotypes were also observed. However, no SNPs were significantly associated with Metabolic syndrome, suggesting that it may not have a strong heritable components as currently defined. Interestingly, from the SNPs associated with the various subphenotypes we are able to select a panel of SNPs that may be valuable for predicting individuals predisposed to metabolic syndrome and ultimately improve its definition.

Use of challenging sample types on oligo aCGH microarrays. *F. Cifuentes[1], M. Nair[1], C. Rizzo[2], R. Taylor[3]* 1) Genomics Marketing, Agilent Technologies, Santa Clara, CA; 2) Genomics Marketing, Agilent Technologies, Wilmington, DE; 3) Genomics Research and Development, Agilent Technologies, Wilm., DE.

The use of microarray technology has been growing rapidly over the past decade. Array comparative genome hybridization (aCGH) has become the method of choice for the detection of copy number changes in tumors and genetic disorders. When working with patient samples, there are often significant challenges to overcome to obtain useful data from microarray experiments. Examples of the more problematic sample types include limited starting material, tissue heterogeneity, and formalin mediated crosslinking of nucleic acids. We have developed an optimized protocol for preparing genomic DNA samples to be hybridized to our 60mer oligonucleotide CGH microarrays. The improved method does not require a DNA amplification step and the digestion and labeling reactions have been streamlined, resulting in a shorter processing time without compromising array performance. The protocol has been validated on our aCGH microarray platform using DNA from commercial sources, DNA isolated from cell lines, and DNA isolated from frozen tissues or formalin-fixed, paraffin-embedded tissues. Analysis of DNA isolated from colon carcinoma cell line HT29 (ATCC) successfully detected previously identified aberrations in chromosomes 8 and 18 when using as little as 200 nanograms of input DNA. A comparison of frozen and FFPE samples from breast tumor tissue identified the same HER2+ aberration among others. In titration experiments in which normal cells and cancer cells were mixed we could reliably identify aberrations at 30% purity using Agilent 44K and 185K Human CGH microarrays and our CGHAnalytics software, demonstrating the high sensitivity of the platform.

Why is the Internal Thoracic Artery Resistant to the Development of Atherosclerosis? *S. Archacki, G. Angheloiu, D. Schmitt, C. Moravec, S. Hazen, Q. Wang, Cleveland Clinic Foundation* Molecular Genetics, Cleveland Clinic, Cleveland, OH.

Why is the Internal Thoracic Artery Resistant to the Development of Atherosclerosis? Stephen R. Archacki, George Angheloiu, Dave Schmitt, Christine S. Moravec, Stan Hazen and Qing Wang/The Cleveland Clinic Foundation. Background: We used genetics as a tool to answer the question: Why is internal thoracic artery (ITA) resistant to atherosclerosis? The answer to this question has remained a mystery, until now. Amazingly, the worldwide incidence of disease is estimated to be less than 1 percent. We hypothesize that the endothelial cells (EC) of the ITA respond differently to agents such as oxidized-LDL. Methods and Results: We used intact, human ITA and compared its expression profile intact proximal LAD coronary arteriesthe site of most life-threatening disease causing sudden death. With this approach, we identified the underlying mechanisms which demonstrate why this artery does not develop atherosclerosis. The EC from ITA had a different expression of PECAM-1 (+3.0-fold; $p < 1.45 \times 10\text{-}5$) and enhanced expression of RGS-5, a vasodilator (+5.9; $p < 0.003$) and testin-2 (+4.3; $p < 0.039$), a marker for endothelial cell regeneration. In smooth muscle cells, the expression of three genes was markedly up-regulated in ITA: HSP-70 (+6.1), NOT-1 (+6.5), and ATF-3 (+8.3), which have been shown to reduce atherosclerosis. When we harvested EC from these tissues and exposed these pure cell lines to ox-LDL, we found a significant down-regulation of cell surface adhesion molecules on the ECs from the ITA: VCAM-1 (-18.1), ICAM-1 (-62.4), P-Selectin (-122.0) and E-Selectin (-15.3)--all of which can contribute to inflammation of the artery and generate atherosclerosis. These findings were also demonstrated with immunostains on these arteries and Western Blots. Finally, we analyzed a subset of these genes in large case-control studies to generalize the findings of this genetic profile to the American population at large. Conclusion: We have solved the mystery and answered the question as to why the ITA does not develop atherosclerosis. It resists inflammation.

Oculoauriculofrontonasal syndrome in a mexican patient. *LE. BECERRA[1,2], L. ARNAUD[1,2], M. DIAZ[1,2], JM. MANTILLA[1,2], JA. NASTASI[1,2], M. ORTIZ[1,2], JE. GARCIA[1,2], LE. FIGUERA[1,2]* 1) Departamento de Genetica, CIBO, IMSS; 2) Doctorado en Genética Humana, CUCS, Universidad de Guadalajara, Guadalajara, Mexico.

Oculoauriculofrontonasal (OAFN) syndrome was first report in 1968. This syndrome displays an association of features of frontonasal dysplasia and Goldenhar syndrome. Here we report an 8 year-old boy with OAFN syndrome. He was product from second pregnancy from young healthy unrelated parents; his mother had a previous miscarriage with a hydrocephalic fetus. The propositus was obtained after a full term uneventful pregnancy; birth weight was 3,000 gr. Height and Apgar were not recorded. At birth, there were evident bilateral preauricular tags, macrostomia and a tumor mass in right frontal region. Psychomotor development was normal. Actually he is studying elementary education. Physical exploration: head circumpherence 51.4 cm (40th percentile), height 120 cm (3-10 th percentile), weight 23.5 kg (20-30 th centile). The head show a scar in coronal region, right frontal bossing by osseous prominence and low hairline on this side, left facial asymmetry, sparse eyebrows, the right has a triangle form, to palpation there is a lost of continuity on supra-orbital bridge, telecanthus, and bilateral epicanthic folds. There are two epibulbar dermoids in both eye, the biggest is on the right eye. The nose has a medial scar; the nasal bridge is prominent, broad and bifid nasal tip, and long philtrum. Mouth, superior lip with partial harelip, oral cavity with high and narrow palate, superior gum with a medial cleft, crowded teeth (central incisors above lateral), macrostomia, long right oral commissure and this is down turned, long chin; large ear lobules. No other abnormalities were identified. Radiographic studies did show not evidence of spinal defects. Renal ultrasound is normal. The present case has features of OAFN syndrome. This rare syndrome has common manifestation from Frontonasal dysplasia and Goldenhar syndrome (telecanthus, broad and bifid nasal tip and ocular dermoids, preauricular skin tags, and macrostomia). It is considerate a different syndrome, however, it has been proposed as a spectrum of a same entity, as our patients demonstrate.

Nucleocytoplasmic shuttling defects in heterotaxy. *J.E.J Bedard, S.M. Ware* Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Missense, frameshift, and nonsense mutations in the zinc finger transcription factor ZIC3 cause heterotaxy and have also been identified in patients with isolated congenital heart disease. Previously, we developed transactivation and subcellular localization assays to test the function of ZIC3 point mutations in vitro. Aberrant cytoplasmic localization in a subset of mutants suggested that the pathogenesis of ZIC3 mutations results, at least in part, from failure of appropriate cellular trafficking. To further investigate this hypothesis, the nucleocytoplasmic shuttling properties of ZIC3 were examined. Subcellular localization assays designed to span the entire open reading frame of wild type and mutant proteins identified the presence of nucleocytoplasmic transport signals. Three potential nuclear localization signals (NLS) were identified in ZIC3 and site directed mutagenesis was utilized to alter critical residues. The results demonstrate failure of nuclear localization, with the largest decrease occurring when all three NLS sites were mutated, suggesting that these elements may operate jointly to facilitate proper nuclear targeting. Additionally, we identify a cryptic CRM1-dependent nuclear export signal and identify a mutation within this region in a patient with heterotaxy. These results provide the first evidence that control of cellular trafficking of ZIC3 is critical for function and suggest a possible mechanism for transcriptional control during left-right patterning. Identification of mutations in mapped NLS or NES domains in heterotaxy patients demonstrates the functional importance of these domains in cardiac morphogenesis and allows for integration of structural analysis with developmental function.

The Apoptotic Pathway and Idiopathic Talipes Equinovarus. *A.R. Ester[1], X. Tang[1], A. Scott[2], S.H. Blanton[3], J.T. Hecht[1,2]* 1) University of Texas Medical School at Houston, Houston, TX; 2) Shriners Hospital for Children, Houston, TX; 3) Duke University Medical Center, Durham, NC.

Idiopathic Talipes Equinovarus (ITEV), also known as clubfoot, is a common birth defect occurring in 1/700-1000 live births. Approximately 4000 newborns are diagnosed in the US each year. ITEV is a complex disorder in which multiple genes and environmental factors are postulated to play an etiologic role. Several chromosomal deletion regions, 2q31-33, 3q23-24, 4p14-16, 7p22, 13q33-34 and 18q22-23, have been associated with ITEV; these chromosomal regions may harbor genes that contribute to the phenotype of ITEV. Previously, eight microsatellite markers spanning the 2q31-33 deletion region were genotyped and two, GATA149B10 and D2S1371, showed linkage with association to ITEV. Three candidate genes near GATA149B10, caspase 8 (CASP8), 10 (CASP10) and CFLAR, which are involved in the regulation and activation of apoptosis, were genotyped. rs3731714 in CASP10 showed significant linkage with association (p-value of 0.002). These results suggested that variation in genes in the apoptotic pathway, which are important in limb morphogenesis, play a role in the development ITEV. To test this hypothesis the genes downstream of the CASP8 and CASP10 in the apoptotic pathway were identified: CASP3, CASP9, Apaf-1 and Bid (pro-apoptotic genes) and Bcl-2 (anti-apoptotic gene). Eight SNPs in CASP3, 6 in CASP9, 7 in Apaf-1, 8 in Bid, and 5 in Bcl2 were genotyped in 156 multiplex families with ITEV and 255 simplex trios. The Caucasian and Hispanic allele frequencies were different except for the SNPs in CASP9 and CASP10. All of the SNPs except those in CASP 9 were in HWE. Suggestive results for one or more SNPs were found in the following genes: BID, CASP3, CASP9 and CASP10. Additional analyses are underway and gene interactions are being tested.

Mutation Analysis of the Retinoic Acid Receptor Alpha Gene (RARA) in Autism. *A. Eran[1,4], I. Eisenberg[1,2], D. Pinedo[1,4], K. Graham[1,2], M. Galdzicki[1,4], I.A. Holm[1,3], I.S. Kohane[1,3,4], L.M. Kunkel[1,2,3]* 1) Children's Hospital Boston; 2) Howard Hughes Medical Institute; 3) Harvard Medical School; 4) Harvard-MIT Division of Health Sciences and Technology.

Autism is a common neurodevelopmental disorder with a significant genetic component, characterized by a spectrum of social deficits and repetitive behaviors. Retinoic acid plays a crucial role during development of the vertebrate nervous system through interactions with retinoic acid receptors. The retinoic acid receptor alpha (RARA), located on 17q21, controls cell function by directly regulating gene expression when bound to retinoic acid. A strong linkage peak for autism has been recently fine-mapped to 17q21.

To test whether genetic variations in RARA contribute to this linkage peak, we sequenced RARAs conserved elements in autistic and normal individuals, with the autistic population subdivided into individuals that did and did not contribute to this 17q21 linkage peak. Evolutionary conservation, as an index of functionality, was determined by multiple whole genome alignments; Functionality, in the sense of regulatory mechanisms, was predicted computationally. Screened conserved sequences include both non-coding and coding elements, such as microRNA binding sites, transcription factor binding sites (TFBSs), exons, and splice junctions.

Preliminary results suggest that mutations in TFBSs upstream of the RARA gene might be associated with autism. Unknown variants were found in several individuals that contribute to the 17q21 autism linkage peak. We are presently validating these and other findings in a larger number of individuals.

Fine linkage mapping of a cognitive trait to chromosome 14. *S. Buyske[1, 2, 3], M.E. Bates[2, 5], N. Gharani[3], T.C. Matise[3], J.A. Tischfield[2, 3, 4, 5], P. Manowitz[5]* 1) Statistics Dept, Rutgers Univ, Piscataway, NJ; 2) Center of Alcohol Studies, Rutgers Univ, Piscataway, NJ; 3) Genetics Dept, Rutgers Univ, Piscataway, NJ; 4) Pediatrics Dept, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ; 5) Psychiatry Dept, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ.

The Digit Symbol Substitution Test (WAIS-R) is a test of cognitive processes and relies on working memory, sustained attention, perceptual scan, and visual-motor coordination. In one of the first genome scans of a non-language cognitive trait (Behavior Genetics 36(1):65-76, 2006), we previously reported that a 10cM genome linkage scan yielded significant lod scores on chromosome 14 for the Digit Symbol test in the Collaborative Study on the Genetics of Alcoholism (COGA) sample.

We have since followed up with much denser SNP genotype data on a subset of the original sample. Genotyping was provided by Affymetrix and Illumina, originally for the Genetic Analysis Workshop 14. The sample consisted of 1193 individuals in 111 families, averaging 10.8 individuals in 2.8 generations per family. About 62% of the individuals were phenotyped and 79% at least partially genotyped. The mean inter-marker distance was .35 cM.

Using Merlin-Regress with linkage disequilibrium modeling, we found a peak lod score of 3.5 on 14q31 in a well-defined peak with locus-specific heritability of 38%. The 2-unit-of-lod support interval, 53 cM in the previous study, is now 10 cM or about 6.0 megabases wide. Lod scores are above 1.0 over much of chromosome 14 and we are investigating epistatic effects on this chromosome. This data provides further support for the presence of a specific gene(s) involved in human cognition on chromosome 14; identification of such genes will further our understanding of cognitive functioning under both normal and psychiatric conditions.

Clinical diagnostic testing for autosomal recessive polycystic kidney disease. *J. Dong[1], M.A. Chen[1], S. Nolet[1], M.P. Herbst[1], S. Yao[1], Y. Su[1], R. Sayer[1], L. Guay-Woodford[2], L.M. Messiaen[1]* 1) Dept of Genetics, University of Alabama at Birmingham, Birmingham, AL; 2) UAB Depts. of Medical Genetics and Translational Medicine, Birmingham, AL.

Autosomal recessive polycystic kidney disease (ARPKD) (OMIM #263200) is an hereditary renal cystic disease with an incidence of 1 in 20,000 to 1 in 40,000. The disorder occurs primarily in infancy and childhood and is characterized by enlarged kidneys and congenital hepatic fibrosis. There is no evidence for genetic heterogeneity and *PKHD1* is the only gene known to be associated with ARPKD. The *PKHD1* gene spans approximately 470 kb of genomic DNA on 6p and encodes a 4074 amino acids transmembrane protein known as fibrocystin or polyductin. Due to the severe course of the disease, there is a great need for sensitive and reliable genetic testing in families with a child who has the disease. Linkage based testing using 7 microsatellite markers encompassing a 5 cM region of the *PKHD1* locus is available, but can only be applied when the clinical diagnosis was firmly established in the proband. If in doubt about the clinical diagnosis or if no material was stored for DNA testing from the deceased proband, only mutation analysis of the *PKHD1* gene may allow preparation for prenatal diagnosis in the future. So far, 22 unrelated families (including 20 probands and four obligate carrier parents of deceased individuals) have been tested, in which the diagnosis of ARPKD was suspected based on clinical findings. Genomic DNA was extracted from various tissues, including lymphocytes, fresh or frozen liver and kidney samples, and cultured amniocytes. By PCR and bidirectional sequencing of the longest *PKHD1* open reading frame, 23 mutations were identified. These included 2 nonsense, 12 missense, 2 splice site, and 6 frameshift mutations (FS). 4 new potential pathogenic missense alterations were found so far. The algorithms used to evaluate these novel missense alterations for their pathogenic effect will be discussed.

Complex chromosome 10q rearrangement with terminal deletion in a patient with multiple congenital anomalies.
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De novo chromosome 10q terminal deletions are rare. Wulfsberg et al. (1989) proposed a chromosome 10q deletion syndrome that includes patients with terminal deletions, interstitial deletions, and other chromosome rearrangements involving chromosome 10q; however, it has been difficult to characterize due to the small number of patients and wide variation in clinical features. Scigliano et al. (2004) reviewed 41 cases of *de novo* 10qter deletions, excluding interstitial deletions of 10qter and deletions associated with other chromosome rearrangements. The patients showed characteristic prominence of the nasal bridge; hypertelorism; abnormal ears; congenital heart disease; anogenital, renal tract, and limb abnormalities; and behavior problems. We report a 7-month-old female with global developmental delays; she was not yet smiling or rolling. She also had a high pain tolerance, chronic otitis media, and shaking spells. EEG was negative and brain MRI showed Chiari malformation. On physical examination, her weight was below the 5th centile. She had a prominent forehead; deep set eyes with trichiasis; kyphosis; scoliosis; sacral dimple; lower extremity spasticity with hammertoe of the great toes; and two café-au-lait macules. Chromosome analysis of metaphase cells showed extra material on chromosome 10q. Subtelomeric FISH analysis proved a *de novo* deletion of 10q. Parental karyotypes were normal. Further investigation with G-banding and whole chromosome painting revealed the extra material is possibly a tandem duplication of 10q26. The atypical phenotypic manifestations of this patient may be due to the breakpoint locus of the terminal deletion or possible 10q26 tandem duplication. *FGFR2* is part of a recombination cluster on 10q26 and has been associated with craniofacial features seen in our patient (Chiari malformation, high forehead, eyelid and lacrimal anomalies, and chronic otitis media). Further characterization of the deletion and possible duplication with molecular cytogenetic studies may provide insight into the specific presentation of the chromosome 10q deletion syndrome.

Analysis of Transforming Growth Factor 3 gene (*TGFB3*) in Pierre Robin Sequence patients. *D. Bueno[1], L.D. Lopes[2], C.E.R. Amaral[3], M.R. Passos-Bueno[1]* 1) Instituto de Biociencias, USP, Sao Paulo,Brazil; 2) Centro de Reabilitacao das Deformidades Faciais,Brazil; 3) SOBRAPAR,Campinas,Brazil.

Pierre Robin sequence (PRS) consists of at least one of these clinical findings: micrognathia, glossoptosis, obstructive apnea, and cleft palate. The pattern of inheritance of the non-syndromic forms is unknown, and both multifactorial inheritance or rare mutations can be responsible for a proportion of the cases. This etiopathological heterogeneity of PRS has made it difficult to identify genes associated to PRS. In an effort to find these genes, we screened the *TGFB3* gene, a transforming growth factor involved in palatogenesis and Meckel cartilage and bone formation, in patients with non-syndromic PRS. The Tgfb3 knockout animal was described as a model for isolated cleft palate associated with respiratory difficulties. Association between polymorphism in *TGFB3* and cleft lip and/or palate remains controversial. Considering the expression pattern of *TGFB3* and the knockout phenotype, we considered *TGFB3* as a good candidate for PRS. Our sample consisted of 48 unrelated patients with non-syndromic PRS. All of them were excluded for deletions at 22q11 (Velocardiofacial syndrome) by microsatellite marker analysis. All the 7 *TGFB3* exons and their neighboring intronic regions were analyzed by dHPLC or SSCP and abnormal patterns were sequenced. We found three rare altered migrating patterns. Sequence analysis of these last ones revealed that they correspond to two previously described mutations: c.1501GA (rs4252346), detected in two of our patients, and c.1500 CT (rs3917215), found in another patient. Although these mutations were not found in 200 control alleles of our population, a frequency of 1,2% and 0,7 % respectively are described by others (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Snp). We have analysed the parents of each of these affected patients and we verified that all of them were *de novo* changes. Considering the rarity of these mutations and that they are *de novo*, it is possible that they are involved with PRS. Further studies will be performed to elucidate its possible functional effect. CEPID/FAPESP- CNPq.

Genetic heterogeneity in schizophrenia evidenced by co-occurring obsessions and compulsions: A candidate gene study. *P.L. Belmonte[1], M.D. Fallin[2], G. Nestadt[3], A.E. Pulver[3], P.P. Zandi[1]* 1) Mental Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 2) Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 3) Psychiatry and Behavioral Sciences, Johns Hopkins School of Medicine, Baltimore, MD.

Schizophrenia is a highly heritable psychiatric disorder for which no susceptibility genes have been definitively identified. One reason for difficulty in identifying disease genes is genetic heterogeneity. Obsessions and compulsions frequently co-occur with schizophrenia and obsessive-compulsive spectrum disorders aggregate in schizophrenia families. We used obsessive-compulsive disorder (OCD) and symptoms (OCS) as markers of genetic heterogeneity in schizophrenia and tested candidate genes for association with schizophrenia in the presence of such heterogeneity. Our sample included 261 Ashkenazi Jewish case-parent trios from the Epidemiology-Genetics Program in Psychiatry at Johns Hopkins University. A total of 64 genes were selected for testing based on prior linkage, association, or biological plausibility for schizophrenia or bipolar disorder. Of these, 16 are involved with the glutamate system. These are of particular interest as glutamate has been implicated in both schizophrenia and OCD. A total of 440 single-nucleotide polymorphisms (SNPs) were typed with an average of 6.9 SNPs per gene. We performed single-SNP and haplotype-based transmission-disequilibrium tests. Analyses were performed using stratification by OCD or OCS status and differences in association were confirmed by tests of interaction. In stratified analyses, we found highly suggestive evidence (p0.01) of association for the gene *TUBA8* with schizophrenia in those with OCD. Associations of *GRID1* and *GRIN2B* with schizophrenia were highly suggestive in those with OCS. Of the non-glutamate genes, we found highly suggestive evidence of association with schizophrenia in those with OCD or OCS for *SCA1*, *KIF13A*, *ADRA1A*, *DPYSL2* and *GRFA1* (all p0.01). Associations of *PNOC* with schizophrenia was highly suggestive in those without OCD or OCS. These results suggest genes contributing to the risk of schizophrenia may differ for those with and without OCD/OCS.

Frequency of Fabry disease family histories in a prenatal genetic counseling population. *D. Cutillo[1], H. Travers[2], E. O'Rourke[2], E.R. Wassman[3], D. Ramsey[1], A.E. Donnenfeld[1]* 1) Genetic Services, Genzyme , Philadelphia, PA; 2) Genzyme Therapeutics, Cambridge, MA; 3) Genzyme, Westborough, MA.

Recent developments in enzyme replacement therapy (ERT) for Fabry disease have had a significant impact on the treatment of patients with this disorder. Therefore, patients who have a family history of this disorder need to be identified and counseled appropriately. One recent newborn screening study indicates that the incidence of Fabry disease may be as high as 1 in 2750. The purpose of this abstract is to report on the frequency of genetic counseling sessions in a prenatal population in which a family history of Fabry disease is discovered. Between January 2003 and April 2006, 197,475 patients were seen at Genzyme Genetics for prenatal genetic counseling at geographically diverse locations within the United States. The patients were referred for genetic counseling from obstetric or perinatal physician practices. There was no referral relationship or selection bias of patients between the product team or clinical providers of ERT and the genetic counseling service. During this time period a total of 50 patients were counseled for a family history of Fabry disease. This yields a frequency of approximately 1/4000 prenatal patients. In 23/50 families there was a first or second degree relative of the patient affected with Fabry disease. In 27/50 families the patient or partner (40.7%) or a first degree relative (59.3%) was reported to be a carrier for Fabry disease. Only 14/50 patients were aware of ERT availability for Fabrys disease prior to the counseling session. Of all the patients counseled for a family history of Fabry disease, 50.0% of patients were referred for another indication and only 28.0% of patients had some advance knowledge of enzyme replacement therapy for Fabry disease. We conclude that in an unselected population of prenatal patients, a family history of Fabrys disease will be uncovered in approximately 1/4000 families. Primary health care providers and genetic counselors should be kept up-to-date on advances in treatment for Fabry disease and be familiar with specialized referral centers as a resource for these patients and their families.

Very complex, de novo chromosomal abnormality in a newborn with multiple congenital defects - a counseling challenge. *A. El-Hattab[1], M. Velinov[2], G. Valencia[1], A. Perenyi[1]* 1) Department of Pediatrics, SUNY Downstate Medical Center, Brooklyn, NY; 2) Department of Gene and Cell medicine, Mount Sinai School of Medicine, New York, NY.

Introduction: A case of complex, de novo chromosomal anomalies (terminal 1q and interstitial 2p deletions and 1p duplication) in a newborn with dysmorphic features, agenesis of corpus callosum, cholestasis, and vesicouretral reflux. **Clinical report:** This was the third pregnancy of a 32 years- old healthy mother with unremarkable family history. Prenatal Ultrasound revealed absence of corpus callosum. Amniosentesis chromosomal analysis revealed 46,XY,der(1)del(1)(q42) dup(1)(p22p34) dup(1)(p36.1p36.3) t(1:14)(p22:q32) der(2)del(2)(p23) ins(2:1)(q31:p36.1p36.3) ins(2:1)(q35:?) inv(2)(p23q31). Baby boy was born at term, with birth weight of 3.115 Kg (25th-50th centile) and HC=35 cm (75th-90th centile). Dysmorphic features include high forehead, ridged metopic suture, coarse and rounded face, hypertelorism, prominent eyes, micrognathia, broad depressed nasal bridge, long flat philtrum, low set ears, short neck, prominent occiput, polydactyly, clinodactaly, flexion deformities of the hand fingers, and small hypoplastic nails of the toes. Eye exam revealed retinosclerosis. VCUG revealed bilateral grade 3 vesicouretral reflux. Direct hyperbilirubinemia developed, HIDA scan was suggestive of biliary atresis. **Discussion:** Terminal chromosome 1q deletion, chromosome 1p duplication and chromosome 2p interstitial deletion each is a definable syndrome with few reports on each. This case is the first reported case that has the combination of all these chromosomal abnormalities and features from these syndromes plus other features- such as the cholestasis- that were never reported with any of these syndromes. This very rare/unique case illustrates the difficulties that such complex chromosomal abnormality may present to genetic counseling and care, since it would be difficult/impossible to predict the phenotypic consequences of this abnormality. Further molecular evaluations using Comparative Genome Hybridization may contribute for more precise determination of the chromosomal unbalance in such cases.

A rare single nucleotide polymorphism within mature miR-125a alters its biogenesis. *R. Duan[1], C. Pak[1,2], P. Jin[1,2]*
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microRNAs (miRNAs) are ~22-nucleotide non-coding RNAs that inhibit expression of specific target genes at the posttranscriptional level. miRNAs are initially transcribed as long primary transcripts (pri-miRNAs) that are then processed to ~65 nucleotide hairpin precursor miRNAs (pre-miRNA). pre-miRNAs are subsequently exported from the nucleus and cleaved by the RNaseIII Dicer to generate mature miRNAs. Since polymorphisms within miRNA genes could influence the biogenesis and/or target selection of miRNAs, we screened all known miRNAs in the human genome for single nucleotide polymorphisms (SNPs). Of the 462 known human miRNAs we have identified the presence of 14 SNPs. Interestingly, a G-T polymorphism was identified at the eighth nucleotide of mature mir-125a (mir-125a-SNP). While the 2^{nd}-8^{th} nucleotides of mature miRNAs have been proposed to act as seed sequences critical for miRNA-mediated translational control, we have found that this SNP does not influence mir-125a-mediated translational modulation. However, subsequent expreiments revealed a significant reduction in mature mir-125a-SNP levels *in vivo*. RNA folding analysis indicated altered secondary structure of pri- and pre-mir125a-SNP that may affect the biogenesis of this miRNA. Consistent with this prediction, we have found that this SNP alters processing of pri-mir-125a-SNP to pre-mir-125a-SNP. To further validate the role of the altered secondary structure in miRNA processing we generated a double mutant (mir-125a-DM) that contained the G-T polymorphism in addition to a complementary C-A base change that should preserve the seconday structure of pri- and pre-mir-125a. We have found the mir-125a-DM is processed normally. Together, these data suggest that instead of altering translational modulation, the SNP associated with mature mir-125a could alter the secondary structure of pri- and pre-mir-125a and specifically affect processing of pri- to pre-mir-125a. Furthermore, identification of SNPs associated with miRNAs will facilitate understanding of the biogenesis of miRNAs and miRNA-mediated gene regulation.

Linkage study of familial thoracic aortic aneurysms and dissections to known chromosomal loci. *D.C. Guo[1], H. Pannu[1], V.T. Tran-Fadulu[1], N. Avidan[1], R.K. Yu[3], A.L. Estrera[2], H.J. Safi[2], M.C. Willing[5], D. Divecha[1], J.H. Chen[1], J. Yuan[1], R. He[6], S.E. Scherer[4], S. Shete[3], D.M. Milewicz[1]* 1) Dept Internal Medicine, Univ Texas/Houston Med Sch, Houston, TX; 2) Dept Cardiovascular Surgery, Univ Texas/Houston Med Sch, Houston, TX; 3) Dept Epidemiology, Univ Texas M.D. Anderson Cancer Center, Houston, TX; 4) Human Genome Sequencing Center, Baylor College of Medicine, Houston, Texas; 5) Dept Pediatrics, Univ Iowa, Iowa City, Iowa; 6) Dept Internal Medicine, Medical College of Shanghai Jiaotong Univ, Shanghai, China.

Aortic aneurysms and dissections are the major diseases affecting the aorta, and a leading cause of morbidity and mortality in the United States. Genetic studies of familial thoracic aortic aneurysms and dissections (TAADs) have identified five chromosomal loci, TAAD1, TAAD2 (TGFBR2), TAAD3 (15q24-26), FAA1, and TAAD/PDA (MYH11) and three genes, TGFBR1, TGFBR2, and MYH11 that are responses to familial TAAD. To investigate genetic conditions predispose individuals to TAADs, our laboratory has recruited 345 TAAD families with 2 or more affected family numbers. Twenty percent of families have no living affected family members and half of the families have only 1 affected family member or are parent-child combination. Multipoint linkage analysis was performed on 59 families that have DNA samples collected from more than three affected individuals on known TAAD loci including TAAD1, TAAD3, and FAA1. Based on families with lod scores greater than 0.6 for these loci, 10% of families show linkage to TAAD1, 5% to TAAD3, and one family to FAA1, with total lod scores of 5.5, 5.05, and 0.6, respectively. Twenty-two families showed low positive lod scores (<0.6) to at least one locus. In addition, sequencing analysis was performed for TGFBR1, TGFBR2, and MYH11 on sampled collected from 100, 200, and 96 affected individuals from unrelated TAAD families, respectively. Mutations were found in 1 family for TGFBR1, 6 families for TGFBR2, and 2 families for MYH11. Interestedly, in 11 families, no linkage to any known loci provides evidence indicating 1 or more TAAD loci yet to be identified.

Molecular and clinical characterization of patients with putative Li Fraumeni Syndrome sent for p53 molecular diagnostic testing. *K.D. Gonzalez, C.H. Buzin, K.A. Noltner, D. Gu, W.A. Scaringe* Dept of Molecular Diagnosis, City of Hope Natl Medical Center, Duarte, CA.

Li Fraumeni Syndrome (LFS) is an autosomal dominant inherited cancer predisposition syndrome caused by germline mutations in the p53 gene. Classic LFS is characterized by a proband with a bone or soft tissue sarcoma diagnosed under 45, a first degree relative with cancer diagnosed under 45, and another first or second degree relative with cancer under 45 or a sarcoma at any age. Patients with a germline p53 mutation have a 90% risk by age 60. There are less strict clinical criteria which have been developed for Li Fraumeni-like syndrome (LFL; Birch criteria, Eeles criteria). We analyzed clinical samples that were submitted to the Clinical Molecular Diagnostic Laboratory (CMDL) at City of Hope for diagnostic testing of the p53 gene. We identified 91 positive families from 550 submitted samples, and clinically categorized 264 families (61 positives, 204 negatives). Several interesting and novel findings have emerged. All positive families had one of four core cancers: adrenocortical carcinoma, choroid plexus carcinoma, sarcoma, or breast cancer. All families which lacked these core cancers were negative. Among all families who met classic LFS, 62.5% were positive and among families meeting the Birch and Eeles criteria (LFL), 16% and 19%, respectively, were positive. Interestingly, 20% of families who did not meet LFS or LFL criteria had a p53 mutation. **Four de novo mutations were identified.** All have either an adrenocortical carcinoma or a choroid plexus carcinoma, or both. Additionally, there are nine other possible new mutations based on negative family histories. We conclude that new mutations are more prevalent than previously thought. In addition, choroid plexus tumors are due to p53 germline mutations until shown otherwise. All eight patients (100%) with choroid plexus carcinoma had a p53 mutation and all had negative family histories. Based on the experience gained from this largest single set of patients with identified p53 mutations, we propose a comprehensive set of clinical criteria for predicting the likelihood of p53 germline mutations.

Is Ménières disease associated with polymorphisms in KCNE1 or KCNE3 in the United States? *C.A. Campbell*[1,2], *C.C. Della Santina*[3], *N.B. Smith*[3], *J.P. Carey*[3], *L.B. Minor*[3], *R.J.H. Smith*[1,2] 1) Dept of Otolaryngology, University of Iowa, Iowa City, IA; 2) Interdepartmental PhD Program in Genetics, University of Iowa, Iowa City, IA; 3) Dept of Otolaryngology, Dept of Biomedical Engineering, and Dept of Neuroscience, The Johns Hopkins University, Baltimore, MD.

Ménières disease (MD) is a complex disorder of unknown etiology characterized by the symptoms of vertigo, sensorineural hearing loss and tinnitus. Its incidence in Caucasians is 1-2 per 10,000 and in the Japanese, 35-160 per 1,000,000 (Morrison 1995). Although candidate genes studies focused on COCH (coagulation factor C homology), ATQ1 (antiquitin) and AQP2 (aquaporin 2) have been unsuccessful in identifying disease-causing allele variants of these genes, Doi and colleagues have reported that two single nucleotide polymorphisms (SNPs) in KCNE1 and KCNE3 are associated with MD in Japanese patients (Doi et al. 2005). These two genes encode potassium channels that are expressed in the stria vascularis and endolymphatic sac, respectively. Their role in ion transport and their expression pattern suggest that they may be important in inner ear homeostasis.

To establish whether a similar association exists in the Caucasian MD population, we sequenced the coding regions and exon-intron boundaries of both genes in ~90 persons with MD and compared results to 168 ethnically matched CEPH controls. Neither of the two reported SNPs were significantly associated with MD (KCNE1, p=0.49; KCNE3, p=0.70). Furthermore, comparison of allele frequencies between the Japanese MD population and our study population revealed no significant difference between groups (KCNE1, p= 0.72; KCNE3, p=0.40), suggesting that the significant differences reported in the Japanese study arose from their control population. Consistent with this possibility, we found allele frequencies between the two control populations to be significantly different (KCNE1, p=0.00; KCNE3, p=0.0008). A comparison of the KCNE3 SNP allele frequency in their control population and the HapMap JPT population is also highly significant (p=0.00). Our data show that SNPs in KCNE1 and KCNE3 are not associated with MD in Caucasians.

Function Follows Form: Differential respiratory capacity of mitochondrial complex I mutants in *C. elegans*. *M.J. Falk[1,2], J. Rosenjack[3], M.A. O'Riordan[2], M.M. Sedensky[1,3], P.G. Morgan[1,3,4]* 1) Depts of Genetics; 2) Pediatrics; 3) Anesthesiology; 4) Pharmacology, CASE SOM and Univ Hosp of Cleveland, Cleveland, OH.

Nuclear gene mutations are postulated to be the major cause for mitochondrial oxidative phosphorylation disorders. However, the majority of causative genes are not yet identified and the mechanisms by which their dysfunction results in clinical disease are not known. The nematode, C. elegans, is a useful translational model in which to study the genetic and biochemical basis of mitochondrial dysfunction. We used this model to investigate whether respiratory dysfunction is differentially caused by particular nuclear DNA encoded components of mitochondrial complex I, the largest and most commonly implicated complex in human mitochondrial disease. At least 82% of the 39 nuclear genes encoding human complex I subunits share extensive homology in C. elegans. Utilizing a feeding RNA interference gene knockdown approach, we generated C. elegans mutants for 13 complex I structural subunits. RNA knockdown was confirmed by qRT-PCR. Integrated respiratory capacity of freshly isolated mitochondria from complex I mutants was assayed by polarography. We show that individual nuclear-encoded complex I subunits vary significantly in their impact on integrated complex I-dependent oxidative phosphorylation capacity. Reactive oxygen species damage, as assayed by mitochondrial protein 4-hydroxynonenol antibody staining, also varies by subunit. Importantly, function appears to follow form. Subunits with the greatest impact on respiratory capacity appear to localize to subcomplexes involved in electron transport through complex I. In contrast, subunits known to localize to the membrane-bound arm of complex I minimally impact respiratory capacity. This model organism approach is useful to aid in the rational identification of candidate gene subsets to investigate in human patients with biochemical evidence of mitochondrial complex I dysfunction. It further permits investigation into mechanisms by which individual nuclear genes, when mutated, contribute to human mitochondrial disease.

Functional characterization of an R488H mutation in the plasma membrane carnitine transporter OCTN2, identified in a patient with moderate but symptomatic carnitine deficiency. *R. Gallagher[1, 2], T. Urban[1], C. Morgan[2], S. Packman[2], K. Giacomini[1]* 1) Department of Biopharmaceutical Sciences, Univ California, San Francisco, San Francisco, CA; 2) Department of Pediatrics, Univ California, San Francisco, San Francisco, CA.

During an investigation of the etiology of carnitine deficiency in a symptomatic patient R488H was identified in one OCTN2 allele. R488H had been identified previously only in *cis* with another mutation, A142S. The doubly mutant transporter was reported to be retained in the Golgi and to have very low transport activity in CHO cells. We hypothesized that R488H resulted in the mislocalization. We developed HEK cell lines stably transfected with wild-type and mutant OCTN2 transporters fused to GFP. Transporter localization was assessed by confocal microscopy. Transport assays were performed to determine function.

Both wild-type and R488H transporters localized to the plasma membrane. In contrast, A142S and A142S/R488H transporters showed *both* intracellular retention *and* plasma membrane staining, indicating a partial trafficking defect in HEK cells. This suggests that the mislocalization of the double mutant is due primarily to A142S.

In transport assays the single mutant OCTN2 transporters had mildly reduced function; the double mutant showed an additive effect: A142S: 65% of wild-type, R488H: 74%, A142S/R488H: 48%. Thus, in HEK cells, in contrast to CHO cells, the doubly mutant transporter had significant residual function.

Fractional excretion of carnitine in the parents of the proband indicated that the non-R488H allele caused a more marked reduction in function than the R488H allele. This suggests that the non-R448H allele contributed more significantly to the patient phenotype.

Our results demonstrate the importance of functional assays in establishing the pathogenicity of a nucleotide change. We further conclude that different heterologous cell systems may yield different results, and that these must be correlated with *in vivo* data.

A human fetal cartilage-specific gene expression profile. *V. FUNARI[1], A. DAY[2], D. KRAKOW[1], Z. COHN[1], S. NELSON[2], D. COHN[1,2]* 1) Medical Genetics Institute at Cedars-Sinai Medical Center, LA, CA; 2) Dept. of Human Genetics at UCLA School of Medicine, LA, CA.

Cartilage plays a critical role in human skeletal development, and mutations in many genes uniquely expressed in cartilage are associated with skeletal dysplasias. However, surprisingly little is known about developing cartilage gene expression. To identify genes differentially regulated in human fetal cartilage, 6 fetal cartilage expression profiles were compared to expression profiles from 15 different organs using 46 Affymetrix U133 Plus 2.0 microarrays. Using Significance Analysis of Microarrays, 2,337 probes were identified with significantly higher expression in cartilage. These probes were further validated by comparing their expression in two fetal cartilage samples to 32 non-cartilage containing tissues using 223 Affymetrix U133A/B and 2.0 Plus microarrays. For validation, the signal in cartilage was required to be well measured (intensity >100), and at least five-fold higher than the median expression in non-cartilage tissues. Probes were then ranked by cartilage-specificity, using an analog of coefficient of variation (CV) derived from the non-cartilage tissues. Only probes with a CV of <50% were considered cartilage-specific. By this measure, 177/2,337 probes, representing 146 genes were validated as specifically expressed in cartilage. Many of these genes encode known cartilage-specific extracellular matrix structural proteins and transcripts previously identified in a fetal cartilage EST library, providing confidence that these methods identified fetal cartilage-specific genes. Notably, 12/146 cartilage-specific genes are regulated by NF-KB, including BMP2, LIF, FN1, iNOS, and RELB. This unique expression profile suggests an important but poorly characterized NF-KB regulatory network in growth plate cartilage. Importantly, 23%(33) of the cartilage-specific genes encode unannotated proteins. Overall, these data indicate that the set of genes preferentially or exclusively expressed in cartilage has been greatly underestimated. Many of these genes are likely to have important roles in skeletogenesis and may be candidate genes for skeletal disorders.

Identification of a New Class of 1p36 Deletion Patients Using Array-CGH. *C.A. Bacino[1], S.-H.L. Kang[1], A. Scheffer[2], P.A. Eng[1], J.T. Appleberry[1], J. Li[1], S. Vacha[2], E.R. Roeder[3], V.B. Enciso[3], S. Braddock[4], S.W. Cheung[1]* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Agilent Laboratories, Palo Alto, CA; 3) Division of Genetics and Metabolic Disorders, University of Texas Health Science Center at San Antonio, San Antonio, TX; 4) Division of Medical Genetics, University of Missouri, Columbia, MO.

Monosomy 1p36 is the most common terminal deletion syndrome with an estimated occurrence of 1 in 5000 live births. This deletion results in mental retardation, developmental delay, hearing loss, seizures, cardiac anomalies, in addition to distinct facial features such as large anterior fontanel, deep-set eyes, flat nasal bridge, asymmetric ears, and pointed chin. The rearrangements causing 1p36- are typically observed cytogenetically as a telomeric deletion spanning <10 Mb from 1pter-1p36.23. We report four patients with atypical interstitial deletions proximal to band 1p36.23 detected by array-comparative genomic hybridization (array-CGH). Three patients carry large overlapping deletions of approximately 11.39-14.69 Mb. One patient however, carries a small 2.97 Mb deletion that overlaps with the larger deletions observed in the other three patients. Interestingly these patients manifest clinical characteristics that are different from those seen in classical Monosomy 1p36 deletion syndrome. The clinical presentations in our patients included: prenatal growth deficiency, poor post-natal growth and failure to thrive, feeding difficulties necessitating gastrostomy or NG feedings in some instances, seizures, developmental delay, congenital heart disease, microcephaly, dysmorphic features and limb anomalies including brachydactyly, digital contractures, and/or 5th finger clinodactyly. The dysmorphic features included frontal and parietal bossing, abnormally shaped and posteriorly rotated ears, hypertelorism, arched eyebrows, and prominent and/or broad nose. All children also displayed hirsutism. Similar features have been described in previously affected children with such interstitial deletions suggesting that this chromosomal abnormality may constitute yet another deletion syndrome distinct from the classical distal 1p36 deletions.

Single Nucleotides Polymorphisms (SNPs) in head and neck cancers. *M.T. Ruiz[1], M. Floria-Santos[2], L.M. Alvarenga[1], E.C. Pavarino-Bertelli[1], P.E.M. Guimarães[3], E. Dias-Neto[3], M.J.C. Ruback[1], J.V. Maniglia[1], E.H. Tajara[1], E.M. Goloni-Bertollo[1]* 1) Molecular Biology Department, FAMERP, Sao Jose do Rio Preto, Brazil; 2) The University of Sao Paulo College of Nursing at Ribeirao Preto, Brazil; 3) The University of Sao Paulo School of Medicine, Brazil.

Purpose: To evaluate SNPs from KISS-1 (GA), NINJURIN (A C) and TAX1BP1 (T A) genes in patients with head and neck cancers, and to compare their frequencies within a control population. Methods: We evaluated 186 patients with head and neck cancer and 175 without neoplasia history. The molecular analysis was done after extract DNA from peripheral blood using PCR-SSCP and PCR-RFLP techniques. We used descriptive statistics and Fisher test. Results: Variables analysis showed that the majority of the cases was men (82.07%), Caucasoid (83.33%), 88.72% used tobacco, and 64.4% used alcohol. The most frequent primary site was oral cavity (38.36 %). We did not find statistical differences for the allelic variants from the studied genes, between patients and the control group (KISS-1 gene p=0.2025; NINJURIN gene p=0.1269; TAX1BP1 gene p=0.8434). Conclusion: We did not find association between the studied polymorphisms and head and neck cancers. Therefore, we recommended that the sample number should be increased in order to investigate those variants. The understanding of the mechanisms involved on the tumorigenic process can contribute for individualized treatments and disease prevention. Supported by: CNPq, FAMERP-FUNFARME and FAPESP.

Duodenal Atresia: Prenatal Imaging and Autopsy Findings. *t. Ben-Omran, a. Moore, e. Cutz, r. Windrim, s. Viero, d. Chitayat* Clinical and Metabolic Genetic, The Hospital for Sick Children, toronto, on, Canada.

Duodenal atresia (DA) is the most common type of small bowel atresia with an incidence of 1:10,000 births. DA is a malformation and associated with other anomalies in approximately 30-50% of cases, including gastrointestinal, cardiac, renal, and skeletal abnormalities. DA can be diagnosed prenatally, as early as 18 weeks gestation; however, it may be delayed until after 24 weeks. Mitchell et al., (2004) reported two families with a hitherto new autosomal recessive condition including IUGR, duodenal/ jejunal atresia, annular pancreas, absent gall bladder, neonatal diabetes and dysmorphic features. Here, we report the antenatal ultrasound and MRI findings done on one of the families reported which diagnosed a recurrence. This was the fourth pregnancy of this couple who already lost two children with this condition. Fetal ultrasound done at 18 weeks gestation showed a "double-bubble" sign as an indication of DA. Fetal MRI revealed DA and an absent gall bladder which suggest that this fetus likely have the same condition. Attempts are made to find the gene associated with this autosomal recessive condition but in the meantime the only way we can diagnose the condition prenatally is by fetal ultrasound and MRI which seem to be reliable.

Alternative pathway therapy, nutritional support, and hemodialysis and/or continuous renal replacement therapy (CRRT) in the treatment of neonatal hyperammonemia. *T.H. Cushing[1], N. Baugh[2], C. Wong[1], S.R. Alexander[1], G.M. Enns[1]* 1) Pediatrics, Stanford University, Stanford, CA; 2) Clinical Nutrition, Lucile Packard Children's Hospital, Stanford, CA.

Neonatal hyperammonemic crises are associated with high morbidity and mortality. Alternative pathway therapy (APT), high-calorie nutritional support and hemodialysis (HD) are the mainstays of treatment. In order to understand better the therapeutic effects of these modalities, we describe the clinical course of 15 hyperammonemic neonates treated between 1998 and 2006. Eight patients had organic acidemias (OA)(5 MMA, 3 IVA) and 7 had urea cycle defects (UCD)(2 CPS, 2 citrullinemia, 2 OTC females, 1 OTC male). Patients were treated with protein restriction, high concentration IV dextrose, and intralipids to provide approximately 100 to 120 kcal/kg/day. Three of 8 OA patients required continuous renal replacement therapy (CRRT). We used continuous veno-venous hemodiafiltration to optimize and sustain ammonia removal without rebound. Three of 7 UCD patients required HD, CRRT, or both. No complications occurred with HD or CRRT. Peak ammonia levels ranged from 75 mol/L (IVA patient) to 2653 mol/L (OTC male). The highest ammonia level that responded to therapy without HD/CRRT was 486 mol/L for UCD and 627 mol/L for OA. Two of 15 patients (OTC female and CPS) died in the neonatal period, giving an overall neonatal survival rate of 87%. Two of 13 surviving neonates died in early childhood (MMA and OTC male). Two MMA patients were lost to follow-up. Six patients showed mild (OTC female, CPS, IVA) to severe (3 MMA) developmental delays on follow-up from 8 months to 5 years and 4 were normal on follow-up from 7 months to 6 years. Although survival is good for both groups, patients with UCDs appear to have better developmental outcome. We stress the importance of immediate transport of hyperammonemic neonates to centers with experience in all aspects of therapy, including APT, nutritional support, and HD/CRRT. Based on our limited cohort, supportive care, APT and nutritional support have the potential to be effective at ammonia levels around 600 mol/L in neonates.

Neonatal RDS and Polymorphisms in the Corticosteroid Metabolism Pathway. *K.S. Borowski[1], J.M. Dagle[2], J.C. Murray[2]* 1) Obstetrics and Gynecology, University of Iowa, Iowa City, IA; 2) Neonatology, University of Iowa, Iowa City, IA.

Respiratory distress syndrome (RDS) is a clinical diagnosis made in newborn infants with symptoms of respiratory distress, characteristic x-ray findings and an oxygen requirement. The incidence of RDS is inversely related to the gestational age at delivery. Rates of RDS are affected by the rate of premature delivery as well as by antenatal corticosteroid admninistration. Risk factors for RDS also include maternal diabetes, race and gender. A genetic component to RDS has been clearly identified with gene mutations in SP-B and ABCA3 leading to fatal RDS. Given the known genetic associations with RDS, as well as a decreased incidence with corticosteroid administration, we hypothesized that genetic variability in the glucocorticoid pathway in mothers and infants may alter the risk of neonatal RDS.

Using a candidate gene approach, 5 genes in the glucocorticoid metabolism pathway were analyzed using tagging single nucleotide polymorphisms (SNPs)-NR3C1, HSD11B1, HSD11B2, CYP3A4 and SerpinA6. Inclusion criteria were enrollment in the Disease Variability in the Newborn study at the University of Iowa from 2/01 to 3/06 with available DNA, diagnosis of RDS and gestational age 33-41 weeks. Genotyping was performed using TaqMan assays from Applied Biosystems. 241 affected neonates were analyzed, along with 225 mothers of affected neonates. A sample of anonymous term control infants and mothers were also used for case-control analysis. The results were analyzed with a transmission disequilibrium test (FBAT) as well as with Fisher's exact test for the case-control data. FBAT was performed for all SNPs with none reaching significance. Significance was found in the infant cases compared to controls for variations in the following SNPs: HSD11B2 rs5479 ($p=0.002$), CYP3A4 rs2242480 ($p=0.000012$) and HSD11B1 rs2235543 ($p=0.0016$). Significance was found in the maternal cases with CYP3A4 rs2242480 ($p=0.004$), HSD11B1 rs2235543 ($p=0.0076$) and HSD11B1 rs2282739 ($p=0.0076$). These results suggest that genetic variability in the glucocorticoid metabolism pathway may alter the risk of neonatal RDS.

Expression profiling of blood in Autism. *M. Galdzicki, A. Eran, I. Eisenberg-Loebl, A.T. Kho, H. Peters, K. Graham, S. Brewster, R. Hundley, E. Hanson, J. Ware, L. Rappaport, I.A. Holm, L.M. Kunkel, I.S. Kohane* Children's Hospital Boston, Boston, MA.

Autism Spectrum Disorder (ASD) is a developmental disorder which includes impairment of communication skills, social interactions and repetitive and stereotyped behavior. Twin and family studies give strong evidence suggesting a genetic susceptibility to ASD. We expect an interplay between genetic and environmental factors to be the underlying cause. As part of our integrative genomic approach to understand the causative mechanism responsible for ASD we have established genome-wide gene expression profiling study in children with ASD using in Peripheral Blood Mononuclear Cells (PBMCs) from affected children and control individuals.

More than fifty blood samples from children diagnosed as ASD affected, based on the ADOS/ ADI-R criteria, were collected to date and we expect five-hundred by the end of the year. Twelve affected samples were processed as a preliminary data set on Affymetrix U133 Plus 2.0 microarrays. We were able to distinguish two separate classes of ASD samples based on their expression signature. A short list of differentially expressed transcripts and pathways was found in the analysis and will be further discussed.

With increased sample numbers we expect to find a set of genes that can be used to help diagnose ASD and further divide the ASD into subcategories based on experimentally quantifiable means. We expect that the analysis will lead to the identification of individual genes and/or entire molecular pathways that are abnormally expressed in the affected population indicating possible pathological mechanisms. The identified genes will be examined as high priority candidates for sequencing. Ideally, our goal is to collect upwards of 1000 samples in each group. Parental samples will be classified in the future among the subcategories of ASD determined.

The Development of a High-Throughput Pharmacogenomic Warfarin Genotyping Assay. *A.M.K. Brown, I. Mongrain, Y. Renaud, M.S. Phillips* Université de Montréal Pharmacogenomics Centre, Montréal, Québec, CANADA.

The drug Warfarin is a frequently used medication that reduces the formation of blood clots in patients who have suffered a heart attack or stroke. Experience and routine blood work are currently the only tools physicians have at their disposal to assist in bringing blood concentrations to a therapeutic range. Presently, wide ranges of therapeutic doses are observed in Warfarin patients and traditional indicators such as age, sex and body weight, only account for about half of this variation. It has been shown that polymorphisms in two genes in particular can explain most of the genetic variation observed in patients. The first gene, cytochrome P450 enzyme 2C9 (CYP2C9), is the primary drug-metabolizing enzyme that is responsible for the pharmacokinetic effects of the drug. Reduced function in this gene due to specific polymorphisms can account for a lower required initial dose. The second gene encoding the Vitamin K oxide receptor complex-1 (VKORC1) is the target receptor of Warfarin. Low expression of the receptor due to allelic variation also accounts for lower initial dose. Recently, the FDA has issued guidance for Warfarin use in patients that recommends that genetic screening should be utilized to assist in determining initial dose. Thus, we have developed several high-throughput genotyping assays for multiple technological platforms that can test for the major differences in the CYP2C9 and VKORC1 genes simultaneously. We are presently assessing the cost effectiveness of each of these technologies. Further still, we have determined the haplotype structures found in different populations by testing hundreds of samples from diverse populations, including family trios in some instances, to create a software analysis tool that can be predictive of an individuals proposed phenotype. A molecular profiling assay such as ours will revolutionize the way in which Warfarin is prescribed. The resulting data will provide guidance to physicians as the trend of pharmacogenomics aiding in the therapeutics of medication grows and continues to increase efficacy and reduce adverse drug reactions.

Bidirectional expression of the SCA8 mutation: evidence for polyglutamine inclusions and CUG RNA gain-of-function effects. *R.S. Daughters[1], Y. Ikeda[1], D.L. Tuttle[2], T. Zu[1], M.L. Moseley[1], J.W. Day[1], M.S. Swanson[2], L.P.W. Ranum[1]* 1) Institute of Human Genetics, University of Minnesota, Minneapolis, MN; 2) University of Florida, Gainesville, Florida.

We previously reported that a (CTG)n expansion causes spinocerebellar ataxia type 8 (SCA8). SCA8 BAC-Exp mice show a loss of cerebellar GABAergic inhibition and similar to human patients have 1C2-positive Purkinje cell inclusions. Recently we discovered transcripts spanning the repeat are expressed in both directions (CUG and CAG) and CAG transcripts encode a pure polyglutamine expansion protein (ataxin 8). The expression of non-coding CUG transcripts and previous work in myotonic dystrophy suggest that SCA8 CUG expansion transcripts may contribute to disease pathogenesis by dysregulation of Mbnl and CELF proteins through an RNA gain of function mechanism. To understand the role of SCA8 CUG expansion transcripts on Mbnl dysregulation we crossed SCA8-BAC-Exp mice to heterozygous Mbnl1 isoform knockout mice. Doubly mutant $SCA8^{+/-}$;$Mbnl1^{+/E3}$ animals show significantly enhanced rotorod deficits compared to single mutant $Mbnl1^{+/E3}$ ($p=0.003$) and SCA8-BAC-$Exp^{+/-}$ ($p<0.001$) mice. In addition, we have begun to investigate downstream effects of the CUG expansion on genes normally regulated by MBNL and CELF proteins. The loss of GABAergic inhibition in our SCA8 mice led to the prioritization of a gene identified as a CUG-BP1 binding target by crosslinking/immunoprecipitation (CLIP) analysis, the *GABA-A transporter 4* (*Gabt4*) gene. If upregulated, Gabt4 could lead to decreased cerebellar inhibition by reducing synaptic GABA. Consistent with this hypothesis, cerebellar Gabt4 is significantly upregulated at the RNA ($p<0.001$) and protein ($p<0.001$) levels in both SCA8 BAC-$Exp^{+/-}$ and $Mbnl1^{E3/E3}$ knockout animals and in SCA8 human brain. Additionally, transient transfections using human SK-N-SH cells show upregulation of endogenous GAT4 is triggered by SCA8 CUG, but not SCA8 CAG, expansion transcripts ($p<0.001$). These data provide functional evidence in mice, humans and cell culture that CUG expansions can trigger GAT4/Gabt4 dysregulation through an RNA gain-of-function mechanism in SCA8.

Searching for novel genes predisposing to breast cancer: a high-density linkage scan at the telomeres. *S. Casadei, T. Walsh, J. Higgins, K. Roach, S. Stray, M.K. Lee, M.-C. King* Departments of Medicine and Genome Sciences, University of Washington, Seattle, WA.

Genes involved in inherited predisposition to breast cancer include *BRCA1* and *BRCA2*, *CHEK2*, *TP53*, and *PTEN*. The genes differ in associated phenotypes and penetrance. A substantial fraction of families with four or more cases of breast cancer are wildtype at all these genes, strongly suggesting the existence of other genes responsible for breast cancer predisposition. Previous genome-wide scans for breast cancer genes have not yielded consistent regions of linkage. While this may reflect extraordinary locus heterogeneity of remaining breast cancer susceptibility genes, it is also possible that genomic problems have led to linkages being missed. In particular, recombination rates are high near telomeres, so that conventional linkage scans fail to adequately interrogate these genomic regions. To address this problem, we set up a high-density linkage scan at the telomeres and subtelomeres. We developed sets of 3 to 6 microsatellite markers at 500 kb to 1.0 MB density at the ends of all chromosomes. Our first scan is being carried out on 16 extended kindreds, each including 4 or more cases of breast cancer and yielding potential lods scores greater than 1.0 per family. Mutations in all known breast cancer genes have been excluded by sequencing, MLPA and related methods, and linkage. Promising regions of linkage identified in these families will be followed by targeted scans in more than 300 additional high-risk families.

Testing for association between type 2 diabetes and 225 candidate genes in 2357 Finnish cases and controls. *K.J. Gaulton[1], K.N. Conneely[2], Y. Li[2], A.U. Jackson[2], L.J. Scott[2], W.L. Duren[2], P.S. Chines[3], N. Narisu[3], L. Bonnycastle[3], A. Swift[3], T.T. Valle[4], J. Tuomilehto[4], R.N. Bergman[5], F.S. Collins[3], M. Boehnke[2], K.L. Mohlke[1]* 1) U North Carolina, Chapel Hill, NC; 2) U Michigan, Ann Arbor, MI; 3) NHGRI, Bethesda, MD; 4) Natl Public Health Inst., Helsinki, Finland; 5) U Southern California, Los Angeles, CA.

Type 2 diabetes (T2D) is a complex disorder with a strong genetic component, although the underlying genes are incompletely known. As stage 1 of a 2-stage T2D association study (which includes a genome-wide association (GWA) study, see abstract from Scott *et al.*), we are genotyping 3378 SNPs from 225 candidate genes in 1171 T2D cases and 1186 normal glucose tolerant controls from the Finland-United States Investigation of NIDDM Genetics (FUSION) study. Genes were selected using CAESAR, our algorithm that uses text and data mining to integrate annotations from 11 databases and rank human genes based on their potential function. To test the ability of CAESAR to successfully select complex trait genes, we used the algorithm to rank genes for 11 complex traits. Of 17 tested genes previously implicated in these traits, 8 were present in the top 2% of ranked genes. We used CAESAR to select 209 candidate genes for T2D and genotyped SNPs in these and 16 other genes based on prior evidence of association in FUSION. 1850 SNPs within 10 kb upstream and 5 kb downstream of these genes are present on the GWA panel. 1528 additional SNPs were selected to more completely cover variation in these genes based on linkage disequilibrium (r^2>.8) and annotation. The 3378 SNPs tag an additional 7099 SNPs using the same r^2 threshold. Preliminary results using the 1850 GWA SNPs in 885 cases and 885 controls indicate that the most significant SNP lies in the *ARID2* gene and has an unadjusted p-value of 1.7×10^{-4} under a multiplicative model. Within each gene, we adjusted for the number of SNPs and the correlation between them and found that 3 genes exhibit p-values<.005, which is consistent with the 1.1 gene expected by chance (p=.1). Our completed stage 1 study will include all SNPs and 2357 samples to further determine the significance of these genes in T2D.

SNP fine mapping in 10q22-23, a region with previous linkage to schizophrenia. *P-L. Chen[1], V.K. Lasseter[2], D. Avramopoulos[2], M.D. Fallin[3], A. Pulver[2], D. Valle[1, 4]* 1) Inst Genetic Medicine, Johns Hopkins Sch Medicine, Baltimore, MD; 2) Dept Psychiatry; 3) Dept Epidemiology, School of Public Health; 4) Howard Hughes Medical Institute.

Schizophrenia, with a prevalence ~1% worldwide, is a complex psychiatric disorder with a strong genetic component. We previously performed a genomewide linkage scan on 29 multiplex families of Ashkenazi Jewish (AJ) descent (Fallin *et al*. Am J Hum Genet 73:601, 2003) with the strongest linkage signal at chromosome 10q22 (NPL score: 4.27, empirical p= 0.00002). *NRG3* (neuregulin 3) and *GRID1* (glutamate receptor, ionotropic, delta1) are strong biological candidate genes in this region. To further narrow down the susceptibility gene(s)/allele(s), we obtained a SNP-based fine mapping study with 1536 SNPs across the 12.5 Mb region in 1771 individuals at CIDR using the Illumina BeadArray system. All subjects are of AJ background and were analyzed as 304 trios in family-based or 478 cases and 531 controls in population-based study. We used the UNPHASED analysis package (Dudbridge, Genet Epidemiol 25:115, 2003) for population-based association and transmission disequilibrium test (TDT) studies, using individual SNP genotypes or constructed haplotypes. We also performed exhaustive allelic TDT (Lin *et al*. Nat Genet 36:1181, 2004) to capture information extending beyond 1 LD block. Around *NRG3*, the strongest association signal was at a 6-SNP haplotype (spanning 49 kb) in intron 1 (nominal p=0.00019) and there were many haplotypes between the 5 upstream region and intron 1 of *NRG3* with association (nominal p<0.01). Around *GRID1*, the best signal (nominal p=0.00049) was a 47-SNP haplotype (spanning 436 kb) 3 downstream to the gene and many haplotypes over the 3region of the gene showed association (nominal p<0.01). Intriguingly, there was a cluster of 8 genes flanked by gene deserts between *NRG3* and *GRID1* that also showed association (nominal p<0.01). We are currently investigating these association signals with independent samples and independent statistical methods.

Familial Aortic and Cerebral Aneurysms: A Common Genetic Basis in a Subset of Aortic Aneurysm/Dissection Families. *V. Fadulu, S.E. Smart, J.H. Chen, H. Pannu, L. Dechtenberg, D. Guo, D.M. Milewicz* IM-Medical Genetics, Univ Texas Medical Sch, Houston, TX.

Aneurysms occur most commonly in the aorta & cerebral arteries. Predisposition to aortic and intracranial aneurysms/dissections can be inherited. Familial intracranial aneurysms (ICA) are linked to 3 loci, and familial thoracic aortic aneurysms/dissections (TAAD) are linked to 5 loci TAAD1, TAAD2, TAAD3, FAA1 and MYH11. We recruited 345 families with 2+ persons w/TAAD involving the ascending aorta. The majority of these families showed autosomal dominant inheritance w/variable expression & decreased penetrance in women (men=66% vs. women= 34%). 57% of families had 1+ person with vascular disease extending beyond the ascending aorta.Medical records on families with TAAD & ICA were reviewed and 37 families (11%) were confirmed to exhibit TAAD & ICA within 1 family and/or within at least 1 family member. The majority of persons with ICA were women (63%). 13 people had both TAAD & ICA (equal number of men (n=7) and women (n=6)). Three unrelated men were diagnosed with aortic dissections at ages 27, 37, and 53. They underwent successful repair of their aorta and died suddenly of an ICA at ages 33, 49 and 56, respectively. 37 families consisted of 46 persons with ICA and 197 persons with TAAD. 57% of families had a parent-child with TAAD/ICA. This suggests that in some families, TAAD & ICA segregate together w/variable expression. Mutation & linkage analysis of the known TAAD loci revealed only 1 family with a TGFBR2 mutation (R460C). The remaining families were not linked to any of the known loci for familial TAAD. These families showed a unique phenotype of both TAAD and ICA.Genetic studies indicate that a novel locus is responsible for this phenotype. First-time occurrence of a deadly ICA in families with initially purely TAAD often causes anger, grief and shock in family members; especially when the family adhered to surveillance guidelines and recommendations for only TAAD. Families with TAAD & ICA should be recommended cerebral & aortic imaging. Individuals in these families with successful repair of TAAD should be imaged for ICAs. A large TAA-ICA family will be described.

Identification of genes contributing to obesity associated cardiovascular disease (OCARD). *Y. Dementieva[1], P. Wehner[1], T.L. Green[1], D.A. Primerano[1], J. Denvir[1], L. Wei[1], M.R. Flood[2], D. Calica[2], B. Freeman[2], M. Huff[2], S. Dodson[2], C. Hill[2], A. Frances[3], C. Taylor[3], B. Connors[3], K. McIntyre[3], R. Kreisberg[3], M. Davis[4], H.M. Lee[4]* 1) Marshall University, Huntington, WV; 2) Fairmont State University, Fairmont, WV; 3) West Liberty State College, West Liberty, WV; 4) West Virginia University, Morgantown, WV.

Obesity is a known risk factor in the development of coronary artery disease (CAD). Based on this finding, we hypothesized that specific genes in overweight or obese individuals potentiate the development of CAD and refer to these modifier genes as obesity associated cardiovascular disease (OCARD) genes. The objective of our study is to identify human OCARD genes using population-based association methods.

In order to identify OCARD genes, we performed case-control studies in groups of overweight [(BMI) 25] and obese [BMI 30] Caucasian individuals from West Virginia. Haploview software was used to select the minimal number of tagging SNPs to be genotyped in each candidate gene. SNP genotypes were determined using either pyrosequencing or TaqMan allelic discrimination. Logistic regression analyses were used to test for association between disease phenotype and SNPs with age, gender, BMI, and smoking as covariates.

We determined the genotypes of 54 SNPs representing 20 cardiovascular candidate genes in 265 human subjects with and without atherosclerosis. Three SNPs [Cholesterol Ester Transfer Protein (CETP) rs4784744 (P=0.014, OR 95% CI 1.14 - 3.11), Low Density Lipoprotein-Related Protein 1B (LRP1B) rs1816608 (P=0.016, OR 95% CI 1.25 - 9.27), and Lipase A Precursor (LIPA) rs1556478 (P=0.013, OR 95% CI 1.17 - 3.83)] met the 0.05 significance level for association with CAD.

CETP, LRP1B, and LIPA are known to play critical roles in lipoprotein metabolism. Our results suggest that defects in these genes may predispose to CAD in overweight and obese individuals. However, further investigations in larger populations and functional studies are required to confirm these findings.

Losartan decreases aortic root dilation in Marfan syndrome. *B.S. Brooke, J.P. Habashi, H.C. Dietz* HHMI & Instit. of Genetic Medicine, Johns Hopkins School of Medicine, Baltimore, MD.

The Marfan Syndrome (MFS) is an autosomal dominant connective tissue disorder caused by mutations in fibrillin-1. The most severe complication of MFS is progressive aortic root enlargement, which leads to dissection or rupture unless surgical repair is undertaken. -blockers are standard medical therapy in these patients and slow aortic growth, but do not prevent the need for surgery. Up to a third of MFS patients are intolerant to -blockers and get switched to other blood-pressure reducing agents during the course of treatment. Recently, data from a mouse model of MFS suggested that over-expression of TGF-signaling contributes to the pathogenesis of aneurysm formation. In this model, aortic dilation was arrested by the use of the angiotensin II type-1 receptor blocker losartan, an antihypertensive medication known to inhibit TGF- signaling. Here, we report the results of an observational study designed to evaluate the effectiveness of losartan therapy in MFS patients with progressive aortic dilation. A total of 12 MFS patients (6 males, 6 females, mean age 86 years) were identified from an institutional database that had been switched from -blocker to losartan between August of 1998 and May of 2006. Serial echocardiographic measurements (mean 10 per pt.) of aortic diameter and corresponding Z-scores were compared for each patient while on -blocker and after initiation of losartan. The mean rate of aortic growth (0.007 cm/yr vs. 0.31 cm/yr; $P<0.005$) and the mean rate of change in Z-score (-1.40 Z-score/yr. vs. +0.81 Z-score/yr.; $P<0.01$) were both significantly decreased for individuals on losartan, as compared to their prior performance on -blockers. After adjusting for confounders using multiple linear regression, losartan therapy independently reduced the rate of aortic growth by 0.28 cm per year [95%CI:-0.46 to -0.09; $P<0.005$]) and reduced the Z-score by 2.14 per year [95%CI:-4.03 to -0.24; $P<0.05$]. In conclusion, these data suggest that losartan therapy offers a significant advantage over -blockers for the treatment of MFS. A prospective randomized trial will be initiated soon and be important to confirm the results of this study.

Lysosomal Disease Network launches research web site. *D. Erickson[1], J. Barranger[2], J. Muenzer[3], C. Eng[4], G. Grabowski[5], J. O'Brien[6], G. Pastores[7], E. Shapiro[1], R. Steiner[8], W. Wilcox[9], S. Walkley[10], B. Davidson[10], S. Patel[10], B. Wedehase[10], C. Whitley[1]* 1) Lysosomal Disease Network, Minneapolis, MN; 2) University of Pittsburgh, Pittsburgh, PA; 3) University of North Carolina, Chapel Hill, NC; 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) Oregon Health & Science University, Portland, OR; 9) Cedars-Sinai Medical Center, Los Angeles, CA; 10) and other LDN member institutions.

The Lysosomal Disease Network 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 (LD). 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. In its initial year, the web site has announced its 3rd annual scientific meeting, the WORLD Symposium (Dec. 7-9, 2006) in Orlando, FL, USA. Through the web site, participants have been 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 LD 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 support has been provided by NIH, NINDS, NIDDK, and ORD, NS49950.

Saturation SNP coverage across the GABRB3 locus refines association to autism. *R.J. Delahanty[1], J.A. Smart[1], J.S. Sutcliffe[1,2]* 1) Department of Molecular Physiology & Biophysics, Centers for Human Genetics Research, Vanderbilt University, Nashville, TN; 2) Center for Molecular Neuroscience, Vanderbilt University, Nashville, TN.

Autism is a neurodevelopmental disorder affecting greater than 1 in 500 individuals with deficits in development and use of language, social reciprocity, and patterns of repetitive behaviors and restricted interests. Despite a recognized complex genetic etiology, locus heterogeneity has thwarted efforts to identify loci broadly contributing to the idiopathic condition. Chromosomal duplications, linkage and association findings have implicated the 15q 11-q13 region that harbors a cluster of $GABA_A$ receptor subunit genes (GABRB3, GABRA5, GABRG3). Data have most consistently implicated the GABRB3 gene, although no single region based on linkage disequilibrium (LD) structure has been identified. We previously reported an LD-map spanning the entire 1-Mb $GABA_A$ subunit cluster and identified three LD blocks showing evidence for allelic association (McCauley et al, 2004). Last year, Curran et al (ASHG program #1692, 2005) reported additional evidence for association to SNPs in GABRB3, further suggesting involvement of this gene in autism susceptibility. We report a second-generation analysis of LD and allelic association to autism with a specific focus on the GABRB3 locus. We selected and genotyped 48 SNPs covering a 231kb interval across GABRB3, with an average spacing of 1 SNP per 4.9 kb. SNPs were chosen to thoroughly index all common alleles across at GABRB3, potentially functional SNPs, and markers we previously identified as associated. SNPs were analyzed in 644 combined multiplex and parent-child trio autism families. Analyses to date show that association to a region in intron 3 of the GABRB3 gene is replicated in a much larger set of families than previously assessed by McCauley et al. We will present detailed analysis of LD, single marker and haplotype-based association at GABRB3, as well as analysis of trait-subsets of autism to explore genotype-phenotype relationships. Our results continue to support involvement of GABRB3 in autism susceptibility.

Homozygosity mapping discard genetic linkage to CNGA3, CNGB3 and GNAT2 in a large Colombian family with Achromatopsia. *L.F. Cuesta, M. Gordillo, H. Vega* Universidad Nacional de Colombia, Bogota, Colombia.

Achromatopsia, also referred to as total colorblindness or rod monochromacy is a rare (prevalence of 1 in 30,000) autosomal recessive disorder characterized by loss of function of all cone classes, low visual acuity, severe photophobia under daylight conditions, infantile nystagmus and normal funduscopic examination. This entity is genetically heterogeneous. To date, three loci have been associated to achromatopsia: the alpha-subunit of the cone photoreceptor cGMP-gated cation channel (CNGA3) on 2q11, the beta-subunit of the cGMP-gated cation channel (CNGB3) on 8q21-q22, and the cone photoreceptor-specific alpha subunit of transducin (GNAT2) on 1p13. Together, mutations in these genes are thought to be responsible for only 60 % of patients affected with this disorder suggesting the presence of additional genetic heterogeneity in this disease. We identified a large consanguineous family from an isolated village in Colombia with clinical findings compatible with the diagnosis of achromatopsia. This multigenerational pedigree, including six affected individuals was investigated for association with CNGA3, CNGB3 and GNAT2 by homozygosity mapping. At least three microsatellite markers with the highest LOD scores reported previously during the mapping of each locus and surrounding each of the affected genes were evaluated to identify a region of homozygosity in the affected individuals. We found no evidence of homozygosity at any of the three loci as no more than two patients presented homozygosity for the same chromosomal region. These data discard with a very high probability that mutations in CNGA3, CNGB3 and GNAT2 are responsible for achromatopsia in this family and support the notion that other loci for achromatopsia remain to be identified.

Treatment of CNS lysosomal storage with high-dose intravenous enzyme replacement therapy in tolerant MPS I dogs. *P. Dickson[1], C. Vogler[2], B. Levy[2], M. McEntee[3], M. Passage[1], S. Le[1], S. Snider[1], H. Manuel[1], E. Kakkis[4]* 1) Dept Pediatrics, Harbor-UCLA Medical Ctr, Torrance, CA; 2) Dept. of Pathol., St. Louis Univ., St. Louis, MO; 3) Dept. of Pathol., Coll. of Vet. Med., Univ. of Tenn., Knoxville, TN; 4) Biomarin Pharmaceutical, Inc., Novato, CA, USA.

Intravenous (IV) enzyme replacement therapy had been thought not to cross the blood brain barrier (1,2). Recently, Vogler et al. showed that high dose IV ERT reduced brain storage in a tolerant mouse model of MPS VII (3). In order to assess whether this approach would work in a large animal model of MPS I, we gave 2 mg/kg weekly IV recombinant human -L-iduronidase (rhIDU) to 3 tolerant and 3 nontolerant MPS I dogs age 8-20 months. The MPS I dogs were first tolerized with a novel procedure. All MPS I dogs were then treated for 11-13 weeks and their brains studied 48 hours after the final dose with biochemical assays for iduronidase and glycosaminoglycan (GAG) storage. Iduronidase was present in the brains of MPS I dogs treated with high-dose IV rhIDU. Iduronidase levels in tolerant dogs were 0.165 0.016 U/mg (2% of normal), vs. nontolerant dogs 0.077 0.051 U/mg (1% of normal, p=0.046; Normal 8.26 0.746, Untreated MPS I 0.032 0.004). Brain GAG storage was reduced in tolerant IV-treated MPS I dogs. Brain GAG levels in tolerant dogs were 6.03 0.446 g/mg, near the normal value of 5.40 1.82 and less than that in nontolerant dogs (8.27 0.468, p=0.0039; Untreated MPS I: 8.26 1.23). Blinded ultrastructural evaluation showed reduction in neocortical neuronal storage in 2 of 3 tolerant MPS I dogs receiving high-dose IV rhIDU. High dose IV enzyme replacement therapy treats lysosomal storage in the brain in tolerant MPS I dogs, confirming results in MPS VII mice. It may be possible to use IV ERT to treat lysosomal storage in the CNS.

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Prenatal diagnosis of lysosomal storage disorders using biochemical testing: Importance of obtaining parental control samples for correct result interpretation. *M.J. Fietz, S. Chin, P. Clements, B. Fong, P. Leane, V. Muller, K. Nelson, C. Nicholls, J.M. Fletcher* Dept of Genetic Medicine, Women's & Children's Hospital, North Adelaide, SA, Australia.

Aim: To evaluate the need for parental samples in the provision of accurate prenatal diagnoses for lysosomal storage diseases (LSD). Setting: The National Referral Laboratory (NRL), Adelaide Womens & Childrens Hospital. The NRL has provided a specialist and comprehensive diagnostic service for lysosomal storage disorders (LSD) to both Australian and overseas patients for over 30 years. During that period, we have performed over 650 prenatal diagnoses for a total of 29 different LSD, with the most frequently tested disorders including MPS-I, MPS-II, Krabbe disease, Pompe disease, metachromatic leucodystrophy (MLD), GM1-gangliosidosis and mucolipidosis type II (I-cell disease). Our standard procedure is to request parental samples prior to performing prenatal testing. This has been thought to be particularly relevant due to the presence of pseudo-deficiency alleles in disorders such as Krabbe disease and MLD. Cases: In case 1, direct enzyme testing for Krabbe disease on chorionic villus (CV) tissue yielded a value intermediate to our previous affected and non-affected reference ranges. The presence of clearly normal enzyme activity levels in parental cultured fibroblasts allowed us to conclude that the fetus was affected by Krabbe disease. In case 2, biochemical testing for Krabbe disease on cultured CV cells was performed, yielding normal results. However, VNTR analysis performed on DNA from both parents and the fetus clearly indicated that the fetus had an identical VNTR pattern to the mother. Therefore, we concluded that the sample was of maternal origin. Amniocentesis was then performed and the assay repeated on cultured amniocytes. Conclusion: Even in the year 2006, despite increasing availability of mutation data, biochemical analysis is still required for prenatal diagnosis of LSD. In such cases, the provision of suitable parental samples (skin fibroblasts or leukocytes) is required to enable optimal data interpretation and the determination of an accurate prenatal result.

A splice mutation in the small subunit of the AP-1 complex underlie the Erythrokeratodermia variabilis type 3 (EKV3) syndrome. *S. Côté[1], A. Montpetit[2], E. Brustein[3,4], C. Meloche[1], M. Boudreau[2], R. Drouin[5], P. Drapeau[3,4], T.J. Hudson[2], C.A. Drouin[6], P. Cossette[1,7]* 1) Service de Neurologie, Centre Hospitalier de lUniversité de Montréal-Hopital Notre-Dame, Montréal,Qc,Canada; 2) McGill University and Genome Quebec Innovation Centre, Montreal,Qc,Canada; 3) Centre for Research in Neuroscience and Department of Biology, McGill University, Montreal,Qc,Canada; 4) Pathologie et Biologie Cellulaire, Pavillon Roger-Gaudry, Université de Montréal, Montréal,Qc,Canada; 5) Département de Pédiatrie, Faculté de Médecine, Université de Sherbrooke, Sherbrooke,Qc,Canada; 6) Centre Hospitalier du Grand Portage, Rivière-du-Loup,Qc,Canada; 7) Department of Human Genetics, McGill University, Montreal,Qc, Canada.

Erythrokeratodermia variabilis 3 (EKV3, MIM 609313) is a rare but often fatal autosomal recessive disorder, characterized by typical skin lesions, peripheral neuropathy, psychomotor retardation, hearing loss and severe chronic congenital diarrhea. The EKV3 gene was recently mapped to chromosome 7q22. Here we report the identification of an A to G homozygous mutation in the acceptor splice site of intron 2 of AP1S1, which encodes the small subunit of the AP-1 complex. RT-PCR analysis of mRNA obtained from patients fibroblasts was compatible with skipping of exon 3, predicting a truncated protein lacking most of its clathrin-adaptor domain. These analyses also revealed that a cryptic splice site is used in these patients, resulting in an in-frame mRNA lacking nine base pairs. Knocking-down of AP1S1 in zebrafish using targeted antisense morpholino oligonucleotides (AMO) revealed striking morphological and behavioural phenotypes, including smaller size, loss of pigmentation, and severe impairment of swimming. Co-injection of wild type human AP1S1 mRNA with AP1S1 AMO resulted in a virtually normal phenotype. In contrast, co-injection of the mutant AP1S1 mRNAs with AP1S1 AMO led to similar morphological and motor deficits, suggesting a loss of function effect of these two mutations. These results are consistent with the mutation in AP1S1 being the underlying cause of EKV3.

Segmental duplications on 10q are linked to complex genomic rearrangements associated with neurobehavioral abnormalities. *J. Balciuniene[1], N.-P. Feng[4], B. Hirsch[3], L. Charnas[2], D. Avramopoulos[5], D. Valle[4], L. Schimmenti[2], S. Selleck[1,2]* 1) Dept of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN; 2) Dept of Pediatrics, University of Minnesota, Minneapolis, MN; 3) Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN; 4) Howard Hughes Medical Institute and the Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 5) Dept of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD.

Low copy repeats (LCRs) or segmental duplications are genomic features that affect chromosome stability and can produce disease-associated rearrangements including deletions and duplications. We describe members of 3 families with deletions in 10q22.3-10q23.31, a region harboring a complex set of LCRs, providing evidence that rearrangements in this region are associated with behavioral and neurodevelopmental abnormalities including mental retardation, autism, hyperactivity and possibly psychiatric disease. Cowden disease was observed in one patient with a deletion extending telomerically to include the PTEN gene.

We fine mapped the deletions in members of all 3 families using a 10q oligo array and CGH (NimbleGen). Our results demonstrate a different deletion in each family with variable breakpoints mapping both between and outside the LCRs. In one proband the deletion breakpoints are at 84,148592 - 89,875445 with an insert containing non-contiguous segments of chromosome 10 DNA from regions outside the deletion and within the deletion in an inverted orientation. In the other two families breakpoints have occurred in the same LCRs (proximal at 81.62 Mb and distal at 89.14 Mb). In one of these families, the deletion co-segregates with neurobehavioral abnormalities in members of 3 generations.

These results suggest that LCRs produce not only non-allelic homologous recombination events but also influence the stability of nearby genomic regions and promote complex chromosome rearrangements. Our data provide increasing evidence that this genomic region harbors one or more genes important for neurologic development and function.

Large scale DNase I hypersensitivity mapping of the human CFTR gene region. *M.O. Dorschner[1], J. Goldy[1], M. Weaver[1], A. Shafer[1], K. Lee[1], F. Neri[1], A. Haydock[1], P. Sabo[1], R. Humbert[1], M.S. Kuehn[2], J.A. Stamatoyannopoulos[1]*
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Transcription of the cystic fibrosis transmembrane conductance regulator (CFTR) gene is tightly controlled in a complex temporal and spatial pattern. To date, few cis-acting elements have been shown to regulate expression of CFTR in a tissue-specific manner. To identify new regulatory elements, we performed quantitative chromatin profiling of 10 tissues across a 525 kb region containing CFTR and three flanking genes. Several CF-relevant cell types including primary airway, intestinal, pancreatic, mammary and renal epithelial cells were examined. Conventional DNase I hypersensitivity Southern blots had been employed previously by other investigators to survey over 300 kb of the CFTR region in an effort to locate active chromatin elements. We extended this work by scanning a larger region spanning from 210 kb upstream of CFTR to 125 kb downstream of the last exon with a set of 1845 contiguous ~250 base amplicons. This high-resolution, real-time PCR-based approach (Dorschner et al., Nat. Methods, 2004) allowed us to rapidly and precisely map 294 unique DNase I hypersensitive sites (DHSs). 74% of the DHSs were only found in one tissue, while the remaining 26% were found in two or more cell types. Close examination of the DHS patterns across tissues yielded over 20 DHSs that may harbor putative CFTR cis-regulatory elements. These sites were found in multiple airway cells and/or a combination of other CF-relevant cell types. A comprehensive catalog of CFTR cis-regulatory elements provides potential targets for therapeutic intervention as well as sites for the identification of mutations that cause cystic fibrosis or modify disease phenotype.

The genetics of copy number variation in a founder population. *D. Conrad, M. Abney, C. Ober, J. Pritchard* Dept Human Genetics, Univ Chicago, Chicago, IL.

There are now numerous approaches for genome-wide ascertainment of human copy number variants (CNVs). Much less work has been done to integrate such variation into traditional methods of genetic analysis. We used the Affymetrix 500k SNP genotyping platform to scan for CNVs in 750 individuals from a young founder population, all of whom have been phenotyped for 25 quantitative traits. A complete genealogy relating these individuals has been constructed from a 12,000-member pedigree. We have identified nearly 1,000 CNVs, including duplications and deletions, ranging in size from hundreds of base pairs to over 1 Mb. In this presentation, we describe new methodology for integrating CNVs into the study of genetic traits. We leverage the pedigree information relating our samples to increase our power to classify CNVs, and to gain new insight into biological properties of CNVs such as the rate of *de novo* CNV formation, transmission distortion, the location of duplication events, and the relationship between copy-variable genomic regions and the genetic map. We anticipate that our work will help to establish a framework by which CNVs can be incorporated into studies of Mendelian and complex disease.

De novo ring chromosome(13)(p11q13): a case report and review bof literature. *S. Batish[1], P. Koduru[2], G. Haberman[2], S. Gupta[2]* 1) Dept Pathology & Lab Medicine, Weill Med Col of Cornell Univ, New York, NY; 2) Cell Genetics, Laboratory Medicine,North Shore University Hospital, Manhasset, NY.

Ring chromosome are rare constitutional abnormality. Loss of genetic material during ring formation as well as mechanical desruption of ring chromosome during mitosis results in dynamic mosaicism and General Ring syndrome characterized by moderate mental deficiency, marked growth retardation, borderline to minor dysmorphogenesis and sometimes intact fertility. A 9 year old girl was evaluated for dysmorphism, growth retardation and seizures. peripheral blood karyotype was 45,XX,-13/ 46,XX,r(13)(p11q33). FISH analysis confirmed the loss of 13q34 band as well as subtelomeric sequences in ring (13). Mosaicism for the ring chromosome was confiemed, 20% of peripheral blood cells were monosomic for chromosome 13. Ring (13) was first described in 1968 and incidence is reported at 1/58,000 livebirths. Charactetistic features of ring (13) include mental retardation, microcephly, facial dysmorphism, and hand, foot or toe abnormalities. The phenotype of the proband is compared with reported cases of r(13) and mechnism of ring formation is discussed.

Genotype-phenotype correlations in MCADD. *G.L. Arnold[1], R. Erbe[2], K. Verdassdonk[2], P.A. Galvin-Parton[3], D.F. Kronn[4]* 1) Div Pediatric Genetics, Univ Rochester Sch Medicine, Rochester, NY; 2) SUNY at Buffalo, Buffalo, NY; 3) SUNY at Stony Brook, Stony Brook, NY; 4) Medical College of NY, Valhalla, NY.

Objective: To investigate genotype-phenotype correlations in children diagnosed with MCADD by expanded newborn screening (ENBS).

Methods: Cross sectional chart review of all children diagnosed with MCADD from four New York ENBS follow-up centers. Infants were classified in three groups: eleven K304E homozygotes, ten with variant genotypes (one or no copies of K304E), and three with atypical MCADD (C8 levels of 30 with brittle fasting intolerance or sibling death, but not homozygous for K304E).

Results: The mean C8 level on ENBS was 14.9 (range 0.99-31.7) in the K304E homozygotes, compared to 6.0 (0.86 -23.2) in the neonates with variant genotypes (p0.02). Organic acids were diagnostic in six of seven K304E homozygotes, but only one of seven with variant genotype (p0.01). Follow-up C8 acylcarnitine levels were 5.2 (1.9-11.3) in the homozygous and 2.1 (0.53-5.3) in the variant infants (p0.01), and follow-up hexanoylglycine levels were 24.7 (12.7-49.3) in the homozygous infants and 9.1 (3.8-23.2) in the variant infants (p0.001). Several variant infants required both acylcarnitine and acylglycine analyses and/or DNA to clarify their affected status.

Conclusions: Infants homozygous for the K304E mutation are more likely to have higher levels of diagnostic metabolites. The extent to which this predicts clinical outcome merits further study, however the 3 atypical infants with profound fasting intolerance and C8 levels 30 suggest a relationship between ENBS C8 level and vulnerability to metabolic stress. Infants with variant genotypes typically have normal organic acid analyses, and some may require both plasma acylcarnitine and acylglycine or DNA analysis to separate affected from carrier infants.

Autosomal recessive severe Brachydactyly caused by a novel BMPR1B mutation. *O. Agamy[1], I. Abuelaish[2], R. Ofir[1], O.S. Birk[1,2]* 1) The Morris Kahn Laboratory of Human Molecular Genetics at the National Institute of Biotechnology; 2) the Genetics Institute at Soroka Medical Center, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel.

Brachydactylies (BDs) are a group of inherited malformations characterized by shortening of the digits due to abnormal development of the phalanges and/or the metacarpals, usually manifest as autosomal dominant traits. We describe four individuals in a large inbred, consanguineous Arab Israeli family presenting with severe brachydactyly segregating in an autosomal recessive mode of heredity. We performed linkage analysis using microsatellite markers adjacent to five genes known to be associated with Brachydactyly: GDF5, ROR2, IHH, HOXD13 and BMPR1B. Linkage was ruled out for four of the five genes tested. A region of homozygosity containing the gene BMPR1B (bone morphogenetic protein receptor 1B) was identified. Two point linkage analysis using SUPERLINK demonstrated maximum LOD score of [Zmax] = 4.05 at recombination fraction [] = 0.0 at marker D4S1599. Mutation analysis of the BMPR1B gene revealed a novel missense mutation, G377A, resulting in substitution of cysteine to tyrosine at the conserved amino acid 34 (C34Y) in the extracellular ligand binding domain of BMPR1B. All affected individuals were homozygous for the mutation. Heterozygous carriers showed no signs of the phenotype. BMPR1B is the major receptor for GDF5, a signaling molecule of the BMP family that plays a major role in digit formation, joint development, and chondrocyte differentiation. So far few mutations in the BMPR1B gene were described; two heterozygous missense mutations in BMPR1B were previously associated with brachydactyly type A2 through a dominant negative effect. Lately, a homozygous loss of function mutation (del359-366) within the extracellular ligand binding domain of BMPR1B was shown to cause a new subtype of acromesomelic chondrodysplasia with genital anomalies. Functional analysis is undertaken in order to characterize the mutation. Our findings highlight the extreme phenotypic variability generated by different BMPR1B mutations.

A novel FLCN gene mutation in a patient with bilateral renal tumors and no other clinical features of Birt Hogg Dubé syndrome. *C.T. Dvorak[1,2], Y. Lien[1], G. Pridjian[1,2,3]* 1) Human Genetics Program, Tulane Sch Medicine, New Orleans, LA; 2) Department of Pediatrics, Tulane Sch Medicine, New Orleans, LA; 3) Department of OB/GYN, Tulane Sch Medicine, New Orleans, LA.

Birt Hogg Dubé Syndrome (BHD) was initially described as a genodermatosis syndrome in 1977, and since then a number of other clinical features (including renal tumors and spontaneous pneumothorax) have been observed in a portion of affected individuals. We describe a 46 year old female who presented with bilateral renal tumors of the chromophobe type. An analysis of the folliculin gene (FLCN) in peripheral blood lymphocytes revealed a novel frameshift mutation in exon 11 (c.1286dupA) that was similar in location and effect to previously described mutations in patients with BHD syndrome. However, our patient did not exhibit the commonly described skin findings or other phenotypic features of BHD, suggesting a greater degree of clinical variability than is typically described for the syndrome. We argue that BHD should be included (along with Von Hippel Lindau and other hereditary cancer syndromes involving the kidney) in the differential diagnosis of all patients presenting with bilateral renal tumors, even in the absence of other clinical findings. In addition, individuals considering FLCN mutation testing should be counselled regarding the variable expressivity and reduced penetrance that can be observed even among family members with BHD.

A simple and robust method for estimating allele frequencies in pooled DNA samples. *H.H. Chen[1], Y.S. Jou[2], W.H. Pan[1, 2]* 1) Department of Biochemical Science and Technology, Institute of Microbiology and Biochemistry, College of Life Science, National Taiwan University, Taipei, Taiwan, ROC; 2) Institute of Biomedical Sciences Academia Sinica, Taipei, Taiwan, ROC.

In large-scaled association studies, measuring the relative allelic abundances of markers in pooled DNA samples is a cost-effective strategy to pinpoint potential loci for confirmation. For some heterozygous SNPs, one allele tends to be preferentially amplified in polymerase chain reactions (PCR), the correction is often carried out in pooled DNAs study. However not all SNPs exhibit the phenomenon of one-to-one correspondence between actual allelic frequency and the amplified results. Thus using correction may result in biased estimates of allele frequencies in DNA pooling study. The aim of this study is to apply a novel approach using polynomial standard curves (SC) to estimate allele frequencies. We compared the accuracy between estimates derived from the proposed method and those from the correction for multiple types of SNP markers (A/T, A/C, C/T, A/G, C/G, and G/T) with 7 designated actual minor allele frequencies (AMiAFs) (5%, 10%, 20%, 30%, 40%, 45%, and 50%). For the SNPs with A/T, A/C, C/T, and A/G polymorphisms; estimates of the 7 AMiAFs derived from SC were similar to those from correction. The former was slightly more precise and closer to the known than the latter. For SNPs with C/G polymorphism; apparent differences were observed between the estimates derived from the two methods. Similar phenomenon was observed for SNPs with G/T polymorphism. In general, the estimates derived from the polynomial SC method were closer to AMiAFs than those from the correction particularly for SNPs with G/C and G/T polymorphism in the AMiAFs range of 20 to 40%. Our results demonstrated that SC method is more accurate and precise than correction, not requiring adjustment by complex statistical models.

Correlation among poor prognostic indicators in B-cell chronic lymphocytic leukemia (B-CLL). *A.W. Block[1], P.K. Wallace[2], K.C. Miller[3], M.S. Czuczman[3], A.A. Chanan-Khan[3], S. Padmanabhan[3]* 1) Clinical Cytogenetics Laboratory, Roswell Park Cancer Institute, Buffalo, NY; 2) Laboratory of Flow Cytometry, Roswell Park Cancer Institute, Buffalo, NY; 3) Department of Medicine, Roswell Park Cancer Institute, Buffalo, NY.

B-CLL, the most common leukemia in adults, is a malignant disorder characterized by progressive accumulation of functionally incompetent B-lymphocytes. Clinical staging systems are of prognostic importance, but cannot predict outcome of individual patients in early clinical stages. Factors that are independent predictors of disease progression and outcome in B-CLL include genomic aberrations and somatic mutation status in the expressed immunoglobulin heavy chain variable region (IgVH). Using a FISH panel that identified specific numerical and structural abnormalities (+12, del(13q), del(11q), del(17p), 14q32 abn) and ZAP-70 (70-kD zeta-associated protein, a surrogate for mutation status) expression assessed by flow cytometry, we studied peripheral blood and bone marrow from 37 unselected B-CLL patients (pts) to investigate the concordance of high-risk markers in these pts. 20/37 (54.1%) pts (10 males, 10 females; median age 62, range 49-85 yrs) showed ZAP-70 expression in less than 20% of cells (mutated status), and were considered ZAP-70 negative. Genomic aberrations were detected in 12/20 (60%) pts with del(13q) in 9/12 (75%) pts. High risk (del 11q and 17p) features were seen in 3/12 (25%) abn cases. 17/37 (45.9%) pts (12 males, 5 females; median age 69, range 42-85 yrs) exhibited ZAP-70 expression in 20% of cells and were considered ZAP-70 positive (non-mutated status). Genomic aberrations were detected in 14/17 (82%) pts with high risk deletions observed in 5/14 (35.7%) abn cases. To develop risk-adapted strategies, prognostic factors are needed to allow the prediction of individual patient clinical course. In this study, poor risk patients were defined by advanced clinical stage, increased age, male predominance, non-mutated status and high risk cytogenetic aberrations.

BRLMM: an improved genotyping calling method for Affymetrix mapping arrays. *S. Cawley[1], X. Di[1], E. Hubbell[1], S. Lincoln[1], M. Moorhead[1], W. Short[1], T.P. Speed[2,3], C. Sugnet[1], J. Veitch[1], T. Webster[1], A. Williams[1], G. Yang[1]* 1) Affymetrix, Santa Clara, CA; 2) Department of Statistics, University of California at Berkeley; 3) Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia.

Highly accurate and reliable genotype calling is an essential component of any high-throughput SNP genotyping technology. The Dynamic Model (Di *et al*, Bioinformatics 2005) which has been extensively used for the GeneChip Mapping 100K and Mapping 500K sets has proven to be very effective, however it is possible to do better. Rabbee & Speed recently developed a model called the Robust Linear Model with Mahalanobis distance classifier (RLMM, Rabbee & Speed, Bioinformatics 2005) which provided an improvement over DM on the Mapping 100K set. We present here an extension of the RLMM model which provides a significant improvement over DM in two important areas - it significantly improves overall performance (call rates and accuracy) and it equalizes the performance on homozygous and heterozygous genotypes. The main difference between RLMM and this evolved approach is the addition of a Bayesian step which provides improved estimates of cluster centers and variances, the new model is called BRLMM (pronounced B-realm). The performance improvement is achieved by two main advances over the DM model. Firstly, RLMM and BRLMM perform multiple chip analyses, enabling the simultaneous estimation of probe effects and allele signals for each SNP and resulting in lower variance on allele signal estimates. The second main source of improvement is the estimation of genotypes by a multiple-sample classification, borrowing information as necessary from other SNPs to better predict the properties of the underlying genotype clusters. By contrast, the DM approach calls genotypes by analyzing the probe-level intensities one SNP and one chip at a time, using strong assumptions about the behaviour of the underlying probe intensity patterns in the context of each of the genotypes. RLMM and BRLMM make weaker assumptions about the behavior of probe intensities than does DM, making them far more robust in the presence of real-world data.

Comparative analysis on deletion polymorphisms using gene expression data. *S. Gopalakrishnan, Z.S. Qin* Center for Statistical Genetics, Biostatistics, University of Michigan, Ann Arbor, MI.

Recent studies suggested that deletion polymorphisms (DP) are ubiquitous in human genome (Conrad et al. 2006, Hinds et al. 2006, and McCarrol et al. 2006). Deletions have been implicated in previous researches as playing a significant role in human diseases (e.g., DiGeorge and Prader-Willi syndromes). With the increasing resolution of the human genome, ever smaller DPs are being identified. Data from the HapMap project and other large scale studies made it possible for a genome wide survey on the impacts of DPs. In this study, we use DPs identified and mapped on all the HapMap individuals (McCarrol et al. 2006) to study their effect on the expression levels in different populations. We compare and contrast the significant results in the three HapMap populations, CEU, YRI, CHB+JPT. The gene expression data are obtained from two different sources: Affymetrix (Cheung et al. 2005) and Illumina (Stranger et al. 2006). Multiple QTL association studies were performed to analyze the data: namely, linear regression using the unrelated founder individuals in all populations, variance component TDT analysis on CEU and YRI trios. In the CEU sample, regression analysis reveals 7 deletion polymorphisms that are significantly associated with expression levels. In the CHB, JPT and the YRI samples, the number of DPs significantly associated with expression levels are 8, 8 and 5 respectively. When using a p-value threshold of 0.0001, we found 11 significant associations in the CEU, 17 in the CHB, 14 in the JPT and 7 in the YRI samples. Special attention has been paid to genes located near the DP sites to identify any potential cis acting effects. We plan to follow up with a more extensive study when denser deletion polymorphism data becomes available.

High density SNP screening of the major histocompatibility complex in systemic lupus erythematosus. *L.A. Criswell[1], S.L. Clark[2], P.P. Ramsay[2], M.F. Seldin[3], L.F. Barcellos[2]* 1) Univ of Calif, San Francisco, CA; 2) Univ of Calif, Berkeley, CA; 3) Univ of Calif, Davis, CA.

A substantial genetic contribution to systemic lupus erythematosus (SLE) risk is conferred by gene(s) within the major histocompatibility complex (MHC) on chr. 6p21. The most consistent associations are with class I and II genes, including HLA-A*01, -B*08, -DRB1*0301, -DRB1*1501, and -DRB1*08. Genes within the class III and extended MHC regions have also been strongly implicated, and may be independent of class II. Previous studies of MHC variation and both SLE susceptibility and related phenotypes have lacked statistical power and genetic resolution to fully characterize MHC influences. We recently completed comprehensive MHC region SNP genotyping in 492 Caucasian SLE cases and 151 nuclear family members (N=643 individuals). A total of 2,360 MHC SNPs spanning 4.9 Mb with an average density of one SNP/2 kb were investigated, including variants from 159 MHC region genes (average 10.7 SNPs/gene). Analyses of linkage disequilibrium and haplotype diversity for MHC SNPs revealed a complex architecture. A total of 236 distinct haplotype blocks were identified in SLE cases and family members, ranging in size from 0.25-161 kb; 2-14 haplotypes/block were observed, marking common haplotypic variation across this region. The most common DRB1 haplotypes, *0301 and *1501, were present in cases at a frequency of 20.1% and 13.7%, respectively; 12% of cases were either homozygous for high risk DRB1 haplotypes or carried one copy of each. The extended DRB1*1501 haplotype previously observed in SLE (~500 kb) was comprised of 17 strongly associated blocks (116 SNPs). Initial results suggest that centromeric and telomeric boundaries are marked by BTNL2 and DQA1 (320 kb region). Full characterization of SLE-associated DRB1*0301 and *08 haplotypes is underway. Our large family-based study of high-density MHC SNP variation, in conjunction with classical HLA loci genotyping in 800 SLE families (Total N=2,400 individuals) will provide an opportunity to systematically screen and identify all MHC gene(s) and interactions contributing to SLE risk and related phenotypes, such as lupus nephritis.

Investigation of somatic *NKX2.5* mutations in congenital heart disease. *J.M. Draus[1], M.A. Hauck[1], E.H. Austin[1], A. Tomita-Mitchell[2], M.E. Mitchell[3]* 1) Surgery, University of Louisville, Louisville, KY; 2) Bioengineering, University of Louisville, Louisville, KY; 3) Surgery, Medical College of Wisconsin, Milwaukee, WI.

Recent reports suggest that somatic *NKX2.5* mutations found in formalin-fixed human hearts with septal defects are etiologic. To test the hypothesis that somatic *NKX2.5* mutations contribute to congenital heart malformations, we repeated the above study using fresh-frozen tissue. Blood and cardiac tissue samples were collected from 28 patients. Our cohort included patients with atrial septal defects (ASD = 16), ventricular septal defects (VSD = 4), and both (AVSD = 8). Cardiac samples were collected as surgical discards or from explanted hearts from malformed and normal regions. Tissue samples were immediately frozen in liquid nitrogen and stored at -80ºC. gDNA was isolated, amplified and sequenced from 42 pathologic cardiac tissue samples using methods described in the published reports. A novel non-synonymous mutation (A65G) was identified in an ASD patient. This mutation results in a glu-to-arg (Q22R) amino acid substitution and is located just outside of the TN domain. Two novel synonymous mutations (G543A and C852G) were observed in separate ASD patients; G543A and A65G occurred in the same patient. Finally, a common SNP (A63G) was observed in 16 patients in homozygous and heterozygous forms. All of the genetic variants were also detected in gDNA from the same patients blood. Conclusion: No evidence of somatic mutations was found in the present study although additional germ-line *NKX2.5* mutations were identified. The effects of fixation are not well understood at the molecular level. gDNA yield from fresh-frozen tissue (1.3 g gDNA from 5 mg tissue) was greater than five times that of formalin-fixed hearts (0.5 - 1 g DNA from 25 mg tissue). The mutational spectrum of formalin-fixed hearts was significantly different from inherited and somatic mutation databases including HGMD, *p53*, *HPRT*, as well as inherited *NKX2.5* mutations. The earlier reports may reflect post-mortem artifacts of fixation, incorporated as mutations during PCR because of low gDNA yield and damaged gDNA templates.

A genome-wide non-synonymous SNP panel. *L.M. Galver[1], P.C. Ng[1], S. Hunt[2], J. Whitacre[1], R. Shen[1], P. Deloukas[2], S.S. Murray[1]* 1) Illumina, Inc, San Diego, CA; 2) Wellcome Trust Sanger Institute, Hinxton, United Kingdom.

Approximately 90% of all genetic variation in humans is attributed to single nucleotide polymorphisms (SNPs) (Collins *et al*., 1998). Many SNPs are believed to cause phenotypic differences and can be related to an individuals susceptibility to disease. SNPs in coding regions that cause amino acid changes, non-synonymous SNPs (nsSNPs), as well as SNPs in regulatory regions, are believed to have the highest impact on phenotype as they directly affect protein structure and function (Collins *et al*, 1997). We have developed a genome-wide panel of nsSNPs that will be a valuable resource either as a whole genome screen of nsSNPs only, or as a complement to other whole genome or candidate gene association studies.

SNPs were selected for this study by screening public databases for all annotated nsSNPs. Approximately 50,000 SNPs were screened for assay designability on the Illumina BeadArray platform and approximately 38,000 were screened for functionality on 90 CEU (Caucasian) samples from the HapMap project. Greater than 13,000 functional SNP assays were chosen for the final panel that represents greater than 5,800 genes. The final assay panel was designed utilizing a whole genome genotyping assay on Illuminas multi-sample bead chips and 270 HapMap samples were genotyped for validation.

Preliminary results from the analysis of 270 HapMap samples show an average minor allele frequency (MAF) of 0.18 for the CEU population, 0.16 for CHB/JPT and 0.16 for YRI. We observed a significant percentage of nsSNPs monomorphic in a single population were polymorphic in one of the other two populations indicating population level differences and possibly selection. Comparing the d_N/d_S ratio(Nielsen, *et al*. 2005) to the no. of nsSNPs per gene, we observed that highly conserved genes have fewer nsSNPs. We also confirmed that putative-damaging nsSNPs (predicted by SIFT, Ng and Henikoff, 2002) tended to be present at lower MAFs as has been predicted in previous studies (Livingston *et al*., 2004, Leabman *et al*., 2003).

A high density admixture scan in 1,670 African Americans with hypertension and 387 normotensive controls. *R. Deo[1], N. Patterson[2], A. Tandon[1,2], G. McDonald[1,2], C. Haiman[4], K. Ardlie[5], B. Henderson[4], S. Henderson[3,4], D. Reich[1,2]* 1) Dept. of Genetics, Harvard Medical School, Boston, MA; 2) Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Boston, MA; 3) Department of Emergency Medicine, Keck School of Medicine, Univ. of Southern California, CA; 4) Department of Preventive Medicine, Keck School of Medicine, Univ. of Southern California, CA; 5) Genomics Collaborative, Inc., Cambridge, MA.

Hypertension (HTN) is a devastating disease with a higher incidence in African Americans than European Americans, motivating searches for genetic variants determining this difference. We present results of an admixture scan for genetic determinants of HTN. Admixture mapping involves screening the genome in a population of mixed ancestry such as African Americans, genotyping markers differing in frequency between the ancestral populations to find regions with elevated proportion of ancestry from the population with higher risk for disease. We genotyped 1,165 HTN cases and 387 controls from the Multiethnic Cohort (MEC) and 505 HTN cases from the Genomics Collaborative, Inc. collection (GCI) using a panel of 1,291 polymorphic markers, and evaluated the likelihood of each position as a disease locus using the ANCESTRYMAP program. MEC cases and controls, and GCI cases had respective mean European ancestry of 2414%, 2515%, and 1513%. No significant (LOD5) or suggestive (5>LOD4) loci for association with HTN were found for the MEC or GCI samples, separately, or together. Redefining cases and controls by logistic regression with age and BMI as covariates yielded a suggestive peak at 14q22 (LOD=4.0) for the MEC samples, corresponding to a local excess of 5% European ancestry for cases above their individual mean, and a local decrease of 6% for controls. Inclusion of 199 stringently defined GCI cases decreased the LOD score to 1.2. In a combined MEC/GCI scan, we find limited association with HTN for the two top loci reported in the admixture mapping study by Zhu et al. (Nat Genet. 2005 Feb;37(2):177-81.) with estimated odds ratios for HTN of 0.87 (95% credible interval: 0.77-0.99) and 0.95 (0.83-1.05) for one European allele at the 6q24 and 21q21 loci, respectively.

Crypic out-of-frame translational initiation of TBCE rescues tubulin formation in the syndrome of hypoparathyroidism, mental and physical retardation and dysmorphism (HRD). *G.A. Diaz[1], G. Tian[2], M. Huang[1], R. Parvari[3], N. Cowan[2]* 1) Dept Human Genetics, Mt Sinai Sch Medicine, New York, NY; 2) Dept Biochemistry, NYU Sch Medicine, New York, NY; 3) Dept of Dev Gen and Virology, Ben Gurion Univ of the Negev, Beer Sheva, Israel.

Mutations in the gene encoding TBCE cause a devastating autosomal recessive disorder characterized by hypoparathyroidism, mental and physical retardation, and dysmorphism (HRD syndrome). TBCE is one of five tubulin-specific chaperones termed cofactors A-E (TBCA-E). TBCC, TBCD and TBCE are essential in higher eukaryotes and function as a multimolecular machine that assembles quasi-native - and -tubulin polypeptides into tubulin heterodimers competent to assemble into microtubules. HRD is most common in Middle Eastern individuals who are homozygous for a 12-bp founder deletion in the second coding exon of TBCE that ablates nt 155-166 of the open reading frame. The mutant protein lacks amino acids 52-55 within the cytoskeleton-associated protein glycine-rich (CAP-gly) -tubulin-binding domain. Total tubulin levels in homozygous mutant cells are normal, consistent with residual tubulin chaperone activity in the mutant, but mutations identified in a Belgian pedigree present a more puzzling picture. One allele contains a two-bp deletion at nt 66-67 in the first coding exon and is predicted to yield a 22-amino acid wild type peptide fused to an out-of-frame 26-amino acid nonsense sequence; the second allele contains a nonsense mutation in exon 12 (1113T->A) that would result in a protein lacking the C-terminal 157 (out of 527) amino acids. These compound heterozygous HRD patients would not be expected to produce a functional TBCE protein. Here we identify cryptic translational initiation at each of three out-of-frame AUG codons upstream of the genetic lesion as a unique mechanism that rescues a mutant HRD allele by producing a functional TBCE protein. Our data identify a novel rescue mechanism to explain how affected individuals who would otherwise lack the capacity to make functional TBCE can survive, and point to a limiting capacity to fold tubulin heterodimers de novo as a contributing factor to disease pathogenesis.

Pharmacogenetic analysis of antipsychotics: comprehensive analysis of pharmacokinetic variants. *I. Grossman*[1], *Y. Liu*[2,1], *N. Walley*[1], *J. Dawson*[1], *C. Gumbs*[1], *M. Weale*[1], *P. Sullivan*[3], *D. Goldstein*[1] 1) IGSP Center for Population Genomics and Pharmacogenetics, Duke University, Durham, North Carolina, USA; 2) Bioinformatics Research Center, North Carolina State University, Raleigh, North Carolina, USA; 3) Department of Genetics, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA.

Clinical implementation of pharmacogenetic testing seems achievable with the development of commercially available genotyping platforms. Current options use genetic tests to predict metabolic phenotypes of a few key drug metabolizing enzymes (DMEs), although the potential utility in clinical practice has yet to be thoroughly investigated. This study was designed to describe comprehensively the effects of known functional genetic variations in DMEs on the response to and dosing of antipsychotics. 756 patients who had participated in the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study were genotyped for 26 genetic variants in *CYP2D6, CYP1A2, CYP3A4, CYP3A5, CYP2C9, CYP2C8, CYP2C19, UGT1A4, FMO3* and *ABCB1*. Drug treatments included the conventional antipsychotic perphenazine, and the atypicals olanzapine, risperidone, quetiapine and ziprasidone. Patients treated with each drug were analyzed separately for association with three phenotypes: optimized dose, safety, and efficacy. ANOVA was used to assess whether gene variants predict optimized dose, while association of these genetic polymorphisms with safety and efficacy measurements was tested by way of contingency tables (^{2} and Fisher's exact tests, as appropriate). Potential covariates were broadly evaluated. No clear significant associations were observed between optimized dose and any genetic variants. We could not replicate an association between the *CYP2D6* poor metabolizer (PM) phenotype and Tardive Dyskinesia. However, a trend was detected between relevant polymorphisms and occurrence of side effects for some of the drugs. In conclusion, our results suggest that any effect of relevant genetic polymorphisms in DMEs on antipsychotics response and dosing is modest.

Genome-wide scan for non-syndromic cleft lip and palate in multigenerational Indian families reveals significant evidence for linkage at 13q33.1-34. *S. Beiraghi[1], U. Ratnamala[2], M. Gaines[3], D. Hutchings[3], S.A. Husain[4], P.S. Gambhir[5], J.J. Sheth[6], F.J. Sheth[6], G.K. Chetan[7], M. Naveed[8], J.V. Solanki[9], U.C. Patel[9], R. Memon[2], G.S. Antonarakis[10], S.E. Antonarakis[10], S.K. Nath[3], U. Radhakrishna[2]* 1) Pediatric Dent 6-150 Moos T, Univ Minnesota, Minneapolis, MN; 2) Green Cross bank and Genetic Research Centre, Paldi, Ahmedabad, India; 3) Arthritis and Immunology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, USA; 4) Department of Biosciences, Jamia Millia Islamia Central University, New Delhi,; 5) Department of Pediatrics, B. J. Medical College, Pune,; 6) Foundation for Research in Genetics and Endocrinology, Ahmedabad,; 7) Dept. of Hum Genet, National Institute of Mental Health & Neuro Sci Bangalore, India; 8) Canadian Specialist Hospital, P.O. Box 15881, Dubai, United Arab Emirates; 9) Dept. of Animal Genetics and Breeding, Gujarat Agriculture University, Anand, India; 10) Department of Genetic Medicine and Development, University of Geneva Medical School, Geneva, Switzerland.

Non-syndromic cleft lip with or without cleft palate (CL-P),is a common congenital anomaly with incidence ranging from 1/300 to 1/2500 live births. We have analyzed two Indian pedigrees (UR017 & UR019) with isolated, non-syndromic CL-P in which the anomaly segregates as an autosomal dominant trait. A genome-wide linkage scan using ~10,000 SNPs was performed. Non-parametric linkage analysis identified 11 genomic regions (NPL > 3.5, P-Value <0.005) that could potentially harbor CL-P susceptibility variations. Among those, the most significant evidence was for chromosome 13q33.1-34 at marker rs1830756 (NPL = 5.57 and p=0.00024). This was also supported by parametric linkage; MOD-score (LOD scores maximized over genetic model parameters) analysis favored an autosomal dominant model. The maximum LOD score was 4.45 and HLOD was 4.45 (alpha = 100%).Haplotype analysis with informative crossovers enabled the mapping of the CL-P locus to a region of approximately 20.17cM (7.42Mb) between SNPs rs951095 and rs726455. Thus, we have identified a novel genomic region on 13q33.1-34 that harbors a high risk variant for CL-P in these Indian families.

Evidence of Association Between Polymorphism on UCPs and Type 2 Diabetes in a Northwestern Population From Colombia. *L. Franco[1], C. Duque[1], N. Gallego[1], F. Uribe[2], A. Villegas[2], G. Bedoya[1], A. Ruiz-Linares[1]* 1) Biology, Universidad de Antioquia, Medellín; 2) Hospital Universitario Saqn Vicente de Paul, Universidad de Antioquia, Medellín.

The uncoupling proteins (UCPs) are a family of mitochondrial transport proteins that promote proton leakage across the inner mitochondrial membrane, uncoupling oxidative phosphorylation from adenosine triphosphate (ATP) production and releasing energy as heat. In comparison to the established uncoupling and thermogenic activities of UCP1, UCP2 and UCP3 appear to be involved in the limitation of free radical levels in cells rather than in physiological uncoupling and thermogenesis. Moreover, UCP2 is a regulator of insulin secretion and UCP3 is involved in fatty acid metabolism. Variation in these genes has been associated with Type 2 diabetes (T2D). We genotyped six polymorphisms in UCP1, UCP2 and UCP3 in a sample of 190 type 2 diabetic patients and 149 nondiabetic participants. Associations between -866G/A (p=0.0025) of UCP2 and -55C/T (p=0.0005) of UCP3 polymorphisms and T2D were found. Additionally, it was found that haplotype AIAGAC was significantly associated with DM2 (p<<<0.0005 and O.R.= 5.19). These results support a role for UCPs in the development of DM2. This research is funded by COLCIENCIAS 11150416451.

Exclusion of MSX1 and PAX9 gene mutations in four families with anodontia. *P. Gambhir[1], S.K. Nath[2], J.V. Solanki[3], U.C. Patel[3], R. Memon[4], U. Ratnamala[4], U. Radhakrishna[4]* 1) Department of Pediatrics, B. J. Medical College, Pune, India; 2) Arthritis and Immunology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, USA; 3) Departments of Animal Genetics and Breeding, Gujarat Agriculture University, Anand, India; 4) Green Cross bank and Genetic Research Centre, Paldi, Ahmedabad, India.

Anodontia is one of the common genetic disorders characterized by partial or complete absence of the primary or permanent teeth. Its genetic basis of development is poorly understood. It is generally associated with a group of non-progressive skin and nerve syndromes. Anodontia is usually part of a syndrome and seldom occurs as an isolated entity. Over 50 syndromes may include tooth agenesis. Most commonly affected are third molars (wisdom teeth), followed by either upper lateral incisors or lower second premolars; agenesis involving first and second molars is very rare. Several studies have shown that MSX1 as well as PAX9 genes play a role in early tooth development (Nature Genet. 24: 18-19, 2000; Nature Genet. 13: 417-421, 1996). We have studied four non-syndromic anodontia families including two with complete and two with partial anodontia. The mode of inheritance is autosomal dominant and the severity of the phenotype was quite variable among and between these families. There was 100% penetrance in these families as no skipping of generation was observed. Some of the partially affected individuals lacked mandibular second premolars as well as mandibular central incisors. Thus far, we have analyzed MSX1 and PAX9 gene coding regions and splice junctions by direct sequencing and excluded these genes as candidates in these four families. This study reveals that genetic heterogeneity exists within anodontia.

Multiple perineural thoracic cysts presenting as "neurofibromas". *K. Gardner[1], J. Boardman[2]* 1) Veterans Administration Pittsburgh Healthcare System, Dept Neurology, Univ Pittsburgh, Pittsburgh, PA; 2) Dept of Neuroradiology, Univ Pittsburgh, Pittsburgh, PA.

We report a case of isolated perineural cysts found along multiple, bilateral thoracic spinal nerve roots that were initially thought to represent neurofibromas. No other conditions associated with dural ectasia / perineural cysts were identified. Localized congenital and post-traumatic or surgical perineural cysts have been described previously though never involving multiple levels as in the case presented here. Incidental "Tarlov" cysts have been described primarily in lumbosacral levels though the frequency of these is quite low according to a recent large sampling of lumbosacral MRI images (1.5%).Dural ectasia, arachnoid, and perineural dilation around multiple spinal roots can also be associated with Neurofibromatosis-1 (NF1) or with Marfan Syndrome. Dural ectasia is one of the major diagnostic criteria for Marfan Syndrome using Ghent nosology, also found usually in lumbo-sacral regions. With NF1 perineural cysts and dural ectasia can occur at any spinal level, are associated with other clinical features for NF1, and are sometimes associated with plexiform spinal neurofibromas. Careful evaluation of MRI imaging signal characteristics is critical to first differentiate cysts from tumors in patients presenting with thickened spinal nerve sheaths. T2 signal should match CSF signal for perineural cysts and T1 signal should match that of nerve root in the case of tumor expansion of nerve sheath (with or without enhancement). Those found to have perineural cysts without enlargement of nerve roots should then be examined carefully to exclude associated conditions looking for stigmata of Neurofibromatosis-1 (NF1) and for connective tissue disorders such as Marfan Syndrome. If thickened nerve roots are truly present then the differential for paraspinous tumor includes NF1, NF2, and schwannomatosis depending on presence or absence of characteristic skin findings, vestibular schwannomas, and other diagnostic features.

A common $_2$ adrenergic receptor haplotype is associated with protection against decrease in receptor sensitivity among smoking men. *X. Bao[1], P.J. Mills[2], J.E. Dimsdale[2], M.G. Ziegler[1]* 1) Dept Medicine, Univ California, San Diego, CA; 2) Dept Psychiatry, Univ California, San Diego, CA.

Cigarette smokers are more likely to develop hypertension than nonsmokers. Blunted $_2$ adrenergic receptor (ADRB2) mediated vasodilation has been implicated in the pathogenesis of hypertension. Both smoking and ADRB2 polymorphisms are associated with vascular responsiveness. However, whether they have interactive effect on the intermediate phenotype of $_2$ adrenergic receptor function is unclear. To investigate this, 10 common single-nucleotide polymorphisms (SNPs) in the promoter and coding regions of ADRB2 were genotyped, and lymphocyte $_2$ adrenergic receptor functions were measured in an unrelated cohort of 187 white men. ADRB2 haplotypes were estimated by expectation maximization (EM) algorithm-based methods. Three common haplotypes (frequency > 5%) were identified. The frequency of the most common haplotype (haplotype 1) is 43%. Lymphocyte $_2$ adrenergic receptor sensitivity did not differ between smokers and nonsmokers who were carrying haplotype 1 (5.1 0.6 vs. 6.0 1.7 pmol/L per 10^6 cells), but was significantly lower in smokers vs. nonsmokers who were not carrying haplotype 1 (3.0 0.4 vs. 7.3 1.4 pmol/L per 10^6 cells, $p < 0.01$). No differences in lymphocyte $_2$ adrenergic receptor density and desensitization were seen between smokers and nonsmoker carrying haplotype 1 or not. Our data indicate that smoking reduces $_2$ adrenergic receptor sensitivity but only in subjects carrying no haplotype 1. Therefore, the common $_2$ adrenergic receptor haplotype 1 is associated with protection against smoking-related decrease in receptor responsiveness, and thus may prevent development of hypertension among smokers.

Widely distributed non-coding selection in the human genome. *S. Asthana[1], W.S. Noble[2], G. Kryukov[1], S. Sunyaev[1], J.A. Stamatoyannopoulos[2]* 1) Division of Genetics, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; 2) Dept. of Genome Sciences, Box 357730, Univ. of Washington, 1705 NE Pacific Street, Seattle, WA 98195.

Evolutionarily conserved non-coding sequences (CNSs) in the human genome have attracted considerable attention for their potential to simplify the search for functional elements and phenotypically important human alleles. A major outstanding question is whether CNSs accurately capture the distribution of functionally significant non-coding variation. We used recently available whole-genome sequence data from chimp, dog, mouse, and rat, to partition the human genome sequence at the nucleotide level into conserved and non-conserved positions based on conservation across four non-human species. Conserved bases are widely distributed, with a majority occurring outside of CNSs. Here we show using analysis of comprehensive human resequencing data that the selective differences observed between CNSs and non-CNS non-coding sequences are recapitulated at the level of individual conserved and non-conserved bases outside of CNSs. Individually conserved positions have lower nucleotide diversity within human population. SNPs in these positions tend to have lower derived allele frequency. This result holds for individually conserved bases not simply outside of genes and conventional CNSs but also in regions with extremely poor overall conservation level. The results indicate that a substantial fraction of active selection in human non-coding sequences is occurring outside of CNSs. This fraction is widely distributed, suggesting that a surprisingly large proportion of the human non-coding genome may be under selection. Analysis in the framework of the infinite number of sites model shows that even assuming very strong heterogeneity of mutation rates at all possible scales and any possible distribution of selection coefficients cannot explain the above observations if less than 20% of individually conserved positions are under selective pressure. Limiting the search for phenotypically important human non-coding variation to CNSs may overlook a large reservoir of functional alleles.

Craniometaphyseal dysplasia associated with de novo complex chromosomal rearrangement 46, XY, der(1)t(1;6;14)(q32;q13q22;q22), der(6)t(1;6),der(14)t(6;14)(q22;q22). *B. Afroze[1], Y.S. Choy[1], A. Mekesat[2], Ruziana[2]* 1) Genetics and Metabolism, Pediatric Institute, Kuala Lumpur Hospital, Kuala Lumpur, Malaysia; 2) Cytogenetic Unit, Kuala Lumpur Hospital, Kuala Lumpur, Malaysia.

Craniometaphyseal dysplasia is a craniotubular bone dysplasia syndrome characterized by craniofacial anomalies presenting in early childhood. It is genetically heterogenous and two loci has been identified. The autosomal dominant type is due to mutations in ANKH gene encoding a protein regulating transmembrane transport of pyrophosphate, mapped to 5p14.1p15.2. The second gene locus responsible for the more severe type was mapped to 6q21-q22 in a large inbred family but not cloned yet. We report here a patient presenting to us at the age of 18 months with craniofacial anomalies typical of craniometaphyseal dysplasia. He had frontal bossing and dolicocephaly with severe hypertelorism, telecanthus, broad nasal bridge and but small short nose. The anterior fontanelle was still widely open. His developmental milestones and growth had been apropriate for age. He was the second twin of a non-consanguineous couple and the first twin was perfectly normal. High resolution karyotype revealed a de novo complex chromosomal rearrangement 46, XY, der(1)t(1;6;14)(q32;q13q22;q22), der(6)t(1;6),der(14)t(6;14)(q22;q22). FISH that was carried out using wcp 1, 6 and 14 probes comfirmed the rearrangement. Karyotype for the parents and the other twin was normal. We concluded that the breakpoint at 6q22 had probably disrupted the gene responsible for craniometaphyseal dysplasia.

SIL1 and SARA2 mutations in a family with Marinesco-Sjogren and Chylomicron Retention Diseases. *G. Annesi[1], P. Tarantino[1], F. Annesi[1], E.V. De Marco[1], D. Civitelli[1], A. Torroni[2], A. Quattrone[1,3]* 1) Inst Neurological Sci, National Research Council, Cosenza, Italy; 2) Dipartimento di Genetica e Microbiologia ,Università di Pavia, Italy; 3) Institute of Neurology, University Magna Graecia, Catanzaro, Italy.

Marinesco-Sjogren Syndrome (MSS) is an autosomal recessive disorder, characterised by cataracts, ataxia, and growth and mental retardation. Recently, Anthonen et al identified the linkage of the MSS phenotype to 5q31 chromosome in a Finnish family, and identified a homozygous 4-nucleotide duplication 506_509dupAAGA 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 mutations in SIL1 gene in individuals with MSS. In the current study, we further investigated a small Italian pedigree, previously described elsewhere. In this family, two brothers had MSS syndrome, with a very low serum concentration of vitamin E and absence of postprandial chylomicrons, a finding consistent with chylomicron retention disease (CMRD). On a subsequent study , we demonstrated that these two brothers carried a mutation in the SARA2 gene belonging to the Sar1-ADP-ribosylation factor family of small GTPases. On the basis of the recent data on SL1 in MSS, we analyzed the SL1 gene in our patients with MSS and CMRD, in their healthy parents, and in 50 healthy controls from Calabria. Sequencing of the SIL1 gene revealed a C-T homozygous mutation at position 331 in exon 4 resulting in a premature stop codon at amino acid 111 (R111X) in the affected brothers, while both parents were heterozygous for the mutation, and all 100 chromosomes from control subjects were wild-type. In our previous study, we hypothesized that both MSS and CRMD found in the two Calabrian brothers could be due to defects in a gene crucial to the assembly or secretion of the chylomicron particle, leading to a very low serum concentration of vitamin E. The present results, however, demonstrate that CMRD and MSS observed in our patients are distinct diseases due to defects in two different genes, SARA2 and SIL1.

Association of m2 and m4 CYP1A1 polymorphisms with Mexican lung cancer patients. *M. Gallegos[1], V. Peralta[1], AM. Puebla[2], GM. Zúñiga[3]* 1) Laboratorios de Genetica Molecular; 2) Inmunofarmacologia; 3) Laboratorio de Mutagenesis, Division de Medicina Mutagenesis, Div. Med. Mol, CIBO, IMSS. Guadalajara, Jalisco. Mexico.

Lung cancer is the leading cause of death among cancers in Mexicans. Although the etiology of lung cancer has yet to be defined, genetic variability in activities of metabolic enzymes has been correlated with lung cancer. In the present study, the possibility of association of CYP1A1 (m2 and m4) genetic polymorphisms with lung cancer was examined among 159 lung cancer patients and 220 controls in Mexicans. Significant association was observed for m2 and m4 CYP1A1 polymorphism with odds ratio 2.19; confidence interval (CI) 0.89 -5.60) and 13.05 (CI 3.01-117.5) respectively. Our results suggest that the m2 and m4 CYP1A1 polymorphisms are an important risk factor for Mexican lung cancer patients.

High Myopia: A Genetic and Clinical Study Among Egyptian Patients. *H. Elbastawisy, M. Salah* Research Institute of Ophthalmology. Giza, Egypt.

Background: In cases of high or pathological myopia, have errors of -6.00D or greater in one or both eyes. Although in many cases myopia appears to be genetically determined, its precise pathophysiology and development have yet to be elucidated. Both, autosomal dominant and recessive transmissions have been reported for the pathological myopia. Methods: forty-two families (group- I) participate in the study with onset of myopia at < 12 years of age in all affected subjects (parents & offspring); myopia of -6.00 D., and two or more affected generations. Group-II, include cases of high myopia as a part of a known genetic syndrome. Full ocular examination as well as axial length measurements was done. The mode of inheritance was thoroughly studied. Results: The (group-I) high myopia families showed a likely autosomal dominant pattern of inheritance, but were far away from autosomal recessive or X-linked inheritance; while high myopia as a part of a syndrome follows the inheritance of the syndrome itself. Ophthalmologic findings showed in (group-I) that (33.3 %) had high myopia ranging from (- 6.00D : -14.00 D), and (48.8%) were extreme myopes (>-15.00D). Most common fundus associations were peri-papillary temporal crescent (95.2%), RPE-atrophy (83%), posterior vitreous detachment (59.5%), typical lattice degeneration (47.6%), and posterior staphyloma (38.1%); while other visual affecting associations were macular hemorrhage (9.5%), choroidal neo-vessels (7.1%), rhegmatogenous R.D. (4.8%) & macular hole (2.4%). Conclusion: The inheritance of isolated high myopia is mostly autosomal dominant in Egyptian cases, while high myopia as a part of a syndrome is inherited as the mode of inheritance of that syndrome. The higher the degree of myopia, the more the severity of an associated ocular degenerative changes & /or complications.

Frequent tetraploid mosaicism in products of conception. *E.M. Davis[1], T. Alexander[2,3], J. Malone[1], R.V. Lebo[1,3]* 1) Pathology, Akron Children's Hospital, Akron, OH; 2) Pathology, Akron City Hospital, Akron, OH; 3) Northeast Ohio Coll Med. Rootstown, OH.

Tetraploid mosaicism defines some cells with 92 chromosomes that arise by doubling the normal number of 46 and is anticipated to result in abnormality. A 40% frequency of tetraploid mosaicism has been reported in 8-cell preimplantation embryos which are considered to be nonviable. During prenatal diagnosis, most mosaic tetraploid cultured amniocytes are considered to have occurred during tissue culture and are not reported. Our laboratory found mosaic tetraploid chorionic villus cells with unique fetal chromosome patterns in 6% to 8% of the 320 products of conception (POCs)-substantially higher than previously reported. To establish whether these tetraploid fetal cells existed in the spontaneous abortion, tissues were studied prior to cell culture. Initially only a few cells in a small fraction of fixed paraffin embedded fetal tissues could be studied by fluorescence in situ hybridization (FISH) because of substantial DNA degradation. Subsequently flow cytometric analysis analyzed thousands of whole nuclei for tetraploid mosaicism from each case. Flow analysis verified tetraploid mosaicism in chorionic villus nuclei in 12 of 13 cases. The single testable fetal tissue in one of these POCs also had mosaic tetraploid cells. These results confirm the high proportion of cases of true tetraploid mosaicism in spontaneously aborted fetal tissues. These data support the conclusion that mosaic tetraploid karyotypes are a biologically important finding in cultures from spontaneously aborted fetuses and should be reported and followed up when found in any cultured fetal tissue.

The genetic susceptibility to retinopathy of prematurity. *M. Bizzarro[1], N. Hussain[2], B. Jonsson[3], R. Feng[4], L. Ment[1], J. Gruen[1,5], H. Zhang[4], V. Bhandari[1]* 1) Dept Pediatrics, Yale Univ Sch Medicine, New Haven, CT; 2) Division of Neonatology, University of Connecticut Health Center, Farmington, CT; 3) Department of Neonatology, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden; 4) Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT; 5) Department of Genetics and the Yale Child Health Research Center, New Haven, CT.

Background: Retinopathy of prematurity (ROP) accounts for significant morbidity in preterm neonates. In addition to prematurity and environmental factors, we hypothesized that genetic factors play a significant role in this disease process.

Objective:To isolate and estimate the genetic susceptibility to ROP

Patients and Methods: A retrospective study (1994-2004) from 3 centers was performed using zygosity data from premature twins born at 32 weeks gestational age (GA) and surviving beyond 36 weeks postmenstrual age. ROP was diagnosed and staged by pediatric ophthalmologists at each center. Data analyses were performed using mixed effect logistic regression (MELR) analysis and latent variable probit modeling.

Results: 63 monozygotic (MZ) and 137 dizygotic (DZ) twin pairs were identified and analyzed. Demographic data for GA, birth weight, gender, respiratory distress syndrome, ROP, bronchopulmonary dysplasia, duration of ventilation and supplemental oxygen use, and length of stay were comparable between MZ and DZ twins. In the MELR for ROP, GA (p=0.024, OR 0.65; 95% CI=0.45-0.94) and duration of supplemental oxygen (p=0.003, OR=1.03; 95%CI=1.01-1.05) were significant covariates. After controlling for known and unknown non-genetic factors, genetic factors accounted for 70.1% (95%CI=0.09-1.00; p=0.026) of the variance in liability for ROP.

Conclusion: Besides environmental factors, there is a strong genetic predisposition to ROP.

Evidence of a Hyperhidrosis Risk Gene at 5q11.2. *R.M. Cantor-Chiu[1], F. Chandra[2], N. Dorrani[2], C. Swartling[3], D. Glaser[4], S. Ahn[2]* 1) Department of Human Genetics,; 2) Department of Surgery, David Geffen School of Medicine at UCLA, Los Angeles, CA; 3) University of Uppsala, Sweden; 4) Washington University, St Louis, MO.

Hyperhidrosis, a disabling lifelong disorder identified by excessive sweating, is familial and common, afflicting as much as 3% of the general population. Although available surgical and drug treatments can reduce excessive sweating, the etiology of this disorder remains unknown. Segregation analysis of families ascertained through a proband with hyperhidrosis revealed a dominant genetic predisposition with reduced penetrance, indicating genetic complexity. Given this evidence of a genetic contribution, a full genome scan of 500 multiallelic markers was initiated to identify chromosome regions likely to harbor hyperhidrosis risk genes. In the current study, 93 members of 16 3-generation hyperhidrosis families were genotyped and analyzed for multipoint linkage under the model of inheritance revealed by segregation analysis. Evidence of linkage to 5q11.2 was observed with a LOD score of 2.0, and no other locus exhibited a lod score greater than 1.0, making this region the most likely location of a hyperhidrosis risk gene. As is necessary with genetically complex disorders, we are conducting a full genome scan in a second independent hyperhidrosis sample of 93 individuals from this cohort before further molecular studies are conducted to narrow the linked region(s) and assess positional candidate genes.

Linking collagen genotypes to molecular phenotypes. *T.F. Chan[1], N. Addleman[1], A. Poon[1], A. Phong[1], T. Klein[2], P. Byers[3], P.Y. Kwok[1]* 1) Institute of Human Genetics, University of California - San Francisco, San Francisco, CA; 2) Department of Genetics, School of Medicine, Stanford University, Stanford, CA; 3) Department of Pathology, University of Washington, Seattle, WA.

Collagen is a fibrous protein that provides structural and functional integrity in the human body. Types I, II, and III fibril collagens are the most abundant forms and account for 70% of the total body collagens. Mutations in collagen lead to an array of disorders. Osteogenesis imperfecta (OI) is the most common manifestation of mutations in the two type I collagen genes, COL1A1 and COL1A2. Other disorders such as chondrodysplasis and vascular Ehlers-Danlos Syndrome (EDS) are due to alterations in type II collagen (COL2A1) and type III collagen (COL3A1), respectively. Although there is a vast repository of clinical phenotypes of the diseases, relationships between genotypes, molecular and clinical phenotypes are poorly understood. Currently, mutations in the collagen genes are identified using a phenotype-to-genotype paradigm. A patient may be diagnosed with a specific phenotype followed by partial genotyping of the individual and possibly close family members. This provides an incomplete picture and does not allow a systematic analysis in a population. In this study, we are moving to a genotype-to-phenotype paradigm in collagen research. Central to this effort is knowing the baseline sequence variations in an ethnically diverse, non-affected population as well as sequence variations seen in patients with collagen disorders. We have performed deep sequencing of the four collagen genes in 192 individuals from the SOPHIE cohort representing 4 ethnic groups as well as 10 patients with OI. We have identified 415 SNPs in the four collagen genes, with 245 as novel SNPs. Correlating the disease causing mutations in the context of background variations in the genes will help explain differences in disease phenotype and guide our study of sequence-structure-function relationship by molecular-folding simulations. This work is supported by the NIAMS AR051582 (T. Klein, PI).

MECP2 large deletions and exon 1 mutations in RTT patients. *F. Ariani[1], I. Longo[1], F. Mari[1], C. Pescucci[1], K. Sampieri[1], R. Artuso[1], E. Scala[1], R. Caselli[1], M. Bruttini[1], I. Meloni[1], G. Hayek[2], M. Zappella[2], A. Renieri[1]* 1) Medical Genetics, University of Siena, Italy; 2) Child Neuropsychiatry, University of Siena, Italy.

Rett syndrome (RTT) is a neurodevelopmental disorder that represents one of the most common genetic causes of mental retardation in girls. MECP2 mutations account for about 80% of classic RTT cases and for a lower percentage of RTT variants. We investigated 74 clinically well-defined mutation-negative RTT patients by searching for missed MECP2 defects. We searched for MECP2 large rearrangements, not detectable by PCR-based traditional techniques, by Real Time qPCR and for mutations in exon 1, previously considered non coding, by DHPLC. We found 9/74 (12%) patients with MECP2 large deletions and 1/74 (1%) with an exon 1 mutation (c.47_57del). The deletions were found in 7 sporadic cases with classic RTT and in one familial case with two sisters with different phenotypes (one is a classic RTT, while the other is a preserved speech variant). The exon 1 deletion was found in a classic RTT girl. These results indicate that MECP2 large deletions are an important cause of classic RTT and confirm the importance of quantitative studies in a complete diagnostic strategy. Moreover, these data suggest that exon 1 mutations are not common in RTT. We decided to characterize the MECP2 deletions by MLPA. All the deletions involve exon 3 and 4, but not exon 2. Intron 2 contains Alu repeats which probably represent recombinogenic factors. Previous studies found that the distal breakpoint of MECP2 deletions frequently occurs in the DPR (deleted prone region). In contrast, in our patients, MLPA showed that 8/9 deletions extend beyond the DPR. MLPA also showed that the deletion in the two RTT sisters involves exon 3 and partially exon 4, suggesting that they probably harbour the same rearrangement. In this case, modifying factors may explain the different phenotypes. Given that the deletion is absent in parents, in order to explain the origin of the rearrangement in the sisters, we hypothesized that one of the two parents may be a mosaic for the mutation in the gonadic tissue.

Tetrasomy X. Report one Mexican patient. *M. Diaz-Garcia[1], A. García-Huerta,[1], D.M. Mendoza-Ugalde[1], C.F. Martinez-Cruz[2,4], R. Baez-Reyes[3], G. Garcia-Sanchez[1]* 1) Genetica. Instituto Nacional de Rehabilitacion. Mexico, DF; 2) Comunicacion Humana. Instituto Nacional de Perinatologia, Mexico, DF; 3) Genetica. Instituto Nacional de Perinatologia, Mexico, DF; 4) Instituto Mexicano del Seguro Social.Hospital General de Zona 53.

The tetrasomy X is a genetic condition in which females have four X chromosomes instead of the usual two. We present a 9-year-and-4-month-old girl who was referred to genetic because shows significant delay in speech development and articulation, language expression and understanding. Clinical features included speech delay, IQ borderline, skin soft and light laxity, muscle hypotonic, high palate, hypoplasic uvula. Joint fingers hyper laxity and showed bilateral clinodactyly in the fifth finger, dermatoglyphics with alterations. Flutes on the outside of the thighs and buttocks, development of secondary sexual characteristics a seven years-and-6 month-old-age and menarche at eight years-old, normal external genitalia. Chromosome analysis was done in lymphocytes. The GTG banded karyotypes showed one cell line, 48,XXXX in 100% of metaphases analyzed. Tetrasomy X is a type of sex chromosome aneuploidy, which simply means an abnormal number of sex chromosomes. In most cases the extra X chromosomes occur when in cell division, two X chromosomes can remain in the egg. If the same event is repeated at the next division, the egg can end up with three X chromosomes. Fertilized by a single X carrying sperm, the egg will then develop into a tetra X chromosomes. Very occasionally, other processes may be involved, such as a failure of the X chromosome to separate in the normal way during cell division in the fertilized egg after conception. The addition of this extra genetic material in a child affects the major areas of development.

Molecular and biochemical alterations in Human Intrauterine Growth Restriction. *L. Guo[1,2], J. Ferreira[1,2], S. Choufani[1], D. Chitayat[1,3], C. Shuman[1], S. Keating[4], J. Kingdom[3], R. Weksberg[1]* 1) Genetics and Genomic Biology, Hospital for Sick Children, Toronto, ON, Canada; 2) Institute of Medical Sciences, University of Toronto, ON, Canada; 3) Maternal-Fetal Medicine Division, Department of Obstetrics & Gynaecology, Mount Sinai Hospital, Toronto, ON, Canada; 4) Department of Pathology, Mount Sinai Hospital, Toronto, ON, Canada.

We hypothesize that epigenetic programming of the genome during embryonic and fetal development affect gene expression patterns and can cause IUGR. The objective of this study is to verify if in IUGR, there is epigenetic dysregulation of some of the imprinted genes on human chromosome 11p15 that are known to be associated with growth alterations. Following informed written consent, placenta samples and cord vein blood from less than 10th percentile birthweight infants (cases) and controls were collected and DNA/RNA extraction was performed using standard procedures. We assessed the regulation of the imprinted genes on chromosome 11p15 H19 (non-coding RNA) and IGF2 (insulin-like growth factor 2 gene) and the associated imprinting control center (ICR1): methylation status of the genes involved by Southern blotting and bisulphite sequencing, mRNA expression level by quantitative real-time RT-PCR, and allelic specific expression by single-nucleotide primer extension assay (SNaPshot). Our results showed that: 1) IGF2 and H19 expression is downregulated in placentae of infants who are small for gestational age. 2) In both IUGR and controls, the H19 promoter and IGF2 DMR2 are hypomethylated in placentae, but normally methylated in cord blood. 3) ICR1 is hypomethylated in some placentae samples from IUGR and maintain its normal methylation status in blood. The decreased IGF2 and H19 gene expression in IUGR versus control placentae is of great interest since these two genes significantly impact placental growth. This decrease of the expression in human IUGR placentae doesnt seem to be regulated by the methylation status of the chromosome 11p15 region. Thus, the cause for IGF2 and H19 downregulation in IUGR is unknown and may be caused by altered histone modification or dysregulated transacting factors.

Evidence of interaction between DTNBP1 and IL3 in schizophrenia. *T. Edwards[1], X. Wang[2], B. Wormly[2], B. Riley[2], A. O'Neill[3], D. Walsh[4], K. Kendler[2], M. Ritchie[1], X. Chen[2]* 1) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN 37232; 2) Virginia Institute for Psychiatric and Behavioral Genetics and Department of Psychiatry, Virginia Commonwealth University, Richmond, VA 23298; 3) The Department of Psychiatry, The Queens University, Belfast, Northern Ireland, UK; 4) The Health Research Board, Dublin, Ireland.

Schizophrenia is a psychotic mental disorder with a lifetime prevalence of approximately 1%. Heritability estimates of approximately 80% indicate this disease is largely genetic. Reports of risk loci and an epistatic model in the literature suggest a complex genetic etiology. In this study, we explored gene-gene interactions in population-based (657 cases: 414 controls) and family-based (269 families; 654 cases: 737 controls) datasets of English or Irish ancestry. Sixty markers in 8 genes were genotyped in families and thirteen markers in 3 genes were genotyped in population data. Results from the family dataset had an intermediate disease definition and the case control sample used the DSM-IIIR schizophrenia diagnosis or poor outcome schizoaffective disorder. Multifactor Dimensionality Reduction Pedigree Disequilibrium Test (MDR-PDT) was used to explore epistasis in families. A highly significant 3-locus interactive model was identified. The 3-locus family interactive model was between genes IL-3, RGS4, and dystrobrevin-binding protein 1 (DTNBP1) (rs2069803, rs2661319 and rs2619539 respectively), $p = 0.006$. To verify these findings, we used MDR on the case-control dataset containing the same markers typed in the 3 genes. While we could not replicate the 3-locus interaction, we saw evidence of a joint effect of IL3 and DTNBP1 with different markers (rs31400 in IL3 and rs760761 in DTNBP1), $p < 0.001$, odds ratio = 1.25, 95% CI [0.95, 1.65]. This is the first report of gene-gene interaction associated with schizophrenia in both population-based and family-based association studies.

A canine model of pontocerebellar hypoplasia with anterior horn cell disease. *J.C. Fyfe[1], R.J. Castellani[2], D. Rosenstein[1], D. Goldowitz[3], P.S. Henthorn[4]* 1) Laboratory of Comparative Medical Genetics and Small Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI; 2) Dept. of Pathology, University of Maryland School of Medicine, Baltimore, MD; 3) Dept. of Anatomy & Neurobiology, University of Tennessee, Memphis, TN; 4) Section of Medical Genetics, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA.

Pontocerebellar hypoplasia type I is a rare genetic disorder, and there are insufficient patient and family resources for gene identification by linkage analysis. In such cases, a disease model in a species with sufficient genome mapping resources may provide the first opportunity for molecular diagnosis. Onset of fetal akinesis in late gestation was observed in a colony of laboratory dogs. Affected puppies exhibited global stereotypical positioning of limbs, scoliosis, arthrogryposis, and respiratory failure at birth. Regional hypoplasia was apparent grossly in the central nervous system, most strikingly as underdeveloped cerebellum and mid and caudal brainstem, mild microcephaly, and reduced spinal cord diameter with attenuated nerve roots. Histopathologic abnormalities included axonal dystrophy and reactive gliosis throughout the brainstem and tracts of the spinal cord; reduced foliation, patchy loss of Purkinje cells, and failure of external granular cell migration in cerebellum; and a dearth of myelinated axons in ventral spinal roots. The disorder thus appears to be a homologue of human pontocerebellar hypoplasia type I. Breeding studies demonstrated that the canine disorder segregates as a fully penetrant, simple autosomal recessive trait. A whole genome scan for linkage is ongoing. This work has been supported by grants NS41989, RR02512.

Genetic Dissection Of A Complex Trait: Advantages of Using Multivariate Phenotypes Over Clinical Binary End-Points. *S. Ghosh*[1], *L.J. Bierut*[2] 1) Human Genetics Unit, Indian Statistical Inst, Kolkata, India; 2) Dept of Psychiatry, Washington Univ Sch of Medicine, St. Louis, USA.

A complex trait is usually a function of a multivariate phenotype comprising correlated quantitative variables. Since clinical end-point traits are usually binary in nature (affected/unaffected) and hence contain minimal information on variation within trait genotypes, it may be statistically more powerful to use a correlated multivariate phenotype for identifying genes for the complex trait. Mapping a multivariate phenotype traditionally uses some function of quantitative values of sib-pairs or other sets of relatives as a response variable and marker identity-by-descent scores as explanatory variables. In these analyses, linkage inferences depend strongly on the assumed probability distributions of the quantitative variables, particularly for variance components approaches. We propose, along the lines of Sham et al. (2002), a linear regression formulation in which the response and explanatory variables are interchanged. Analyses do not require apriori modeling of the covariance structure of the multivariate phenotype vector or any data reduction technique such as principal components. It can simultaneously incorporate qualitative and quantitative traits and can use data on n siblings as (n-1) independent observations. Monte-Carlo simulations are performed to evaluate the power of the proposed method under different genetic models. We find that this method yields more power than using binary end-point traits as well as the Haseman-Elston regression and the reverse regression approaches based on principal components. An application of the method is illustrated using data on alcoholism related phenotypes from the Collaborative Study On the Genetics Of Alcoholism project, each of which has provided evidence of linkage near the *ADH* gene cluster on Chromosome 4 using univariate analyses.

Characterization of the haplotypes, loss of heterozygosity and expression levels of glycine N-methyltransferase gene in prostate cancer. *Y.M.A. Chen[1], Y.C. Huang[1], C.M. Lee[1], Y.P. Shih[1], M.Y. Chung[2,3], Y.H. Chang[4,5], J.S.W. Huang[4,5], M.T.D. Ho[5,6], C.C. Pan[5,6], T.T. Wu[7], J.M. Hsu[8], S. Yang[8], M.W. Lin[1]* 1) Institute of Public Health, National Yang-Ming University, Taipei, Taiwan; 2) Faculty of Life Sciences and Institute of Genome Sciences, National Yang-Ming University, Taipei, Taiwan; 3) Department of Medical Research and Education, Taipei Veterans General Hospital, Taipei, Taiwan; 4) Division of Urology, Taipei Veterans General Hospital, Taipei, Taiwan; 5) Faculty of Medicine, National Yang-Ming University, Taipei, Taiwan; 6) Department of Pathology and Laboratory Medicine, Taipei Veterans General Hospital, Taipei, Taiwan; 7) Division of Urology, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan; 8) Department of Urology, Mackay Memorial Hospital, Taipei, Taiwan.

Glycine N-methyltransferase (GNMT) affects genetic stability by regulating the DNA methylation level and interacting with environmental carcinogens. Previously, through genotypic analysis, we demonstrated that GNMT is a tumor susceptibility gene for hepatocellular carcinoma. In this report, we used a case-control study to study the association between different haplotypes of GNMT and the susceptibility to prostate cancer (PCa) in Chinese population. The results showed that more than 92% of the subjects had three major GNMT haplotypes: A, 16GAs/DEL/C (58%); B, 10GAs/INS/C (19.9%) and C, 10GAs/INS/T (14.5%). Compared to persons with haplotype A, people carrying haplotype C had significantly lower risk for PCa (OR, 0.68; 95% CI = 0.48-0.95). Phenotypic analysis using luciferase reporter constructs showed that among three haplotypes, haplotype C had highest level of promoter activity and the differences were statistically significant (ANOVA test, p = 0.001). In addition, 38.1% (8/21) of prostatic tumor tissues had loss of heterozygosity of GNMT gene. Immunohistochemical staining showed that GNMT expressed abundantly in benign prostatic hyperplasia tissues, while its expression was diminished in 86% (37/43) of the prostatic tumor tissues. Therefore, GNMT is a tumor susceptibility gene for prostate cancer.

Four novel MLH1 and MSH2 mutations in Korean patients with HNPCC. *J.R. Choi[1], S. Park[1], N. Kim[2], D. Lee[3]* 1) Laboratory Medicine, Yonsei University College of Medicine, Seoul, Korea; 2) General Surgery, Yonsei University College of Medicine, Seoul, Korea; 3) Seoul Clinical Laboratory, Seoul, Korea.

Hereditary non-polyposis colorectal carcinoma (HNPCC) is one of the most common hereditary cancer-susceptible syndromes which inherits in an autosomal dominant pattern and is predisposed to the early development of colorectal and endometrial cancer as well as a range of other tumor types. Germline mutations in the DNA mismatch repair (MMR) genes, particularly MLH1, MSH2, and MSH6, are associated with the clinical phenotype of HNPCC. In this study, we report the analysis of 4 patients including a family with two affected individuals (a mother and her son). All patients had been previously diagnosed with colorectal cancer. Genomic DNA was extracted from whole blood and tested by PCR, DHPLC, and direct sequencing methods. One patient had a novel nonsense mutation in exon 3 of the MLH1 gene (c.256CT) and another patient had a frameshift mutation in exon 9 of the MSH-2 gene (c.1413delA). The mother had a missense mutation in exon 3 of MSH-2 gene (c.505AG) whereas the son had a slightly different missense mutation in exon 3 of MSH-2 gene (c.380AT), both of which have not been previously reported. In addition, both the mother and son had an identical splice mutation that had already been published in databases in exon 13 of the MSH-2 gene (c.2006-6TC). This is the first report of 4 novel mutations, according to the Human Gene Mutation Database (HGMD) in 4 patients with a history of colorectal cancer.

Informatics for next-generation DNA sequencing using single molecule clusters and sequencing-by-synthesis (SBS). *C. Brown, A. Cox, L. Davies, C. Goddard, N. Spiridou, K. Maisinger, M. Parkinson, D. Bentley* Computational Biology & IT, Solexa Ltd, Saffron Walden, United Kingdom. CB10 1XL.

Solexa has developed a new generation of DNA sequencing technology that promises to transform the economics of sequencing, offering advantages in both cost and throughput of two orders of magnitude or more. Recently Solexa successfully validated its Cluster-SBS technology by resequencing first the Phi-X 174 bacteriophage and then a 162 kb region of the human. Eventually the vast majority of differences between an individuals genome and the human reference sequence will be detectible from a single sample of fragmented human DNA either following re-alignment of the resulting reads or re-assembly. We will describe the high-throughput bioinformatics pipeline we have developed to match the unprecedented rate of sequence generation. The raw output of a Solexa machine is a large number of CCD images, from which sequences and associated quality metrics must first be extracted. These reads are then aligned to a reference genome or assembled, allowing for errors. Custom solutions enable us to run these computationally intensive tasks on modest hardware. A final consensus calling stage detects differences between the reference genome and the target genome being sequenced, providing confidence measures for each mutation found and, for a diploid genome such as the human, distinguishing heterozygotic from homozygotic variation. To determine the read length necessary for single-sample human resequencing, we computed a whole-genome alignment for each 25 base fragment in the human genome. This exhaustive study shows that good quality 25 base reads suffice to detect variation in around 82% of the genome, the remaining 18% being largely comprised of known repeats. Importantly the impact of well-calibrated base scoring schemes, coverage biases, error rates and the resulting yield of primary data underpinning these calculations are defined. Solexa collaborates with the European Bioinformatics Institute on the integration of resequencing data into Ensembl and with Imperial College London on exploiting the new possibilities for genetic analysis presented by this data.

Epidermal expression of the Hutchinson-Gilford progeria syndrome mutation in transgenic mice. *M. Eriksson[1], H. Sagelius[1], Y. Rosengardten[1], M. Hanif[1], M.R. Erdos[2], R. Varga[2], B. Rozell[3], F.S. Collins[2]* 1) Karolinska Institutet, Department of Biosciences and Nutrition, Stockholm, Sweden; 2) Genome Technology Branch, NHGRI/NIH, Bethesda, MD; 3) Karolinska Institutet, Department of Laboratory Medicine, Huddinge, Sweden.

Hutchinson-Gilford progeria syndrome (HGPS) is a rare disorder characterized by features of premature aging. Clinical findings in the skin include scleroderma, alopecia, and loss of subcutaneous fat. HGPS is caused by mutations in *LMNA,* a gene that encodes two major proteins of the inner nuclear lamina, lamin A and lamin C. The predominant cause of progeria is a single nucleotide change in exon 11, 1824C>T, which results in the activation of a cryptic splice site that produces a lamin A protein with an internal deletion of 50 amino acids. We have generated tetracycline inducible transgenic lines containing a minigene incorporating exons 1-11, intron 11, and exon 12 of human *LMNA*, under the control of a tet-operon. The two lines differ only in nucleotide 1824, where one minigene carries the wild-type sequence of *LMNA,* and the other carries the mutation. After breeding with a mouse line carrying the K5tTA, expression of the minigene is directed to keratin 5 expressing tissues, including hair follicles and the epidermis. Histopathological examination of transgenic mice expressing the HGPS mutation reveals progressive changes in the skin compared to wild-type minigene transgenic controls. Early stages are characterized by slight epidermal hyperplasia and concomitant mild hypergranulosis and hyperkeratosis. More severe regions show severe epidermal hyperplasia, with extensive immature hyperparakeratos, as well as enlarged and displaced sebaceous glands. The severe changes are associated with dermal inflammatory infiltrates. End stage disease is characterized by epidermal hypoplasia with resting hair follicles and associated hypoplastic sebaceous glands. Severe abnormalities include a well developed fibrosis, absence of detectable hypodermal adipocytes, and premature death. Taken together, this system provides a good model for studying molecular mechanisms leading to skin abnormalities in HGPS and other related disorders.

MCA/MR patients and array CGH analysis: report of 14 cases. *R. Caselli[1], C. Pescucci[1], F. Mari[1], C. Speciale[1], MA. Mencarelli[1], M. Bruttini[1], O. Zuffardi[2], R. Artuso[1], F. Ariani[1], E. Scala[1], A. Renieri[1]* 1) Medical Genetics, University of Siena; 2) Medical Genetics, University of Pavia.

We report here the analysis of 14 cases by array CGH. Patients were selected for having mental retardation and facial dysmorphisms. One major anomaly was present in about half of cases: eye coloboma and polydactily in 1 case, hypospadia and dolichocolon in 1 case, cleft palate and sandal gap in 1 case, and retinoblastoma in 3 cases. In one case a pericentric inversion of chr.15 was inherited from the healthy mother. In this selected population we identified segmental deletions in 6/14 patients (about 40%). In 4 cases we identified known deletion syndromes. Two out of three patients with retinoblastoma had the 13q deletion syndrome (13q13.3-q21.2 and 13q14.11-31.1). In these cases the specific facial feature and the presence of retinoblastoma strongly indicated this diagnosis on clinical ground. On the contrary, the diagnosis of 22q11.2 and 15q11-q13.1 was quite unexpected. The 22q11.2 deletion was found in a 18 year-old girl with eye coloboma (both iris and retina), upper postaxial polydactyly, CLP, IVD and stereotypic hand movements and an inherited pericentric inversion of chr.15 . In this case, the adult age and the uncommon abnormalities of the patient such as eye coloboma have made more difficult the diagnosis on a clinical ground. The 15q11-q13.1 deletion was found in a 4-year old boy with hypospadia, dolichocolon, gastroaesofageal reflux and epilepsy. A posteriori re-evaluation of the patient identified features compatible with Angelman syndrome.In 2 cases there were two uncommon deletion syndrome of the long arm of chr. 2. A 4 year-old girl with cleft palate, sandal gap and epilepsy has a 10 Mb deletion in 2q32.1-32.3. Comparison with other case of the literature suggested that a specific phenotype of this syndrome may be defined. A 14 year-old boy with criptorchidism, aggressiveness and self-mutilation has a 12 Mb deletion in 2q24.3-31.1. Previous studies have demonstrated the association of this region with autism. Recently, four cases with an overlapping deletion have been described. All the patients showed similar facial dysmorphisms and behavior disorders.

GNPTA Disorders: Mutations in 25 patients with Mucolipidosis II and III. *R. Bargal[1], M. Zeigler[1], A. Abu-Libdeh[2], V. Zuri[1], H. Mandel[3], Z. Ben Neriah[1], F. Stewart[4], N. Elcioglu[5], T. Hindi[6], M. Le Merrer[7], M. Penttinen[8], G. Bach[1], A. Raas-Rothschild[1]* 1) Human Genetics , Hadassah Hebrew University Medical Center, Jerusalem, Israel; 2) Department of Pediatrics, Makassed Hospital, Al-Quds University, Jerusalem, Israel; 3) Metabolic Unit, Meyer Childrens Hospital, Rambam Medical Center,Haifa, Israel; 4) Northern Ireland Regional Genetics Service, Belfast City Hospital, Ireland; 5) Department of Pediatric Genetics, Marmara University Hospital, Istanbul, Turkey; 6) Department of Pediatrics, Augusta Victoria Hospital, East Jerusalem, Israel; 7) Departement de Genetique Humaine, Necker Enfants-Malades, Paris, France; 8) Clinical Genetics Unit, Turku University Central Hospital, Turku, Finland.

Mucolipidosis II (MLII) and Mucolipidosis III (MLIII) are autosomal recessive disorders of lysosomal hydrolases trafficking due to the deficiency of the multimeric enzyme, UDP-N-acetylglucosamine-1-phosphotransferase. The / subunits encoded by the GNPTA gene are catalytic subunits while the GNPTAG encodes the recognition gamma subunit. We report on the GNPTA mutations in 22 ML II and 3 ML III patients. 21/22 ML II patients were homozygous and one was compound heterozygous for the disease-causing mutations. The ML II causing mutations were scattered along the GNPTA gene, in the subunit (3 patients) and in the subunit (19 patients). The various GNPTA mutations found in the ML II patients are predicted to damage profoundly the protein structure. Two ML III patients carried the same splice-site mutation IVS17 + 6T>G in compound heterozygosity with two severe mutations [c.3502_3delCT;R1189X]. A third MLIII patient was compound heterozygous for an -subunit missense mutation [c.1196C>T] and a -subunit deletion (c.3502_3delCT(suggesting that there is no GNPTA intragenic complementation. The results of this study show that the severity of the mutations, rather than their localization in the or subunits correlate with the severity of the phenotype and confirm that ML III is genetically heterogeneous. We suggest that MLII and MLIII diseases due to mutations in GNPTA represent the same entity with a severe to moderate clinical continuum.

Facioauriculovertebral spectrum on 250 Mexican patients. *A. Garcia-Huerta[1], L.M. Rosales-Olivarez[2], M. Diaz-Garcia[1], C.F. Martinez-Cruz[3,4], G. Garcia-Sanchez[1]* 1) Genetica. Instituto Nacional de Rehabilitacion, Mexico,DF; 2) Cirugia de Columna. Instituto Nacional de Rehabilitacion. Mexico, DF; 3) Departamento de Seguimiento Pediatrico. Comunicación Humana. Instituto Nacional de Perinatologia Mexico, D.F; 4) Instituto Mexicano del Seguro Social.Hospital General de Zona 53.México, D.F. E mail:guillegs@yahoo.com.mx.

250 Mexican patients with Facioauriculovertebral spectrum. This study compares the clinical, radiologic, audiometric and genealogic findings on 250 patients with aural Facioauriculovertebral spectrum, seen in the Department of Genetics at the National Institute of Rehabilitation, from January 2004 to December 2005. We made clinical story, pedigree, spine X rays and audiometry, on each patient. The range of age was 1 to 35 years old, with a sex ratio: 2:1, been predominant in males. We found bilateral microtia in 41%, atresia of external auditive meatus in 79.8%, facial asymmetry:17.2%, preauricular tags:13.3%, submucous cleft palate:7%, vertebral anomalies 18.8%, severe hearing loss:70.7%, mainly conductive and 41.8% had adverse prenatal factors, such as bleeding during the early pregnancy. We found 38.7% affected relatives with Facioauriculovertebral spectrum, 57.4% were sporadic cases, 26.6% had autosomal recessive inheritance and 12.1% autosomal dominant inheritance. Molecular studies were made on 11.3%, but they were not conclusive. We discuss the clinical differences with previous reports. We also confirm the variability in expression and the need of a detailed clinical examination to all first degree patients relatives in order to find a mild expression of Facioauriculovertebral spectrum, and give them an accurate genetic counseling.

An *EVC2* mutation is associated with Weyers acrofacial dysostosis and severe EvC syndrome in the same pedigree. *M. Galdzicka[1], E.W. Jabs[2], E.I. Ginns[1]* 1) Pediatric Neurology, Univ. of Massachusetts Medical School, Shrewsbury, MA; 2) Institute of Genetic Medicine, Johns Hopkins School of Medicine, Baltimore, MD.

A novel premature stop mutation has been identified in exon 22 of *EVC2* in a four-generation family. The proband has Ellis-van Creveld syndrome (EvC; MIM225500) while other affected individuals have features of Weyers acrofacial dysostosis (MIM193530) (Howard TD et al. 1997). Weyers acrofacial dysostosis is an autosomal dominant condition with dental anomalies, nail dystrophy, postaxial polydactyly, and mild short stature. The autosomal recessive EvC syndrome is similar to Weyers but has additional features of disproportionate dwarfism, thoracic dysplasia, and congenital heart disease. Although the proband's phenotype fits EvC syndrome, the phenotype in other affected family members resembles Weyers acrofacial dysostosis. *EVC* and *EVC2* gene mutations have previously been identified in both disorders (Xiaoqian Y et al. 2006; Galdzicka M et al. 2002), but this is the first instance where a mutation is associated with both phenotypes. Other than predicted transmembrane domains, no homologies/motifs give clues about either protein's function. The genes are divergently transcribed with transcription start sites separated by only 1.6kb, and have multiple start sites and numerous splice variants. These genomic features may reflect a transcriptional regulatory scheme that coordinates expression of these two genes in a way that is critical to normal skeletal and cardiac development. All exons and intron-exon boundaries were amplified from genomic DNA and sequenced. The heterozygous *EVC2* mutation, SER1245STOP (S1245X; 3734C>A; NCBI reference sequence AY185210) was present in 7 affected but not in 12 unaffected family members, and was absent in 100 normal individuals. Even though we anticipated finding a compound heterozygous mutation in the proband, only the S1245X has been identified. Our findings support the hypothesis that mutations in either *EVC* or *EVC2* cause a wide variety of manifestations, ranging from mild Weyers acrofacial dysostosis to the more severe EvC syndrome, and that individuals carrying either *EVC* or *EVC2* mutations can have clinically indistinguishable presentations.

Complex chromosome rearrangement maps ectrodactyly-deafness (OMIM 220600) to chromosome 7q21.13. *B. Dallapiccola[1], L. Bernardini[1], C. Palka[2], C. Ceccarini[1], A. Capalbo[1], R. Mingarelli[1], A. Novelli[1]* 1) CSS-Mendel Institute, Rome, Italy; 2) "La Sapienza" University, Rome, Italy.

Two-chromosomes translocations occur in about 0.1-0.2% individuals, while complex rearrangements with >2 breakpoints are extremely rare. While most of these individuals are healthy and phenotypically normal, clinical abnormalities are found in about 6% of them. We report on a 5-year-old girl, evaluated because of psychomotor delay, deafness, ectrodactyly of right hand and feet, craniofacial dysmorphisms, cleft palate and tetralogy of Fallot (TF). She had also macrocephaly, sparse hairs and eywbrows, wide nasal bridge with piriform nose, long philtrum and micrognathia. Ears were prominent, low-set and abnormally shaped, with crumpled helices. The right 5th finger was short, with hypoplastic palmar and distal interphalangeal flexion creases. Severe bilateral sensorineural deafness was corrected by prosthesis. Standard karyotype suggested a small intrachromosomal duplication of long arm of chromosome 7, but an array-CGH analysis with an average 1 Mb resolution excluded any genomic imbalance. The chromosomal rearrangement was characterized by m-BAND analysis, which disclosed a reciprocal interstitial translocation t(7q21;8q24). FISH analysis using specific clones, and an high resolution array-CGH analysis (44K Agilent chip, Agilent Technologies Inc., Palo Alto, CA) disclosed a paracentric inversion of 7q and a microdeletion of 7q21.13. Parents had normal chromosomes. A few patients with ectrodactyly and other defects, including deafness, have been found presenting with structural rearrangements affecting 7q21-q22. The imbalance found in the present patient narrows the candidate region of ectrodactyly-deafness (OMIM 220600)to sub-band 7q21.13. Interestingly, this girl had some facial features reminescent of tricho-rhino-phalangeal syndrome (TRPS) and one chromosome breakpoint involved band 8q24, where the TRPS locus has been mapped. In addition, FOG1 gene, which had been implicated in a subset of subjects with TF, maps to chromosome 8q23. Thus, cryptic chromosome deletion and, likely, position effects account for the complex phenotype observed in this unique patient.

Retrospective study of Clinical and histological manifestations of IP in Adults. *C. Bodemer[1], A. Rimellla[1], S. Fraitag[2], Y. de Prost[1], J.P. Bonnefont[3], A. Smahi[3], S. Hadj-Rabia[1]* 1) Dermatology,Hôpital Necker-Enfants Malades,Paris,France; 2) Pathology, Hôpital Necker-Enfants Malades,Paris,France; 3) Genetics, Hôpital Necker-Enfants Malades,Paris,France.

Introduction Incontinentia pigmenti (IP) is a rare X dominant genodermatosis related to mutations of NEMO gene. Itt affects mostly female patients and is usually lethal for males in utero. It is a multisystem disorder, primarily of ectodermal origin, accompanied by dental, ocular, and central nervous system disorders. The skin lesions may occur in 4 classically successive diagnostic stages: erythema and vesicles, (stage 1); verrucous lesions (stage 2); linear hyperpigmentation (stage 3); and pallor and scarring (stage 4).In adults, manifestations are skin involvement (stage 4) and teeth and nail anomalies. Patients and Methods Among the 325 patients with molecular diagnosis of IP, the adult females (age over 18 years) were contacted for clinical examination. Skin, ocular, neurological and stomatological manifestations were recorded using a standard form. Skin biopsy was performed. Results 25 patients fulfilled the criteria : the diagnosis was made in adulthood in 52%of the patients. Stage 4 was constant (100%) stage 3 and 2 were found in 11 and 1 patients respectively. Other manifestations were: woolly hair (44%), nails (84%), teeth (92%), ocular (48%), mammary (28%) and neurological (12%) anomalies. Histology shows apoptotic kératinocytes, absence of follicles and sweat glands (stages 3 and 4), hypopigmentation and atrophic epidermis (stage 4). Discussion Diagnosis of IP was delayed in 52% of the patient and based on the constant presence of stage 4 cutaneous lesions. Extracutaneous manifestations, such as teeth anomalies and mammary malformation, are frequent. During the first and second stages of IP, keratinocyte apoptosis is classical on skin biopsy. Interestingly, we demonstrate that keratinocyte apoptosis persists in adults and is associated with absence of both sweat glands and follicles. Histology may be helpful for the diagnosis of IP in adults and should be include in the IP criteria defined by Landy and Donnai. Late apoptosis may explain rare manifestations of IP in adults.

Molecular cytogenetic analysis of 149 breakpoints in 76 cases with apparently balanced chromosome rearrangements. *D.R. FitzPatrick[1], J.A. Fantes[1], E. Borland[2], J. Ramsay[1], D. Donnai[2], J. Clayton-Smith[2], V. van Heyningen[1], G. Black[2]* 1) Medical Genetics Section, MRC Human Genetics Unit, Edinburgh, United Kingdom; 2) Dept of Medical Genetics and Regional Genetic Service, St. Marys Hospital, Hathersage Road, Manchester M13 0JH.

De novo apparently balanced chromosome rearrangements (DN-ABCRs) are detected in 1 in 2000 amniocenteses with a 3-10% risk of serious malformations and learning disability. Currently predicting the phenotype associated with a DN-ABCR is not possible. We ascertained 76 cases (46 cases with clinically significant phenotypes, and 30 cases without) through two UK regional Genetics Centres. A total of 149 breakpoints (BP) were mapped by a FISH strategy scanning 10-20 Mb around each cytogenetic BP using the Sanger 1 Mb clone set to identify flanking clones. A contig of tiling path clones were then used to identify a breakpoint-spanning BAC clone. 23% of all BP were fine-mapped with fosmid clones or long-range PCR products. Analyses of the results from the two groups of patients and have delineated a number of differences:

1. Microdeletions were only identified in cases with a clinical phenotype. Three of the six deletions detected were some distance (6-30 Mb) from the translocation BP.

2. The BP disrupted genes in a similar percentage of cases both with and without a phenotype, however in cases with a phenotype the disrupted genes were more commonly transcription factors or DNA binding proteins.

3. In cases without a phenotype, there are fewer than expected breakpoints mapping to R-bands.

4. BP regions in abnormal cases tend to be more evolutionarily conserved

This is the largest systematic study of DN-ABCRs which includes a control group of clinically normal cases. The four molecular features with discriminant power may be useful in prenatal counselling particularly if our rapid and effective strategy for DN_ABCR mapping can be implemented in clinical cytogenetic labs.

Clinical genetics service for the psychiatric population. *E.W.C. Chow*[1,2], *A.L. Rideout*[1], *A.S. Bassett*[1,2] 1) Centre for Addiction and Mental Health, Toronto, ON, Canada; 2) Department of Psychiatry, University of Toronto, Toronto, ON, Canada.

Psychiatric genetics is an emerging field in clinical genetics that is attracting more and more interest among psychiatric clinicians. Genetic testing for syndromes or conditions associated with psychiatric and behavioral manifestations are increasingly available. The Clinical Genetics Service (CGS) was set up in July 2005 at a psychiatric hospital to address issues in clinical psychiatric genetics including genetic testing, behavioral phenotypes, and genetic counseling. So far, we have provided services to 25 probands (11 M, 14 F; mean age 25.6 years, SD 13.1 years) and/or their families. Primary psychiatric diagnoses for these individuals included schizophrenia (n=6), autistic spectrum disorders (n=6), mental retardation (n=4), anxiety disorders (n=3), depression (n=2) and other diagnoses (n=4). We have assessed 17 individuals for genetic testing; 10 of these individuals were seen for testing for 22q11.2 deletion syndrome (22q11DS), six for fragile X syndrome, and nine for other potential genetic syndromes. In addition, we provided genetic counseling to four families, three of which were for various psychiatric disorders and the fourth due to a history of a familial cardiac condition. Four individuals were referred for a consultation on behavioral phenotype for previously diagnosed genetic syndromes such as 22q11DS, oculo-auriculo-vertebral spectrum and Borjeson-Forssman-Lehmann syndrome. Establishing a psychiatric genetics clinic at a psychiatric hospital has proven a feasible means of providing genetic services to the psychiatric population.

Genotype Fidelity within DNA Extracted from Archival Tissue. *J. Breyer[1], P. Schuyler[2], B. Elmore[1], B. Yaspan[1], K. Bradley[1], D. Page[3], W. Dupont[2], J. Smith[1]* 1) Deptartments of Medicine and Cancer Biology, Vanderbilt University Medical Center, Nashville, TN; 2) Department of Biostatistics, Vanderbilt University Medical Center, Nashville, TN; 3) Department of Pathology, Vanderbilt University Medical Center, Nashville, TN.

Formalin-fixed paraffin embedded human tissue is a rich potential resource for genetic investigation of cancer. However, the limiting quantity and poor quality of DNA extracted from archival tissues presents a challenging obstacle. We have conducted investigations of SNP genotyping accuracy and whole genome amplification fidelity within DNA extracted from archival tissue of the Nashville Breast Cohort. Each of the 7,772 women in this cohort underwent biopsy for benign breast disease and have been followed for over 17 years for the subsequent development of breast cancer. Among them 552 women have progressed to breast cancer. We conducted a matched study of 47 women from the cohort. DNA from archival tissue was compared with DNA of the same subjects obtained from recently collected saliva. We demonstrate that direct genotyping of extracted DNA from the benign entry biopsy is extraordinarily accurate using the high-throughput Illumina GoldenGate assay, even with very limiting amounts of DNA. The genotype concordance rate was 99.7% between DNA from paraffin embedded tissue samples and from recent saliva samples of the same individuals. Of 99,546 genotypes sought from these samples, 99,292 (99.7%) were obtained. We further observe that protocols for whole genome amplification can fail to replicate the source genome for accurate genotyping and highlight the need for improved methods.

A genome-wide scan suggests a region on chromosome 16p is a determinant of serum ferritin after adjusting for *HFE*: The HEIRS Family Study. *R. Acton[1], B. Snively[2], J. Barton[1], C. McLaren[3], P. Adams[4], S. Rich[2], J. Eckfeldt[5], R. Press[6], P. Sholinsky[7], C. Leiendecker-Foster[5], G. McLaren[3,8], M. Speechley[4], E. Harris[9], F. Dawkins[10], V. Gordeuk[10]*
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1/200-1/400 whites of northwestern and central European descent have hemochromatosis (H), of whom 60%-100% are *HFE* C282Y homozygotes whose iron overload (IO) phenotype is variable. We recruited family members of H and IO probands for a genome-wide linkage scan of potential quantitative trait loci (QTL) that contribute to variation in transferrin saturation (TfSat), unsaturated iron-binding capacity (UIBC), and serum ferritin (SF). Participants with elevated TfSat and SF, and all C282Y homozygotes who attended a clinical exam, became probands if a qualifying group of first-degree relatives 19 years old were also available. Linkage scan genotyping utilized 402 microsatellite markers with average spacing of 9 cM; the largest gap between adjacent markers was 18 cM. 943 individuals, 76% of whom were white, were evaluated from 174 families. After adjusting for age, gender, and race/ethnicity, the strongest evidence for linkage with UIBC (LOD = 9.52), TfSat (LOD = 4.78), and SF (LOD = 2.75) was in the Ch6p region containing *HFE* (each P <0.0001). After adjusting for *HFE* genotype, recruitment site, body mass index, menopausal status, history of phlebotomy treatment, hepatitis B or C positivity, alcohol intake, and C-reactive protein, evidence of linkage was also observed for SF in a region of Ch16p (LOD = 2.63, P = 0.0007) that includes the heme oxygenase 2 gene. Thus, we found evidence of QTL(s) on Ch16p that contributes to SF variation, and the expected QTL(s) on Ch6p that contributes to UIBC, TfSat, and SF variation.

Experience of Discrimination Among Persons who Have Undergone Predictive Testing for Huntingtons Disease.

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Little is known about the actual long-term consequences of genetic testing for genetic conditions that may lead to discrimination. The consequences of genetic discrimination generate social, health, and economic burdens for those at genetic risk and for society as a whole. The objective of this study was to better understand experiences of genetic stigmatization and discrimination among persons who have undergone predictive testing for Huntingtons disease (HD). Using a semi-structured interview and computerized qualitative analysis, 15 presymptomatic persons with a positive gene expansion for HD were interviewed with regard to treatment following predictive testing. The sample was comprised of 11 women and 4 men, mostly married, ranging in age from 22 to 62 years. Participants lived in eight U.S. States and resided in urban, rural and suburban settings. Participants reported consequences following disclosure of genetic test results in three main areas: employment, insurance and social relationships. Although the majority of employed participants had revealed their test results to their current employers, nearly all of them reported that they would not disclose this information to future employers. Most of the sample disclosed their test results to their physician, but a similar majority did not disclose their genetic status to insurers. Most participants had openly disclosed the test results to family and peers although patterns of disclosure varied widely. Study findings provide a better understanding of factors influencing individuals acceptance of genetic tests and suggest the need for further examination of this important population. Data collected in this study will guide development of a survey to examine the long-term effects of genetic testing in a larger study cohort. Ultimately, these findings will improve understanding of communication preferences, patient-provider processes, and health outcomes.

Mosaic aneuploidies in the developing and adult brain. *J. Chun* Department of Molecular Biology Helen L. Dorris Child and Adolescent Neuropsychiatric Disorder Institute The Scripps Research Institute 10550 North Torrey Pines Rd ICND-118 La Jolla, CA 92037.

The brain is remarkable for its enormous cellular diversity and complex organization that may be unique for each individual. This uniqueness might in part explain behavioral differences between people, or contribute to the sporadic presentation of most major neurological and psychiatric diseases. A possible contributing mechanism to produce brain uniqueness is the existence of mosaic aneuploidies that have been shown in recent years to be pervasive throughout the developing and adult brain. Although the functions of mosaic aneuploidies in normal brain remain unknown, constitutive aneuploidies, like Trisomy 21 (Down syndrome), have proven associations with cognitive function, providing proof-of-concept for functional consequences of neural aneuploidies in humans. Using human and/or mouse models, aneuploidies have been documented throughout the neuraxis. During neurogenesis, over 1/3 of mitotic, neural progenitor cells are aneuploid, and assessment by spectral karyotyping (SKY) identifies a wide range of different aneuploidies that include chromosome gain, loss and combinations of the two. The number of aneuploid cells can be altered by growth conditions in culture as well as by genetic deletion of DNA repair/surveillance genes like Atm and Xrcc5. The generation of neural aneuploidies can, at least in part, be attributed to well-known chromosome missegregation mechanisms. In postmitotic neurons that do not have discernible chromosomes, the actual karyotype of individual neurons is not known in detail. However, use of FISH with chromosome-specific point probes indicates that even for a single locus, approximately one to several percent of cells are aneuploid, representing a substantial population when accounting for all chromosomes. Aneuploid neurons have been shown to be integrated into functional neural circuits, raising the possibility that these mosaic aneuploid cells can produce distinct neural outputs. Recent data on some of the functional consequences of aneuploidy will be presented. This work was supported in part by the National Institute of Mental Health.

A systematic genome wide association study of 19,779 coding SNPs with putative function identifies a novel susceptibility gene for Crohn disease. *A. Franke[1], J. Hampe[2], P. Rosenstiel[1], A. Till[1], K. Huse[3], M. Albrecht[4], G. Mayr[4], F.M. De La Vega[5], J. Briggs[5], M. Wenz[5], S. Guenther[5], D.A. Gilbert[5], N. Prescott[6], T. Lengauer[4], C. Matthew[6], M. Krawczak[7], S. Schreiber[1]* 1) Institute for Clinical Molecular Biology, Christian-Albrechts-University, Kiel, Germany; 2) Department of General Internal Medicine, Kiel, Germany; 3) Leibniz Institute for Age Research - Fritz Lipmann Institute, Jena, Germany; 4) Max Planck Institute for Informatics, Saarbruecken, Germany; 5) Applied Biosystems, Foster City, CA, USA; 6) King's College London School of Medicine, London, UK; 7) Institute of Medical Statistics and Informatics, Kiel, Germany.

Use of a comprehensive panel of coding SNPs may enable fast identification of susceptibility variants for complex phenotypes. A subset of 19,779 putatively functional SNPs was selected from over 60,000 candidate nsSNPs compiled from public and private sources. Genotyping in 735 patients with Crohn disease and 368 controls by SNPlex identified 7,832 SNPs as being informative in this population, and these were genotyped in an independent German sample of 386 trios, 946 cases and 1091 controls. Hierarchic analysis identified one significantly disease-associated non-synonymous SNP (p=8.4x10-6 single point allelic case-control, OR=1.70 [95% CI: 1.33-2.16], p=2.9x10-5 TDT). The association was replicated in a British (661 controls, 515 cases) and another independent German (736 controls, 736 cases) sample. Full mutation detection failed to detect any other cSNP that explained the association. The novel susceptibility variant interacts with known variants in CARD15 (Breslow Day test, p=0.046 evaluating homozygous and compound heterozygous carriers of CARD15 risk alleles). The predicted function suggests that the variant leads to an impairment of the killing of intracellular bacteria. Direct association analyses with a comprehensive set of nsSNPs can lead to the direct identification of susceptibility variants for complex phenotypes. Genome-wide nsSNP panels are therefore a valuable addition to other LD-based mapping approaches. The identified novel disease gene adds to the understanding of Crohn disease as an innate immune disorder.

Unraveling the genetic aetiology of stroke in Pakistan. *P.M. Frossard, D. Saleheen* Dept Biological & Biomed Sci, Aga Khan University, Karachi, Pakistan.

Stroke, the most common cause of disability and the third cause of death worldwide, represents an enormous public health problem which is rapidly spreading in developing countries. In search of quantitative trait loci underlying the etiology of ischaemic stroke (IS) in Pakistan, we designed an association study at candidate gene loci chosen from various atherosclerotic pathways. Our study population included 210 IS cases and 350 disease-free, age- and gender-matched, controls, recruited at Liaqat National and AKU Hospitals, Karachi, Pakistan. Genotyping of genetic markers (dimorphisms) was done by PCR-RFLP. Associations of the studied dimorphisms with IS were tested by both single-point and haplotype analyses. Three loci are directly associated with clinical IS diagnosis. Phosphodiesterase 4D (PDE4D): we investigated three PDE4D genetic markers (SNP32, 83 and 87, see Gretarsdottir et al., Nat Genet. 2003;35:131-138), and evidenced that TT genotypes of SNP83C>T confer a significant risk for IS on both univariate and multivariate analyses ($P<0.005$). Paraoxonase genes (PON1, 2 and 3): amongst the eight markers included here, PON1 192Q>R, PON2 148G>A and PON2 311C>S are associated with IS, and QAS haplotypes indicate a 1.87-fold increased risk of developing IS ($P=0.01$). ATP-binding cassette transporter A1 gene (ABCA1): we included five intragenic markers, and first established that the RL haplotype (combination of R219K and V825L marker mutations) is strongly associated with decreased HDL-c levels (OR=8.33, $P=0.001$). We then found that allele 774P of mutation T774P showed the strongest direct association with IS (OR=4.06, $P<10\text{-}7$). We further demonstrated that a three-point VPL haplotype (combination of V771M, T774P and V825L markers) is indicative of a very strong increased risk for IS (OR=10.34, $P<10\text{-}7$). Upon applying an overall, single multivariate model, we established that two major gene effects (ABCA1 and PON) and one minor gene effect (PDE4D) account for 65.3% of IS in the studied Pakistani population.

Population-specific abnormal high density of HapMap QC- SNPs in ENCODE regions: from the 1% towards a high-resolution genome-wide analysis of copy number variants (CNVs) of the human genome. *L. Armengol[1], S. Villatoro[1], M. Garcia-Aragones[1], J.R. Gonzalez[1,2], X. Estivill[1,2,3], ENCODE variation analysis group* 1) Genes and Disease Program, Center for Genomic Regulation (CRG), Barcelona, Catalonia, Spain; 2) CeGen Barcelona Node, Barcelona, Catalonia, Spain; 3) Pompeu Fabra University, Barcelona, Catalonia, Spain.

A bulky number of copy number variants (CNVs), ranging from several bases up to many kb, appear to be polymorphic either in copy number or in orientation in different populations. The existence of such structural variants is a putative source of SNP genotyping problems and inconsistencies. When analysing SNPs in such regions, violations of Hardy-Weinberg equilibrium, inconsistencies in Mendelian inheritance, reduced efficiency in genotype calling, discrepancies among different genotyping platforms, and many other problems might arise. The systematic analysis of such SNPs (designated as QC- since they do not fulfil the Quality Control standards) can help in the identification of genomic regions that are polymorphic and that have not yet been uncovered. We have focused on the ENCODE (ENCyclopedia Of Dna Elements) regions to analyse the content of QC- SNPs generated in the HapMap project. We computed the density of QC- along the ENCODE regions using a 25-kb sliding window and identified 42 several population-specific regions having an abnormally high density of QC-. Analysis of these regions using array-based CGH and MLPA provided experimental validation of computational observations. Fifteen of such regions contain transcribed sequences. Fifteen of 30 regions contain CNVs, of which 12 are novel. Four of the newly detected CNVs are polymorphic in the four populations. Population-specific CNV composition differences were detected for the highly polymorphic variants. While this study was initially focussed on 1% of the human genome sequence, the whole genome scan for QC- SNP features in different populations should provide a complete picture of CNVs that cannot be detected by current array screening methods. Supported by Genome Spain and Genome Canada and the Catalan Government. We are in debt with all members of the Hap Map and ENCODE projects.

BBS10 mutations in severe antenatal cases with severe cystic kidneys "Meckel-like" type. *S. Audollent[1], L. Baala[1], R. Khaddour[1], J. Martinovic[1], A. Buenerd[2], G. Leichmeijer[3], M. Le Merrer[1], A. Munnich[1], F. Encha-Razavi[1], M.C. Gubler[4], M. Vekemans[1], T. Attié-Bitach[1]* 1) Genetics, Inserm U-781, Paris, Paris, France; 2) Hopital Edward Herriot, Lyon, France; 3) Klinische Genetica en antropogenetica De Boelelaan, Amsterdam; 4) INSERM U-574 Hopital Necker, Paris France.

Bardet-Biedl syndrome (BBS, OMIM 209900) is a multisystemic disorder characterized by progressive retinal dystrophy, postaxial polydactyly, obesity, hypogonadism, learning difficulty and renal dysfunction. Other manifestations include diabetes mellitus, neurological signs, heart disease, and hepatic fibrosis. The condition is largely genetically heterogeneous and 11 genes have been identified (*BBS1-BBS11*) thus far, mutations of *BBS10* on chromosome 12q21.2 accounting for 30 % of cases. In addition, a complex epistatic inheritance has been established in this disorder, i.e. in some families, 3 mutations at 2 BBS loci are necessary for expression of the disease. We recently showed that fetuses with severe cystic kidneys were indeed prenatal forms of BBS. In the present study, we sequenced the *BBS10* gene in 16 antenatal cases referred as Meckel-like. In 4 cases, we identified a recessive mutation at the *BBS10* locus. Three fetuses had MKS kidney phenotype, whereas liver and brain were normal. As previously reported in foetal BBS cases, although the kidney histopathological findings were similar to MKS, no bile duct proliferation of liver was observed. Interestingly, one case had *situs inversus* and polysplenia suggesting a ciliary dysfunction. In another case, BBS genes screening identified a heterozygous *BBS6* nonsens mutation in accordance with the multigenic inheritance of BBS. These results confirmed that the diagnosis of BBS is underdiagnosed antenatally, and should be systematically suspected in fetuses with severe cystic kidneys leading to oligoamnios and foetal or perinatal death. This study also confirms the high frequency of *BBS10* mutations in BBS.

Analysis of SMS1 mRNA expression in focal cerebral ischemia in rats after treatment with neuropeptide Semax and its C-terminal Pro-Gly-Pro tripeptide. *V.G. Dmitrieva[1], E.V. Torshina[1], O.V. Povarova[2], L.V. Dergunova[1], S.A. Limborska[1], V.I. Skvortsova[2]* 1) Institute of Molecular Genetics RAS, Moscow, Russian Federation; 2) Institute of Stroke RSMU, Moscow, Russian Federation.

It is now well known that ceramide and diacylglycerol (DAG) are signal molecules of apoptotic and antiapoptotic pathways of cell regulation. Being element of sphingomyelin cycle sphingomyelin synthase 1 (SMS1) controls the synthesis of ceramide and diacylglycerol which relative concentrations are responsible for balance between cell death and surviving. It is established that apoptosis is involved in mechanisms of cerebral ischemia. Consisting of a fragment of ACTH4-7 and C-terminal PGP tripeptide neuroprotective polypeptide Semax is used for acute therapy of stroke. To investigate the expression of SMS1 the brains of male Wistar rats were analyzed at three time points following permanent middle cerebral artery occlusion (pMCAO) and treatment either with saline, Semax or PGP: 3, 24, and 72 hours. The intraperitoneal injection of solutions were done at 15 min, 1 h, 4 h and then after every 4 h. Semiquantitative RT-PCR has been used to measure changes in SMS1 expression in the ipsilateral and contralateral frontoparietal cortex and subcortex of rat brains. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal control. In the lesioned cortex, SMS1 mRNA decreased during first 24 h post-MCAO, while at 72 h SMS1 expression returned to control level. During first 24 h the expression of SMS1 in ipsilateral subcortex of sham-operated and ischemic rats was also decreased. The level of SMS1 mRNA in ipsilateral subcortex of ischemic rats continue to be decreased at 72 h post-MCAO. During first 24 h Semax did not change the SMS1 expression neither in frontoparietal cortex nor in subcortex of ipsilateral hemisphere. In contrast to Semax its C-terminal PGP tripeptide during first 24 h increased the level of SMS1 mRNA up to control value. However in the lesioned cortex at 72 h post-MCAO the expression of SMS1 was decreased after treatment either with Semax or PGP. In addition during all three days of treatment Semax decreased SMS1 mRNA expression in subcortex of control rats.

Factor D Deficient Mice as Model of Mesangial Immune Complex Glomerulonephritis. *M.A. Abrera-Abeleda[1,2], Y. Xu[3], M.C. Pickering[4], R.J.H Smith[1,2], S. Sethi[5]* 1) Dept of Otolaryngology and; 2) Interdisciplinary PhD Program in Genetics, University of Iowa, Iowa City, IA; 3) Division of Clinical Immunology & Rheumatology, University of Alabama, Birmingham, AL; 4) Molecular Genetics & Rheumatology Section, Imperial College School of Medicine, London, UK; 5) Dept of Laboratory Medicine and Pathology, The Mayo Clinic, Rochester, MN.

Complement Factor D (CFD) is a serine protease that catalyzes the formation of the C3 convertase of the alternative complement pathway. Mice with a targeted deletion of Cfd (Cfd-/-) have complete inhibition of complement activation through the alternative pathway (Xu 2001 PNAS). Because the complement system plays an important role in the pathogenesis of some renal diseases, we studied the renal pathology in the Cfd-/- mutant mouse and compared findings to renal lesions in the Factor H deficient (Cfh-/-) mouse, which is an established model for membranoproliferative glomerulonephritis (MPGN).

Cfd-/- (n=5), Cfh-/- (n=6) and wildtype (WT) mice (n=5) derived on C57BL/6 background were euthanized at 8 months of age. Prior to euthanasia, renal function was assessed by measuring serum and urine creatinine and albumin, and calculating creatinine clearance. Renal histology was evaluated on formalin-fixed specimens under light microscopy; snap frozen tissue was used for immunofluorescence analysis of C3 and IgM. Ultrastructural comparisons were done by electron microscopy.

In both the Cfd-/- and Cfh-/- mutants, creatinine clearance is decreased and urine albumin is elevated as compared to control animals ($p<0.05$), although the change in renal function is greater in the Cfh-/- mice. Cfd-/- mice had mild-to-moderate mesangial expansion with C3 and IgM deposition in the mesangial region. Cfh-/- mice had mesangial proliferation and thickening of capillary walls with deposition of C3 and IgM, which is characteristic for MPGNII (Dense Deposit Disease). Renal lesions in Cfd-/- are restricted to the mesangium, which suggests that Cfd-/- mutant mice may serve as a model of mesangial immune complex glomerulonephritis.

Family history and electronic medical records in primary care. *W. Feero, E. Piekarz, D. Meyer* Dept Community/Family Med, Maine-Dartmouth Family Pract, Fairfield, ME.

Family history is recognized as an important bridge to the integration of emerging genetic technologies into primary care. Electronic medical records (EMR) are fast becoming the standard of record keeping in primary care, however, little is known about how the use of EMR systems impacts the ascertainment of family history. To examine this, charts of faculty in a rural primary care residency program were reviewed for the quantity of family history data obtained at initial patient visits pre and post EMR implementation. 28 charts total were reviewed using an objective scoring system (0- least information, 4- most information). None of the charts (pre or post EMR) contained a three generation graphical pedigree. Family history was most commonly recorded on a paper- based initial visit questionnaire. Composite family history scores were 2.9 for visits pre- EMR and 2.6 post- EMR implementation. However, if information captured on paper only is excluded for the post-EMR visits, the composite score drops to 1.4. Further studies should be directed at determining if this result can be generalized to other EMR platforms, and if so, how the decrement in the amount of family history obtained in the electronic environment may be avoided.

Examination of Sex and Age of Onset as Important Sources of Variation for Genetic Association Findings in Schizophrenia. *M.D. Fallin[1,6], P. Belmonte[5], V.K. Lasseter[2], N. Cheng[2], D. Avramopoulos[2,3], J. Mulle[2,3], P.S. Wolyniec[2], J.A. McGrath[2], G. Nestadt[2], KY. Liang[6], D. Valle[3,4], A.E. Pulver[2]* 1) Dept Epidemiology, Johns Hopkins School of Public Health, Baltimore, MD; 2) Department of Psychiatry & Behavioral Sciences, Johns Hopkins School of Medicine, Baltimore, MD; 3) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 4) Howard Hughes Medical Institute; 5) Dept of Mental Health, Johns Hopkins School of Public Health, Baltimore, MD; 6) Dept of Biostatistics, Johns Hopkins School of Public Health, Baltimore, MD.

Schizophrenia (SZ) is a common heritable psychiatric disorder, yet few susceptibility genes have been confirmed. We recently performed SNP genotyping on 64 candidate genes (440 SNPs)in 274 SZ/schizoaffective Ashkenazi case-parent trios. We genotyped 6.9 SNPs/gene (1 SNP/11.9 kb), and reported results for SNPs and haplotypes across all 64 genes (Fallin et al 2005). These initial results did not accommodate sources of heterogeneity such as sex of the case and age of onset, yet both may be very important in the genetics of SZ. We have therefore performed interaction analyses in these trios to assess the impact of sex and age of onset on the association findings for these 64 genes, using covariate terms in conditional logistic regression for each SNP. We show 5 genes that gave suggestive evidence for association with SZ in our original analysis whose SNP signal is further refined by consideration of age of onset (PRODH, GRID1, NRG3, ADRA1A, NOS1) and 7 genes where a signal emerges that was not detected in the original marginal results. We also show 1 gene with suggestive evidence in our prior analyses that is refined by the inclusion of case sex (NOS1), and 4 additional genes with evidence of interaction by sex that were not suggestive in marginal analyses (DISC1, CABIN1, DRD4, RTN4R). Our results are consistent with previous findings of increased risk for DISC1 among women (Hennah et al 2003). These SNP findings were confirmed in haplotype analyses stratified by either age of onset or sex.

Evidence of a new QTL for BMI on chromosome 1 in the isolated population of Campora. *M. Ciullo[1], C. Bourgain[2], V. Colonna[1], C. Bellenguez[2], T. Nutile[1], M. Astore[1], A. Calabria[1], M.G. Persico[1]* 1) IGB A. Buzzati-Traverso - CNR Naples, Italy; 2) INSERM U535, Villejuif, France.

Campora is a geographically isolated village of Cilento, South Italy, with a few founders and inbreeding. Recently, Campora population allowed us to identify a new locus strongly linked to hypertension, encouraging the study of this population for the identification of loci involved in other complex traits. A collection of several quantitative traits related to the cardiovascular system have been measured in this population. The present study is the first linkage analysis of a quantitative trait in this population. Body-mass index (BMI), an obesity-related trait and a risk factor for cardiovascular diseases, was calculated for 394 adult individuals in the population. These individuals, all related through a 2947-member pedigree spanning 15 generations, were genotyped for 1122 microsatellites on the genome (average marker distance: 3.6 cM, heterozygosity 0.70). To perform the linkage analysis, we broke the very complex pedigree into 92 families including 366 phenotyped individuals, with an optimized use of the maximum partitioning approach to pedigree breaking proposed by Falchi and collaborators (2004). With the regression-based linkage statistic proposed by Sham and collaborators (2002), we detect a strong linkage on chromosome 1 (position 176.38, LOD= 4.47, pvalue=0.01), robust to the various trait transformations considered. We also replicated linkage for two known QTLs on chromosome 2 (Deng et al. 2002) and on chromosome 6 (Atwood et al. 2002). The linkage is also detected with a variance component analysis. Again, the result is robust to trait transformation. This study suggests that linkage study of sub-pedigrees carefully chosen in the Campora population is a powerful strategy to detect new QTL. Atwood et al (2002) Am.J.Hum.Genet 71:1044 Deng et al (2002) Am.J.Hum.Genet 70:1151 Falchi et al (2004) Am J Hum Genet 75:1015 Sham et al (2002) Am J Hum Genet 71:238.

Estimating Kinship Coefficients from High-Density SNP Genotypes for QTL Mapping and Association. *A. Day, E. Sobel, K. Lange* Dept Human Genetics, Univ California, Los Angeles, Los Angeles, CA.

The power to detect disease genes can be dramatically increased by including knowledge of the true genetic relationships between individuals in the study sample. Unfortunately for some pedigree-based datasets, or even in case-control studies, there are relationships among the individuals that are unknown to the analyst. This can arise for example from purposefully hidden relationships, loss of historical information, or simply the proper question never being asked.

We have developed a simple, closed-form, likelihood-based algorithm to recover the useful data from these cryptic relationships. The algorithm is based on using the number of identity-by-state matches observed in an extended segment of SNPs as an estimate for the expected number, which is a function of the kinship coefficient. Solving for the kinship coefficient provides an unbiased estimator. For smaller segments of SNPs, this method of moments statistic estimates the conditional kinship coefficient. As the segment length grows, the estimate converges to the theoretical kinship coefficient. Using the very large-scale SNP genotyping tools now available, we can quickly calculate a good estimate for the true theoretical kinship coefficient and reasonable estimates for the conditional kinship coefficients between any two individuals. These kinship coefficients estimates can be used in QTL mapping and association analyses for gene localization.

To test our method we used the Affymetrix 100K SNP set as a representative high-density SNP dataset. We present results based on both simulated and real pedigrees. The results from 1000 simulated runs give excellent agreement to the known true values with very small standard errors. The results on a limited number of real datasets also give reasonable estimates. We are currently gathering more real datasets to demonstrate the usefulness of this method.

DUX4 transcriptionally regulates paired-like homeodomain transcription factor 1. *M. Dixit[1], Y.-W. Chen[1,2]* 1) Center for Genetic Medicine Research, Children's National Medical Center, Washington, DC; 2) Department of Pediatrics, George Washington University, Washington, DC.

Fascioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant disease linked to a partial deletion of the 3.3 kb D4Z4 repeat arrays at chromosome 4q35. The only known transcript encoding a double homeodomain protein, DUX4, in each of the repeat unit was shown likely aberrantly expressed in the muscles of the FSHD patients but not in healthy individuals. In this study, we determined whether paired-like homeodomain transcription factor 1 (PITX1) which were shown specifically up-regulated in FHSD was a direct transcriptional target of DUX4. To test if the DUX4 gene can directly interact with the pitx1 promoter, we first amplified a 318 bp fragment flanking a putative DUX4 binding site in an evolutionally conserved region. We then inserted the fragment to the pGL3-basic luciferase reporter vector. The construct was co-transfected with the pCIneo-DUX4 expression vector and luciferase activities were measured at 24 hours post-transfection. Co-transfection with pCIneo-DUX1 (a non-4q35 homolog of DUX4 gene), pCIneo-DUX4c (a 4q35 homolog of DUX4 gene) expression vectors and the insertless vector alone were performed as controls. The results showed a 110-fold increase (n=4, $p=2.4x10^{-17}$) of the luciferase activity when the pGL3-pitx1 was co-transfected with pClneo-DUX4 compared to samples co-transfected with insertless vector. Co-transfection with DUX1 or DUX4c also lead to up-regulation of the luciferase activity, 1.5 and 5 fold ($p=1.6x\ 10^{-11}$ and $p=2.2x10^{-8}$), respectively but the changes were dramatically lower than DUX4 co-transfection. This is the first report on potential transcriptional target of DUX4 in muscle cells. The fact that the target gene, PITX1, was shown specifically up-regulated in FSHD suggested that aberrant expression of DUX4 in the FSHD patients likely to be responsible for the disease-specific up-regulation of PITX1, which activated down-stream pathways contributing to muscle pathologies in the FSHD patients.

The development of an open platform LIMS solution to address the complexity of genomics and systems biology workflows, data management, and analysis. *D. Bronnikov* GenoLogics Life Sciences Software Inc., Victoria, BC, Canada.

Recent breakthroughs in the development of high throughput technology platforms for large scale gene expression and genotyping studies introduced a new challenge to geneticists: efficient use of the avalanche of data produced. It is becoming an increasingly daunting task to archive, retrieve, share and compare the data generated by different technology platforms and software applications for data analysis. We present a genomics-purposed LIMS software that enables efficient data management, automatic acquisition from instrumentation, and processing of diverse data in a highly customizable and user friendly environment, integrating open data standards and an open technology platform to encourage collaboration. Based on an established LIMS platform, we created flexible in silico solution that accelerates the entire procedure of sample tracking, quality control, workflow optimization, ensure accuracy and reproducibility of the results, hence increasing the productivity of researchers conducting experiments in Genomics and emerging discipline of Systems Biology.

Transmission and instability of the Huntington's Disease CAG repeat length in a large multigenerational Venezuelan pedigree. *D. Brocklebank[1], J. Gayán[1], M. Andresen[3], S. Roberts[2], D. Housman[3], L. Cardon[1], N. Wexler[2], The US-Venezuela Collaborative Research Group* 1) Dept Statistical Genetics, University of Oxford, UK; 2) Columbia University, NY 10032 USA; 3) MIT, Cambridge, MA 02139 USA.

Huntington's disease (HD) is a progressive neurodegenerative disorder caused by an unstable trinucleotide (CAG)n repeat expansion in the gene on chromosome 4 which codes for the huntingtin protein. This CAG repeat shows remarkable instability, changing often in number during transmission from parents to offspring. Normal alleles (fewer than 35 repeats) are generally stable, while expanded alleles (35 or more repeats) can exhibit dramatic instability on transmission to subsequent generations. There is a strong paternal sex bias towards expansion. Repeat lengths of 40 CAGs or greater are fully penetrant and will produce symptom onset if a gene carrier lives a normal lifespan. The instability of the repeat length has important implications for HD since it accounts for approximately 70% of variation in age of onset. We analyzed the instability of the repeat length as it is transmitted from parent to offspring in large Venezuelan (Vz) kindreds, with a particular view to determining the heritability of the instability. We followed the transmission of alleles through multiple generations, and calculated the change in length of each transmitted expanded allele. Paternal transmissions usually feature repeat length expansions, sometimes almost doubling in size. In contrast, female transmissions predominantly display very little change, minor expansions balanced by contractions. For paternal transmissions only, we have also observed a significant relationship between the amount of change in the repeat length and its original size. The extensive Vz kindreds provide a unique resource for detecting any familial effects influencing the instability. Using relative-pair correlations and variance component maximum likelihood methods, we have obtained evidence for a highly significant familiality and heritability for the CAG change on transmission from fathers. This suggests that modifier genes may influence the stability of the repeat length on transmission of the expanded allele from father to child.

Assessment of ALOX5AP in two independent coronary artery disease studies. *D.R. Crosslin[1], J. Rose[1], J.J. Connelly[1], C. Haynes[1], S. H. Shah[1,2], T. Wang[1], D.C. Crossman[3], C.B. Granger[1], J. L. Haines[4], C.J.H Jones[5], J.M. Vance[1], P.J. Goldschmidt-Clermont[6], W.E. Kraus[2], S.G. Gregory[1], E.R. Hauser[1]* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Division of Cardiology, Duke University Medical Center, Durham, NC; 3) University of Sheffield, Sheffield, United Kingdom; 4) Vanderbilt University, Nashville, TN; 5) University of Wales College of Medicine, Cardiff, United Kingdom; 6) Miller School of Medicine, University of Miami, Miami, FL.

Leukotrienes are arachidonic acid (AA) derivatives known for their inflammatory properties. In recent years, it has been well-documented that the inflammation process of leukotrienes may play an important role in atherosclerosis and subsequently cardiovascular disease (CAD). Mediating the conversion of AA to an unstable leukotriene precursor (LTA4) is the enzyme 5-lipoxygenase-activating-protein (ALOX5AP, FLAP). Several publications have demonstrated an association between variants in FLAP and other genes in the leukotriene metabolism cascade (Helgadottir, 2004). We performed an association study between these variants in FLAP and CAD in the GENECARD study of early onset coronary artery disease study (438 families) and in the Duke University Hospitals cardiac catheterization CATHGEN (556 Cases with early onset CAD and 256 Controls) sample. Analysis of single SNPs in FLAP showed weak evidence for association (p-val < .10). The four-SNP haplotype (HapA) that conferred a 2 times greater risk of myocardial infarction (MI) and stroke in the Icelandic cohort proved to have a moderate association (global p-val = .03) in the CATHGEN population while controlling for age, race and cardiovascular covariates. This result serves as further validation of the association of HapA in CAD. Interestingly we also detected an association with SNPs in ALOX5 (p-val < .05), the target of FLAP. The GENECARD families do not demonstrate association with either ALOX5 or FLAP. We are exploring additional genes in this pathway, as well as the effects of ethnicity and CAD subphenotype (Helgadottir, 2005). We are also examining evidence for interactions among ALOX5, FLAP and other genes in the AA pathway.

Online Interdisciplinary Healthcare Training: A Model for Genetic Education. *S. DeLany Dixon[1], S. Ashley[1], K. Gordes[2], L. Doucette[2]* 1) Master's in Genetic Counseling Program, University of Maryland,School of Medicine Baltimore, MD; 2) University of Maryland School of Medicine, Baltimore, MD.

Purpose: To increase exposure and understanding of the role of genetic counseling in interdisciplinary healthcare and to educate genetic counseling students in cultural competency, an online interdisciplinary healthcare course with both didactic and clinical components was developed. Allied health graduate students in the medical technology, genetic counseling, and physical therapy programs at the University of Maryland School of Medicine participated. The course focused on areas of health promotion and prevention, geriatrics, long-term care, home health and hospice care, ethics, bioterrorism, multiculturalism, and regulatory updates. Methods: An online interdisciplinary course was offered in the Fall of 2005. Students completed online discussions via Blackboard and written assignments. In the Spring semester, students participated in 10 interdisciplinary wellness fairs targeting the underserved population in inner-city Baltimore. Results: Students completed pre- and post- course tests to evaluate and assess their understanding of interdisciplinary healthcare. Approximately 74% of students demonstrated improvement in the understanding of interdisciplinary healthcare. Additionally, students completed the Inventory for Assessing the Process of Cultural Competence Among Healthcare Professionals-revised (IAPCC-R) to assess their level of cultural competency. Prior to the course, 56% of students were noted to be culturally aware, while 44% of students classified as culturally competent. Following the didactic course and participation in community health fairs, 73% of students improved or maintained their level of cultural competency. Conclusions: Based on evaluation from the students as well as the pre- and post- course exams, online education is effective in training students in interdisciplinary healthcare. Additionally, multicultural educational experiences impact the level of cultural competence in allied health students.

Using next generation technology for human genome sequencing and SNP discovery. *D. Bentley* Solexa Ltd, Little Chesterford, Essex, United Kingdom.

Re-sequencing and characterization of sequence variants in individual human genomes on a large scale is of critical importance for studies of medical genetics and evolution of populations. We have developed a system that is capable of re-sequencing human genomes at two orders of magnitude lower cost and higher throughput than conventional technology. Single molecules of randomly fragmented genomic DNA are attached to a planar, optically transparent surface and amplified in situ to create an ultra-high density array of templates (up to 10 million per cm2) for sequencing. Novel reversible terminators with removable fluorescence are used in a robust four-color DNA sequencing-by-synthesis method. Sequence reads with individual base quality scores are generated and aligned against a reference genome. Genetic differences are called using a specially developed data pipeline. Read alignments, sequence variants and summary statistics are displayed using an implementation of the Ensembl genome browser, and also in the sequence display Staden software gap4. Initial experiments using a human BAC enabled us to generate data with high raw read accuracy (Q20) and to obtain consensus sequence of accuracy 99.99 %, with accurate determination of all homopolymeric tracts, dinucleotide repeats and single base differences when compared to the reference sequence. We are currently sequencing an individual human genome (Yoruba from Ibadan, Nigeria) using a random shotgun approach. Early high-quality datasets matched to the reference sequence reveal an even distribution of reads across all chromosomes. We also demonstrate applicability of the system to generating paired reads, targetted sequencing of human genes and genomic regions and accurate identification and display of single nucleotide polymorphisms.

Clinical Benefit Following Enzyme Replacement Therapy (ERT) with Alglucosidase alpha in Children with Pompe Disease. *D. Corzo[1], C. Spencer[2], B. Byrne[2], M. Nicolino[3], W.L. Hwu[4], N. Leslie[5], H. Mandel[6], E. Wraith[7], P. Kishnani[8]* 1) Genzyme Corp, Cambridge, MA; 2) Shands Hospital at the University of Florida College of Medicine, Gainsville, FL; 3) University Hospital Debrousse, Lyon, France; 4) National Taiwan University Hospital, Taipei, Taiwan; 5) Cincinnati Children's Hospital, Cincinnati, OH; 6) Ramban Medical Center, Haifa, Israel; 7) Royal Manchester Children's Hospital, Manchester, UK; 8) Duke University Medical Center, Durham, NC.

Introduction. Pompe is a metabolic myopathy due to a deficiency of the lysosomal enzyme acid alpha glucosidase. Those with onset of symptoms in the first year of life typically die from cardiac and respiratory failure at a median age of 8.7 months (Kishnani et al., in press). **Methods.** Study 1 enrolled 18 patients <6 months of age; Study 2 enrolled 21 patients >6 to 36 months of age. All patients had onset of symptoms in the first year of life and evidence of cardiomyopathy prior to treatment. **Results.** After 1 year of ERT with rhGAA 18/18 (100%) patients in Study 1 were alive and 15/18 patients (83%) were free of invasive-ventilator support. In Study 2, 16/21 patients (76%) were alive and 11/16 (69%) patients who were free of invasive ventilation at baseline, remained so. All but 2 patients with follow up cardiac echo data demonstrated decrease in LV mass; 2 patients showed LV mass stabilization. Thirteen out of 15 patients (73%) of patients treated at <6 months of age (Study 1) showed acquisition of new motor milestones, in contrast to10/21 patients (48%) of those treated at >6 months of age (Study 2). Thirty five out of 39 patients (90%) developed anti-rhGAA antibodies; in one case antibodies had inhibitory activity (in vitro). **Conclusions.** Administration of alglucosidase alpha to this large cohort of patients with Pompe disease resulted in measurable clinical benefit, even in those patients with advanced stage of the disease at onset of ERT (Study 2). Results from these trials provide further evidence that early initiation of ERT is of paramount importance to maximize the chances of a favorable motor outcome. Patients continue to be treated and followed up in extension studies.

Impairment of the Hypothalamus-Pituitary-Thyroid axis in Patients with Glycogen Storage Disease type I (GSD1): increased prevalence of thyroid autoimmunity and hypothyroidism in GSD1b but not in GSD1a. *G. ANDRIA[1], G. PARENTI[1], R. PIVONELLO[2], M. SIBILIO[1], F. BALIVO[1], R. DELLA CASA[1], R. TAURISANO[1], M. SALERNO[1], G. LOMBARDI[2], G. SEBASTIO[1], A. COLAO[2], D. MELIS[1]* 1) DEPARTMENT OF PEDIATRIC, Federico II University, SERGIO PANSINI 5, Naples, Italy; 2) Department of Molecular and Clinical Endocrinology and Oncology Federico II University, SERGIO PANSINI 5, Naples, Italy.

Background: GSD1 has been supposed to be associated to endocrine dysfunction. Aim: The aim of the current study is to investigate the hypothalamus-pituitary-thyroid axis in patients with GSD1 and to compare it in GSD1a and GSD1b patients. Patients and Methods Ten patients with GSD1a, 7 with GSD1b and 34 sex and age-matched healthy controls entered the study. Results Serum FT4 was significantly lower in GSD1a and GSD1b ($p<0.05$) whereas TSH was significantly higher in GSD1b ($p<0.005$) and slightly higher ($p=0.06$) in GSD1a patients than in controls. Tg and TPO auto-antibodies were significantly higher in GSD1b than in GSD1a patients and controls ($p<0.005$). After TRH stimulation, an enhanced TSH response was found in GSD1a and GSD1b patients ($p<0.005$) compared to controls. The presence of a subclinical or overt hypothyroidism was found in 4/7 GSD1b and in none of GSD1a patients (2=7.47, $p<0.005$), and controls (2=27.2, $p<0.0001$). Conclusions GSD1b patients have an increased prevalence of thyroid autoimmunity hypothyroidism and, whereas GSD1a patients showed only a mild impairment of thyroid function. The results of the study suggest a primitive damage of the thyroid gland although the only slightly elevated TSH levels even in patients with overt hypothyroidism suggest a possible concomitant damage at the level of the hypothalamus or pituitary gland .

Characterization of New SBCAD Gene Sequence Variants in Patients with 2-Methylbutyrylglycinuria Identified by Newborn Screening with Tandem Mass Spectrometry. *J. Alfardan[1,2], N. Majumder[3], D. Matern[5], J. Kant[1,2,4], J. Vockley[1,3,4]* 1) Univ of Pittsburgh School of Medicine/Graduate School of Public Health; 2) Dept Pathology; 3) Dept Pediatrics; 4) Dept Human Genetics; 5) Mayo Clinic School of Medicine, Dept Laboratory Medicine.

Objective: Short/branched chain acyl-CoA dehydrogenase deficiency (*SBCADD*), also known as 2-methylbutyryl-CoA dehydrogenase deficiency, is a recently described autosomal recessive disorder of isoleucine metabolism. Few patients have been reported so far, most due to a founder mutation found in the Hmong Chinese population. While the first reported patients had severe disease, most of the affected Hmong have remained asymptomatic. In this study we describe three non-Hmong patients brought to attention by elevated C5-carnitine in blood spots on MS/MS newborn screening. **Methods:** PCR and bidirectional sequencing was performed on genomic DNA from two of the patients covering the entire *SBCAD* (*ACADSB*) gene sequence of 11 exons along with a control subject. 2-Methylbutyrylcarnitine production by patient fibroblasts was quantitated by incubation with isotopically labeled isoleucine followed by tandem mass spectrometry. **Results:** Fibroblasts from two patients accumulated elevated 2-methylbutyrylcarnitine. Sequence analysis of genomic DNA from each patient identified variations in the *SBCAD* gene not previously reported. The first was a homozygous p.Gln99X nonsense variant in exon 3. The patients parents are first cousins of Indian descent. The second patient was a compound mutant for c.443C>T, p.Thr148Ile in exon 4, and c.1159G>A, p.Glu387Lys in exon 10. Both missense changes are predicted to be deleterious to enzyme function. The patients, now 9 and 10 months respectively, are well and developing normally with no specific therapy. Mutational analysis of fibroblasts from the third patient, who is also well, is pending as are functional studies of the mutant enzymes. **Conclusion:** These findings show *SBCAD* deficiency can be identified through newborn screening by tandem mass spectrometry. Our patients have been well without treatment and call for careful follow-up studies to learn the true clinical impact of this disorder.

Germline CDKN2A coding mutations are rare in the majority of cutaneous melanoma patients. *M.L. Council[1], J. Gardner[1], J. Ivanovich[2], C. Kamp[2], L. Cornelius[3], A. Bowcock[1]* 1) Dept Human Genetics, Washington Univ, St Louis, MO; 2) Hereditary Cancer Core, Washington Univ, St Louis, MO; 3) Div of Dermatology, Washington Univ, St Louis, MO.

Malignant melanoma is the most serious form of skin cancer, and its incidence has tripled in Europeans over the past 30 years. Intensive, episodic UV-exposure is felt to be contributory to this process. Rare germline mutations of CDKN2A, a tumor suppressor located at 9p21, are detected in affected members of some families where cutaneous melanoma is segregating. We have begun a genetic study of cutaneous melanoma at Washington University School of Medicine (St. Louis, Missouri). DNA from a cohort of 110 patients is currently available. These include 75 female and 35 male patients; all are of European origin. Twenty-two patients have at least one other first, second, or third-degree relative with melanoma. The majority (85%) of patients have a first, second, or third-degree relative with a second cancer: breast (40%), lung (27%), and colorectal (25%), most commonly. All patients have been screened for variants in CDKN2A and the overlapping p14 gene with dHPLC and/or direct sequencing. Only one germline mutation was detected within the coding region of CDKN2A (D125H); this mutation has been previously described. As this patient was adopted, we have no additional family history. Two patients had intronic variants of undetermined significance, and one patient harbored an A134A silent change. In summary, less than 1% of our cohort of melanoma patients were found to have a mutation altering the coding sequence of CDKN2A, although 20% of patients have a first, second, or third degree relative with melanoma. This is in agreement with other studies. It suggests that CDKN2A mutations are rare in the majority of melanoma patients and that a search for other susceptibility genes is warranted.

Negative BRCA1 immunohistochemistry and/or reduced RNA expression in ovarian tumors predicts germline BRCA1 mutation status. *A. De Luca[1,2 *], J.Z. Press[3 *], S. Young[2,6], Y. Ridge[6], S. Kalloger[5], D.M. Miller[4], D. Horsman[2,6], C.B. Gilks[2,5], D.G. Huntsman[1,2,5,6]* 1) Center for Translational and Applied Genomics, Provincial Health Services Authority & BC Cancer Agency, Vancouver, BC, Canada; 2) Departments of Pathology and Laboratory Medicine; 3) Obstetrics and Gynaecology; 4) Gynecologic Oncology, University of British Columbia, Vancouver, BC, Canada; 5) Genetic Pathology Evaluation Centre of the Prostate Centre, Vancouver General Hospital, Vancouver, BC, Canada; 6) Hereditary Cancer Program, BC Cancer Agency, Vancouver, BC, Canada.

Germline DNA from 49 consecutive women with ovarian cancer was assessed by dHPLC for BRCA1 mutations, and abnormal peaks were sequenced. BRCA1 RNA expression and loss of BRCA1 protein were assessed by real-time PCR and immunohistochemistry (IHC) [anti-BRCA1 (Ab-1), mouse mAb (MS110)], respectively. Mutation screening revealed 8/49 (16%) germline truncating BRCA1 mutations and 1 somatic BRCA1 mutation. 26/49 (53%) tumors had reduced BRCA1 RNA expression, 23/49 (47%) showed loss of BRCA1 immunoreactivity, and 19/49 (39%) had both. All cases with loss of BRCA1 immunoreactivity were high grade serous or undifferentiated. Frequency of BRCA1 germline mutations was 7/26 (27%) in patients with reduced BRCA1 tumor RNA expression and 8/23 (35%) in patients with negative BRCA1 immunoreactivity. The sole case with a somatic truncating BRCA1 mutation had loss BRCA1 expression at a protein and RNA level. 10/14 of the cancers with loss of BRCA1 immunoreactivity and no mutation had BRCA1 promoter methylation, as detected by methylation-specific PCR after bisulfite treatment. None of the 26 ovarian cancer patients with detectable BRCA1 immunostaining had a BRCA1 germline mutation. The sensitivity and specificity for detection of patients who harbour BRCA1 germline mutations were 7/8 (87%) and 22/41 (54%) for RNA expression, 8/8 (100%) and 26/41 (63%) for IHC. Ovarian carcinomas from BRCA1 germline mutation carriers had loss of BRCA protein expression. BRCA1 IHC in primary ovarian carcinoma specimens could be used to triage women for genetic counseling then BRCA1 mutation testing. *Authors contributed equally to this work.

Partial trisomy 2p with congenital polyvalvular disease. *D.N. Abuelo[1], L. Kochilas[1], U. Tantravahi[2]* 1) Pediatrics, Rhode Island Hospital, Providence, RI; 2) Pathology, Women and Infants'Hospital, Providence, RI.

In congenital polyvalvular disease (CPVD), two or more of the cardiac valves show dysplastic features. It is usually associated with major chromosomal abnormalities, most often trisomy 18, but has been described in other aneuploidy syndromes as well. Here we report CPVD associated with partial trisomy 2p.

The patient was born after an uneventful pregnancy with a birth weight of 2.5 kg. Intermittent cyanosis developed at two hours. Physical examination showed craniofacial dysmorphism (prominent forehead, plagiocephaly, deep-set eyes, unilateral microtia with patent external auditory canal), capillary hemangiomas on the forehead and posterior neck and postaxial polydactyly of the left foot. Cardiac examination showed biventricular and septal hypertrophy, a large conoventricular ventricular septal defect(VSD)and a patent foramen ovale. Both semilunar valves were dysplastic with partially fused and thickened leaflets. Doppler interrogation revealed moderate valvar pulmonary and aortic stenosis, mild aortic regurgitation through the non-coapted dysplastic leaflets and some degree of dynamic subvalvular outflow tract obstruction bilaterally. Because of progressive pulmonic stenosis, she underwent initial pulmonary balloon angioplasty at two months and subsequent pulmonary valvulotomy, VSD closure, resection of infundibular muscle bundles and subaortic septotomy. Psychomotor testing showed global developmental delay for which she receives early intervention services.

Cytogenetic study of peripheral lymphocytes revealed an inverted duplication of bands p24p22 on one chromosome 2, confirmed by FISH analysis using whole chromosome DNA paint probe and 2p telomere DNA probe.

Conclusion: The cardiac abnormalities found in this patient are within the spectrum of defects previously reported in patients with 2p duplication, except for the presence of CPVD. Since our patient has a slightly larger duplicated segment than previously reported cases, we suggest that this additional chromosome region may include genes involved in cardiac valvulogenesis.

Sequence-level population simulations over large genomic regions, with gene conversion, recombination hotspots and selection. *D. Balding[1], J. Whittaker[2], M. De Iorio[1], C. Hoggart[1], T. Clark[1]* 1) Dept Epidemiology/Public Hlth, Imperial College, London, United Kingdom; 2) London School of Hygiene and Tropical Medicine, London, United Kingdom.

Population samples of DNA sequences can be simulated using coalescent-based simulation software, such as Hudsons MS. Coalescent methods work backwards in time, which is computationally efficient but is limited in the amount of recombination that can be incorporated, as well as the flexibility to include important features such as gene conversion and selection. With increased capacity of computers, it is now feasible to implement more flexible, forwards-in-time simulation strategies. We have developed FREGENE, software for forwards-in-time, whole-population simulation of sequence-like data over large genomic intervals. It can incorporate different demographic models, as well as recombination, both crossovers and gene conversions at highly variable rates, and several selection models. As illustrations of its potential uses, we (1) examine the performance of algorithms for identifying recombination hotspots, in the presence of potential confounding by gene conversion and selection, and (2) find approximations for genome-wide significance levels of genetic association studies using high marker density or resequence data, under different assumptions about demography and gene conversion. FREGENE is useful both for population genetics simulation studies, and for realistic power simulations for genetic epidemiology studies.

The characterization of RNA instability mutations in carbamyl phosphate synethetase I (CPSI) through inhibition of nonsense mediated decay. *A. Eeds[1], D. Mortlock[1], R. Wade-Martins[2], M. Summar[1]* 1) Ctr Human Genetic Res, Vanderbilt Univ, Nashville, TN; 2) The Wellcome Trust Centre for Human Genetics, University of Oxford, UK.

Carbamyl phosphate synthetase I (CPSI) is the liver-specific enzyme that catalyzes the first and rate-determining step of the urea cycle. Mutations cause CPSI Deficiency (CPSID), an autosomal recessive disease characterized by hyperammonemia. We have previously identified many patient mutations hypothesized to cause CPSI RNA instability. These genomic variants were present in patient genomic DNA but not detectable in patient RNA, indicating RNA degradation, perhaps due to splicing errors and/or nonsense mediated decay. These mutations included the intronic substitutions c.654-3T>G and c.1210-1G>T, the exonic c.1893T>G mutation which directly creates a nonsense codon, and c.2388C>A which is a translationally silent exonic mutation. Using a model system developed in our lab, we show proper expression of wild-type CPSI and significantly decreased expression of CPSI transcripts containing each mutation, as measured by quantitative RT/PCR assays. To determine if this decrease was due to NMD, it was inhibited by siRNA mediated knockdown of UPF2. Subsequently, relative expression levels of each mutant transcript were increased, indicating these mutations cause degradation via the NMD pathway. In addition, cryptic splice sites activated by intronic mutations were identified. This project supports the proposed prevalence of RNA instability mutations in a severe metabolic disease and demonstrates a mean for examining functional consequences of such mutations including the activation of aberrant splicing and nonsense-mediated decay.

Nucleotide sequence analysis of exon 1 of the MECP2 gene in patients previously found to be negative for mutations in exons 2, 3, and 4. *B. Anderson, F. Quan, A. Buller, M. McGinniss, M. Peng, W. Sun, C. Strom*
Department of Molecular Genetics, Quest Diag Nichols Inst, San Juan Capist, CA.

Rett syndrome (RTT) is a neurodevelopmental disorder most commonly seen in females. RTT is caused by mutations in the X-linked methyl CpG binding protein 2 (MECP2) gene. The MECP2 gene consists of 4 exons and codes for multiple transcripts. The transcript for the MeCP2e2 isoform includes all four exons and utilizes a translation start site in exon 2. This transcript was originally thought to be the only transcript from the MECP2 gene. However, an alternatively spliced transcript that codes for the MeCP2e1 isoform was subsequently identified. This transcript consists only of exons 1, 3, and 4, and uses a translation start site in exon 1. The MeCP2e1 isoform appears to be the predominant isoform in the brain. Most patients with RTT have mutations in exons 3 and 4 of the MECP2 gene, and no patients with mutations in exon 2 have been described to-date. Since exon 1 was originally thought to be non-amino acid coding, we previously performed testing for RTT by nucleotide sequence analysis of exons 2-4 of the MECP2 gene. With the discovery of the transcript for the MeCP2e1 isoform, and the identification of rare RTT patients with mutations in exon 1, we have now modified our assay to include this exon. We report here the results of nucleotide sequence analysis of exon 1 for 400 patients previously found to be negative for mutations in exons 2, 3, and 4 of the MECP2 gene. Our results indicate that mutations in exon 1 detectable by nucleotide sequence analysis are likely not a frequent cause of RTT.

The Guilford Genomic Medicine Initiative (GGMI): Developing a model for personalized medicine. *S. Blanton[1], P. Leitz[2], V. Henrich[3], J. Vance[1], M. Pericak-Vance[1], GGMI Investigators[2,3]* 1) Duke Ctr Human Genetics, Durham, NC; 2) Moses Cone Health Care System, Greensboro, NC; 3) The University of North Carolina at Greensboro.

Genetic and genomic information has the potential to revolutionize medicine by changing it from a discipline that reacts to disease to one that prevents it. The main soldiers of this revolution will be the existing primary and specialty physician practices in the private, not the University, setting. However, in a recent survey, only 8% of medical directors believed they were prepared to provide even the most basic education, decision support and assessment of risks for their patints in a genomic medicine setting. To this end, the Moses Cone Healthcare System of Greensboro, NC, UNC at Greensboro, and the Center for Human Genetics at Duke Univesity have begun the GGMI. Its goal is to create a working model to addresss the specific problems in reengineering current medical systems to be genomic medicine ready. GGMI will create solutions that can be applied to other medical plans, such as the military's. The specific aims are to create first a baseline genomic literacy in the general and medical community and to then use a step-wise implementation of three initial diseases to develop a genomic medicine model. Focus groups, both lay and professional, have been conducted to ascertain the level of education and interest in genomic medicine. For the first aim, a web-based genetic and family history module has been developed for physician education. The diseases considered initially in the second aim are thos in which the genomic inforamtion is clearly and universally agreed to be beneficial as a single test and have wide appeal to the population as a whole. This module inclues acquisition of the patient's medical and family history and risk algorithms which generate recommendations based on the family history. Algorithms for thrombotic disease (Factor V Leiden) and breast and colon cancer have been developed and are being piloted in select private practices. Genomic medicine for pharmacogenetics will be developed next. Update of the progress and model development paradigm will be presented.

Increased prevalence of vitiligo and associated autoimmune diseases in a small inbred Romanian community. *S. Birlea, P.R. Fain, R.A. Spritz* Human Medical Genetics Program, University of Colorado Health Sciences Center, Aurora, CO.

The utility of population isolates for gene mapping and identification is well established, taking advantage of the relative homogeneity of genetic and environmental risk factors in these settings. We have studied a small village in the mountains of northern Romania in which vitiligo, an autoimmune disease characterized by acquired pigmentary loss, occurs at a prevalence of 2.9% (48 cases/1673 inhabitants), ten times more than in the surrounding villages or elsewhere in the world. The village is geographically isolated and was founded ~400 years ago by only three families; three-fourths of villagers today still share those original three family names, with many known consanguineous matings. The vitiligo phenotype in the village is typical, and is highly associated with other autoimmune diseases, including autoimmune thyroid disease, rheumatoid arthritis, and adult-onset autoimmune diabetes mellitus, but the mean age of vitiligo onset (34.8 yrs) is relatively late. There is striking (96%) case clustering among closely related individuals, and most patients derive from two unaffected parents (37/48). The high frequency of vitiligo and associated autoimmune diseases, high rate of consanguinity, and pattern of recurrence among sibs is consistent with multifactorial inheritance involving a major-locus recessive derived from a founder. Genomewide linkage and linkage disequilibrium analysis of this remarkable isolated population provides a unique opportunity to map and identify a major gene conferring susceptibility to vitiligo and associated autoimmune diseases that will likely have application to other populations around the world.

Severe language delay associated with duplication of the Williams-Beuren critical region. *J.S. Berg[1], N. Brunetti-Pierri[1], B. Nowakowska[1,2], E. Obersztyn[2], C-T. Fong[3], A. Summers[4], A. Patel[1], A.L. Beaudet[1], S.W. Cheung[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) Pediatric Genetics, University of Rochester, New York, NY; 4) North Bay Health Unit - Genetics, Ontario, Canada.

The Williams-Beuren Syndrome (WBS) is among the most well-characterized microdeletion syndromes, caused in most cases by recurrent de novo deletions at 7q11.23 mediated by non-allelic homologous recombination between low copy repeats (LCR) flanking this region. Given the presence of numerous LCRs and the frequency with which deletions in the WBS region are observed, one would expect that duplications of the same segment might also occur. This hypothesis was recently demonstrated to be correct with the identification of an eight-year-old male with severe language delay and duplication of the WBS critical region (Somerville, NEJM 2005). However, the degree to which duplications of this region contribute to the overall burden of language delay or other developmental disorders is unclear.

We now report five additional patients with severe language delay associated with 7q11.23 duplications. Two patients with prominent language delay were found to have reciprocal duplications of the classical WBS critical region. One 47,XXY patient with uncharacteristically severe language delay was found to have ~33% mosaicism for a ring chromosome containing material from 7q11.23. Two siblings with dysmorphic features and significant language delay were shown to have ~20% mosaicism for a >10 Mb 7q11-q11.23 duplication. These duplications were all first identified by array-comparative genomic hybridization and confirmed by FISH, thus confirming the initial report of severe language delay seen in duplication of the WBS region and further delineating the phenotypic spectrum of this condition. We propose that duplications of the WBS region may be more frequent than previously suspected and that screening for this duplication is warranted in children with language delay.

Pharmacological Induction of Mitochondrial Biogenesis and Antioxidant Genes in X-linked Adrenoleukodystrophy. *R. Deering[1], G. Dong[2], K.D. Smith[1,2]* 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Kennedy Krieger Institute, Baltimore, MD.

X-linked adrenoleukodystrophy (XALD) is caused by mutations in the peroxisomal membrane protein gene ABCD1. Patients have increased levels of very long chain fatty acids (VLCFA; C>22:0), heterogeneous mitochondrial damage, and increased oxidative stress and damage. They typically present with adrenal insufficiency and cerebral inflammatory demyelination or peripheral axonopathy. Available therapies include bone marrow transplantation or dietary therapy (Lorenzos oil) with variable outcomes. We have been studying 4-phenylbutyrate, trichostatin A (TSA), both histone deacetylase inhibitors and hydroxyurea (HU), a ribonucleotide reductase inhibitor, as possible pharmacological therapies. These drugs reduce VLCFA levels in cultured fibroblasts and XALD mouse models (HU not yet tested), increase mitochondrial biogenesis, and increase peroxisome proliferation. Increased mitochondrial biogenesis is required for reduction of VLCFA levels. In recent RT-PCR studies of XALD fibroblasts, these drugs increased peroxisome proliferator activated receptor gamma coactivator 1 alpha (PGC1) and beta (PGC1), nuclear factor erythroid 2-related 2 (NFE2L2), and basic-leucine zipper transcription factor MAFK mRNA levels. PGC1 is a key transcription factor involved in mitochondrial biogenesis; the role of PGC1 is unknown. Nfe2l2 and Mafk bind to the antioxidant response elements of heme oxygenase (HO-1) and manganese superoxide dismutase genes. Treatment of Nfe2l2 -/- mouse fibroblasts demonstrated that increased mitochondrial biogenesis is independent of Nfe2l2 up regulation. Initial studies of HU and TSA treated XALD fibroblasts, grown under hyperoxic conditions (40% oxygen), reduced HO-1 mRNA levels 2-fold (compared to untreated cells) indicating an increased ability of the cells to handle induced oxidative stress. Understanding how these drugs contribute to organelle biogenesis, alleviate oxidative stress, and understanding the resultant lowering of VLCFA levels may contribute to therapy of XALD and provide means for identifying more effective therapeutic interventions.

High-resolution Melting Curve Analysis - a laboratory tool capable of more than mutation detection only. *J.T. Den Dunnen, R.H.A.M. Vossen, G.J.B. van Ommen* Human & Clinical Genenetics, Leiden University Medical Center, Leiden, Nederland.

Recently, the possibilities to scan DNA sequences for sequence variants with melting curve analysis (MCA) have been improved considerably. Using a combination of improved fluorescent dyes (saturating) and enhanced high-resolution instrumentation, the sensitivity and specificity of the technology have been significantly improved. In our lab hrMCA is developing quickly into a standard laboratory tool, applied to tackle diverse issues. Addition of the intercalating fluorescent dye can be both before and after PCR. Addition prior to the PCR usually demands a small modification of the PCR conditions; usually slightly increasing the Mg^{+2}; concentration and annealing temperature is sufficient. Since MCA is sensitive to variation in DNA concentration and PCR yield, we introduced a post-PCR stabilisation buffer that reduces variation between melting curves and noticeably improves results. Mutation detection in clinical diagnosis was performed in a range of diseases, with excellent results; nearly all known changes could be detected starting with existing PCR amplicons, mostly designed for other mutation detection methods. Using addition of an unlabelled melt-probe we were able to discriminate 10 alleles of a set of 3 SNPs in a 25 bp region. The same approach also facilitated discrimination between VNTR alleles varying between 14 and 21 CA-repeats. Results with hrMCA in relation to SNP discovery were excellent; scanning is simple and simple, and the throughput excellent (over 1600 samples per hour). In addition, upon discovery, it is immediately clear whether hrMCA can also be used for subsequent high-throughput SNP-scoring without the need to design a specific assay. Finally, hrMCA was used successfully to determine the complexity of sets of DNA-clones and -fragments, incl. identifying mutation carrying clones, to follow the efficiency of DNA bisulfite treatment and in relation to clone selection protocols (e.g. phage-display selections).

ArrayCGH reveals complex chromosome 8 rearrangement in a patient with supravalvular pulmonary stenosis.
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Recurrent constitutional chromosome 8p rearrangements have been described, in particular the inversion duplication and t(4;8)(p16;p23). For these rearrangements, involvement of olfactory receptor (OR)-gene clusters has been demonstrated. Here we describe yet another chromosomal rearrangement involving both the chromosome 8 short and long arm and which is complex in nature. The proband was born after an uncomplicated pregnancy of 38 weeks. Birth weight was 3150 g (P50), length 49 cm (P90), head circumference 32 cm (P10). A heart murmur was detected at birth. Echocardiography showed a supravalvular pulmonary stenosis. Physical examination at the age of 3 months revealed a baby in good general condition. There was an impression of hypertelorism, strabismus of the left eye, upslanting palpebral fissures and blue sclerae. The hands showed bilateral simian crease. The ears were posteriorly rotated with a preauricular appendix on the left side. There was an intergluteal hairy dimple. Neurological examination was normal and the child showed a normal psychomotor development. Karyotyping revealed an aberrant chromosome 8 with extra material on the short arm. FISH analysis with telomeric probes showed extra material of 8qter on 8pter and a deletion of the distal 8p segment. This was indicative for a recombinant chromosome resulting from a balanced pericentric inversion. However, chromosomal analysis of both parents gave normal results. An arrayCGH analysis was performed in order to delineate the breakpoints. The deletion of 8p and duplication of 8qter material was confirmed. However, an additional gain of material was seen on 8p22. The karyotype was rewritten as 46,XX,der(8)(qterq24.1::p23qter).arr cgh 8p23.1(pterCTD-2629I16)x1, 8p22(RP11-148E1RP11-545M21)x3, 8q24.13(RP11-158K1qter)x3. Further molecular investigation is ongoing to define the exact nature of this unusual rearrangement and particular attention will be given to the possible role of the genomic architecture of 8p which is known to mediate recurrent imbalances such as inv dup(8p).

Screening for APC gene mutations in 23 Chilean families with familial adenomatous polyposis: analysis of extracolonic manifestations. *K. Alvarez[1, 2], M. De la Fuente[2], A. Letelier[2], M. Acuña[2], F. Bellolio[1], F. León[1], F. López-Kostner[1], M.P. Carvallo[2]* 1) Laboratorio de Oncología y Genética Molecular, Departamento de Cirugía Digestiva, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile; 2) Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile.

Familial adenomatous polyposis (FAP) is an autosomal dominant disease with complete penetrance. It is characterized by the development of hundreds to thousands of adenomatous polyps present mainly in the colon and rectum, and the development of colorectal cancer at 35-40 years old. FAP is caused by mutations in the tumor suppressor gene APC (5q21-22). Different studies in FAP patients have shown a correlation between the site of the mutation and the clinical phenotype, such as the degree of severity and/or the presence of extracolonic manifestations. Desmoid tumours constitute the most frequent extracolonic manifestations in FAP patients, associated with mutations present in exon 15 between codons 1310-2011. We performed the screening of the APC gene in twenty three Chilean families apparently not related. All patients were selected by standard criteria. Exons and intron-exon boundaries of the APC gene were analyzed through Single Strand Conformation Polymorphisms, Protein Truncation Test, and DNA sequencing. Our study revealed 17 different truncating mutations present in 21/23 families (91%), nine of these novel. Fourteen mutations were found in exon 15 between codons 829 and 1570, one in exon 6, one in exon 7 and one in exon 14. Eight families (35%) showed almost one patient affected with desmoid tumours. In these families we found seven mutations among codons 849 and 1533. Four mutations were inside the region associated with desmoid tumours. Three mutations were found upstream of the described region; two of them present in families with almost 100% penetrance for desmoid tumours among relatives. By means of the use of conventional techniques our study has demonstrated a high detection rate of mutations in this gene. FONDECYT 1040827.

A Robust, Scaleable Solution for High Throughput Data Production Using Affymetrix 500K SNP Arrays. *B. Blumenstiel[1], M. DeFelice[1], M. Parkin[1], W. Winslow[1], C. Healy[1], D. Mirel[1], P. Lin[1], B. Handsaker[1], M. Nizzari[1], P. DeBakker[1], M. Daly[1,2], D. Altshuler[1,2], S. Gabriel[1]* 1) Broad Institute, Cambridge, MA; 2) Center for Genome Research, Massachusetts General Hospital, Boston, MA.

Over the past year robust, comprehensive tools for genome-wide association studies have become available. In order to apply these tools to perform association studies of disease it is critical to produce data at high scale and high quality. To meet this need, we have developed an automated lab process for target prep, data management and tracking system to support the Affymetrix 500K SNP array. Initially at a scale of 384 arrays per week in March 2006 the pipeline reached full scale in May 2006 generating 1536 arrays per week. In total over 6,000 arrays (1.5 billion genotypes) have been generated, with quality and accuracy of data maintained throughout the scale up. Overall call rates across different datasets exceed 99% on average and accuracy as assessed by segregation tests in family samples and comparison to the Hap Map is approximately 99.5%. We have implemented quality control tests to take advantage of the product content and the dense genotype data. Routine tests include chip to chip comparison on overlap SNPs and tests for unexpected allele sharing which suggests sample mix up or contamination.

Enzyme replacement therapy in a murine model of mucopolysaccharidosis IVA. *M.A. Gutierrez[1], J.H. Grubb[2], T. Nishioka[1], G. Trandafirescu[1], S. Tomatsu[1]* 1) Department of Pediatrics, Saint Louis University, Saint Louis, MO; 2) Department of Biochemistry and Molecular Biology,Saint Louis University, Saint Louis, MO.

Mucopolysaccharidosis IVA (MPS IVA) is an autosomal recessive disorder caused by a deficiency of N-acetylgalactosamine-6-sulfate sulfatase (GALNS), leading to accumulation of keratan sulfate and chrondroitin-6-sulfate. Preclinical studies of enzyme-replacement therapy for MPS IVA were performed in MPS IVA mice. The pharmacokinetics and biodistribution were determined for recombinant human N-acetylgalactosamine-6-sulfate sulfatase (rhGALNS). The plasma half-life of the phosphorylated rhGALNS was 3 min and disappeared from the systemic circulation of the animals in two phases. After intravenous doses of 250 units/g body weight were administered, the enzyme was primarily recovered in the liver, with detectable activity in other tissues including bone and bone marrow but not in brain. Greater activity levels were reconstituted in various tissues after a high dose (1,500 units/g body weight). Even a single rhGALNS injection reduced remarkably the amount of storage material in the liver and spleen of MPS IVA mice. After intravenous dose of 250 units/g body weight was administered for 12 weeks, MPS IVA mice showed 80-90% reduction of storage in visceral organs, bone marrow cells, osteoblasts, osteocytes, ligaments, and connective tissues surrounding the bone, while the growth plate region showed a little improvement. These preclinical mouse studies demonstrate the clearance of tissue by administered rhGALNS, thereby providing the in vivo rationale and the critical pharmacokinetic and pharmacodynamic datafor the design of enzyme-replacement trials in patients with MPS IVA.

A voxelation map of gene expression in a mouse brain section. *M.H. Chin[1], A. Geng[1], A. Khan[1], W.J. Qian[2], S. Levy[3], R.D. Smith[2], R.M. Leahy[4], D.J. Smith[1]* 1) Depts. Molecular and Medical Pharmacology & Human Genetics, Sch Med, Univ California, Los Angeles, Los Angeles, CA; 2) Biological Sciences Division and Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA; 3) Biomedical Informatics Department, Vanderbilt University, Nashville, TN, 37232, USA; 4) Signal & Image Processing Institute, University of Southern California, Los Angeles, CA.

Gene expression signatures in the mammalian brain hold the key to understanding neural development and neurological diseases. In order to address this problem, we employed voxelation, which involves dicing the brain into spatially registered voxels (cubes) and then assaying gene expression from each voxel using microarrays. Multiple volumetric images of brain gene expression are created, similar to those obtained from biomedical imaging systems. We have reconstructed 2-dimensional images of gene expression for 20,000 genes in a coronal slice of the mouse brain using microarrays in combination with voxelation at a resolution of 1mm. Good reliability of the microarray results were confirmed using multiple replicates, subsequent quantitative RT-PCR, proteomics and publicly available in situ hybridization data. Clustering analysis identified known and novel genes with expression patterns localized to defined substructures within the brain. In addition, genes with unexpected patterns were identified. The genome-scale maps of gene expression obtained using voxelation will be a valuable tool for the neuroscience community.

Identification of cis sequence effects on gene transcription using multiple methods. *A. Bergen[1], A. Baccarelli[1,2], T. McDaniel[3], K. Kuhn[3], E. Chudin[3], P. Bender[4], R. Pfeiffer[1], D. Garcia-Rossi[1,5], K. Jacobs[1,5], B. Packer[1,5], S.J. Chanock[1,5,6], M. Yeager[1,5]* 1) Division of Cancer Epidemiology and Genetics, NCI, Bethesda, MD; 2) School of Public Health, Harvard University, Boston, MA; 3) Illumina, San Diego, CA; 4) Coriell Institute for Medical Research, Camden, NJ; 5) Core Genotyping Facility, NCI, Gaithersburg, MD; 6) Section on Genomic Variation, Pediatric Oncology Branch, CCR, NCI, Bethesda, MD.

We investigated the role of sequence variation in cis on gene expression and identified associated SNPs and/or haplotypes in a group of genes commonly studied in cancer research. Three replicate cell cultures of thirty unique individual cell lines drawn from the Caucasian subsample of the SNP500Cancer resource were grown with same-lot cell culture reagents in parallel. Total RNA purified from each sample was amplified and labeled by the method of Eberwine, and gene expression profiling was performed using a custom Illumina Sentrix Array Matrix-96 array. N=32 genes had sequenced verified SNPs in the SNP500Cancer database and sufficient gene expression signal and variance. Association analysis of the quantitative trait of rank-invariant normalized mean RNA transcript level and SNP alleles focused on genes with 2 to 15 tag SNPs. We used 1) the single-point additive model of Mander, 2) SNP-wise regression, and 3) a phylogenetic method (TreeScan), using haplotypes and phylogenetic reconstruction via neighbor joining to evaluate association between partitions of the haplotype phylogeny and gene expression signal. For genes analyzed by all three methods (N=21 genes encompassing N=104 tag SNPs), 3, 5 and 4 genes (a total of N=7 genes encompassing N=43 SNPs) exhibited significant ($p<0.05$) association between one or more SNP genotypes and/or haplotype partitions and RNA transcript level. Two genes were identified using all methods, one gene was identified by the two SNP-wise methods, two genes were identified by the phylogenetic method, and two genes were identified via SNP-wise regression. These results identify candidate SNPs involved in the regulation of transcription and identify strengths and limitations of SNP-wise and haplotype-wise analytical methods.

ANTERIOR CERVICAL HYPERTRICHOSIS: A CASE REPORT. *D. Garcia-Cruz, M.G. Lopez-Cardona, J.R. Corona-Rivera* Instituto de Genetica Humana, Centro Universitario de Ciencias de la Salud, Guadalajara, Jalisco, Mexico.

Introduction: Anterior cervical hypertrichosis (ACH) (OMIM 600457) is a rare form of localized hypertrichosis, Tsukahara and Kajii (1992) described the first Japanese family with 7 affected members in 3 generations, posteriorly Braddock et al. (1995) reported a similar case of anterior cervical hypertrichosis without other abnormalities. Also it has been reports associated to hereditary motor and sensory neuropathy (HMSN), (OMIM 239840), severe chorioretinal degeneration, optic atrophy and recently we described a case with ACH associated with unusual features: moderate mental retardation, abnormal EEG, and mild microcephaly. Methods: We describe clinically a 24 month-old Mexican boy with congenital ACH. Results: Case Report: the propositus is a 24 month-old Mexican boy with congenital ACH, he was the product of a full-term pregnancy and non-consanguineous parents. Clinically he showed hypertrichosis on the forehead, cheeks, cervical anterior just cephalad to the laryngeal prominence, which was noted since birth, low posterior hairline on neck and fine hypertrichosis on posterior thorax. Radiological examination showed megacolon. Discussion: ACH has been considered to be an autosomal dominant phenotype, and/or X-linked. Up to date it is not clear if isolated ACH, ACH-HMSN, or other associated findings reported in some patients with ACH, are part of ACH or fortuitous associations. ACH syndrome is not yet well delineated mainly by the small number of affected patients.

Incorporating linkage information in testing strategies for genome-wide association studies in family-based designs. *D. Fardo[1], S.T. Weiss[2], C. Lange[1]* 1) Deptartment of Biostatistics, Harvard School of Public Health, Boston, MA; 2) Channing Laboratory, Brigham and Women's Hospital, Boston, MA.

Genome-wide association studies have come to the forefront of statistical genetics. A number of analysis approaches for such studies have been suggested but have not yet proven successful. Van Steen et al (2005) suggested a testing strategy that allows the same data set to be used for the screening step and the replication step, bypassing the multiple testing problem and minimizing the effects of study heterogeneity. The approach has been successfully applied to identify a novel SNP for BMI that replicated in 5 additional studies (Herbert et al, in press). One limitation of the testing strategy by Van Steen et al (2005), however, is that linkage information is ignored, which, in practice, will lead to a substantial reduction in power. Our new methodology incorporates linkage information and can use the same data set for both the screening step and the replication step. The approach enables a more powerful analysis of genome-wide association studies in order to detect genetic factors for complex diseases. We assess the power of our approach by a series of simulation studies at a genome-wide level and show that the power gains are of practical relevance. We illustrate the approach by an application to an asthma study.

The genetics of lateral fusion defects during female reproductive tract development. *D.R. Cordero[1,3], H. Kim[2,3], D.J. Donovan[1,3], C.C. Morton[1,3], B.J. Quade[1,3]* 1) Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital; 2) Department of Molecular Genetics, Massachusetts General Hospital; 3) Harvard Medical School.

Developmental abnormalities involving the female reproductive tract (FRT) manifest at different ages as a heterogeneous group of disorders and may have significant psychological, sexual and reproductive implications. One common group of FRT abnormalities is those secondary to defects in lateral fusion of the Mullerian ducts. However, little attention has been given to the genetic and molecular regulatory mechanism controlling lateral fusion of the Mullerian ducts. We obtained a lymphoblastoid cell line (LCL) from a patient with a didelphic uterus, cleft palate and a structurally abnormal cerebellum who had a balanced translocation involving chromosomes X and 17 (46,X,t(X;17)(p11.2;p11.2) enrolled in the Developmental Genome Anatomy Project (dgap.harvard.edu). Metaphase and interphase chromosomes from the LCL were used for Fluorescence in situ hybridization (FISH) using BAC and fosmid clones to narrow the breakpoints. Refined mapping of the breakpoint region on the X chromosome by Southern blot analysis has narrowed the breakpoint region to a 4kb region on the short arm of the X chromosome. This highly conserved region is several kb away from known genes and may be involved in important gene regulatory functions. Future directions will include suppression PCR to determine the junction fragment. The DNA sequence data obtained from the junction fragment will allow further analysis to determine the contributions of genes on chromosomes X or 17 to normal and abnormal lateral fusion of the mullerian ducts.

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Genotype-calling algorithm for high-throughput chip-based TaqMan SNP assays. *B.Q. Doan[1], B. Carvalho[2], S.N. Liu-Cordero[3], B. Bishe[1], A. Fink[1], A. Chakravarti[1], R. Irizarry[2], D.E. Arking[1]* 1) IGM/JHMI,Baltimore,MD; 2) Biostat/JHSPH,Baltimore,MD; 3) BioTrove,Woburn,MA.

With the International HapMap Project and discovery of millions of single nucleotide polymorphisms (SNPs) within the human genome, the ability to conduct large-scale genome-wide association studies is being realized. Chip-based technologies and genotype calling software have made possible the systematic generation of genotype data consisting of 1,000s- 100,000s of SNPs. However, little is available for large-scale fine mapping studies that focus on regions needing <100 SNPs, which typically have relied on individual TaqMan assays that are relatively labor intensive with genotype calling heavily reliant upon manual calling. In our study we focus on using a chip-based OpenArray SNP genotyping technology developed by BioTrove using the TaqMan assay to follow up on regions identified from a genome-wide association study on the QT interval. Two advantages are the small amount of DNA required (2.3 ng/sample/SNP) and its high throughput (up 100,000 genotypes/day), allowing for the efficient genotyping of 32 SNPs in 25,000 subjects done in duplicate. To accommodate the throughput, we develop an automated genotype-calling algorithm for the raw data (VIC and FAM dye fluorescence intensities) to systematically control for unwanted sources of variation. First to adjust for differences in the intensities based on the dye, we normalize VIC to FAM. Then we further normalize by samples to adjust for variations in DNA concentrations, and by arrays to adjust for variations in chip performance. We examine several methods including quantile, median, and mean normalizations. Genotype clusters are defined based on known genotypes from HapMap sample controls, and the mean of the log2(ratio of normalized intensities) for each cluster is defined. Study sample genotypes are called from the known genotype cluster mean with the smallest distance to the sample log2(ratio). We are further developing our algorithm to control for other possible systematic variations such as differences in sample well location and in the assay PCR efficiency (based on GC content of the primers, and/or product length).

Reproductive Genetics And Responsibility: The Patient Standpoint. *C. Bouffard* Dept Pediatrics, Univ Sherbrooke, Sherbrooke, PQ, Canada.

The concept of « responsibility » used in bioethics has been neglected, with respect to the patients standpoint. Interest, for the most part, has been directed almost exclusively, so far, toward the impact of genetics on society, as opposed to that of society on genetics. As a sequel to my previous work, this study proposes to examine: 1)different ways in which patients view the notion of responsibility when consulting for reproduction-related genetic problems, 2)the representation they entertain towards these responsibilities, 3)the repercussions of these responsibilities on their decision-making processes, on their lives and that of their unborn child and, 4) the socio-cultural factors that generate these responsibilities. Beyond the acquisition of new information, the consequences of a selective abortion or the birth of a severely ill offspring shall determine responsibilities, which are more of a socio-cultural than genetics in nature: Responsibilities assumed by patients undergoing genetic counselling with respect to reproductive issues, as well as the representations such patients entertain toward these responsibilities, are multiple and determined by cultural, social, institutional, economic, familial and individual factors that are associated more with the collective concept of quality of life than with genetics. Ethnographic multi-sited research design based on participative observation, taped and videotaped interviews of 48 research subjects (or until the data is saturated). Methodology: comparative analysis of resultants from multiple sources like literature review, participative observation, semi-structured interviews, content and discourse analysis. So far, my work showed 7 levels of responsibility from the patients perspective: 1) Assimilating genetic information and knowledge; 2) Making decisions after a genetic counselling session intended to promote an informed consent; 3) Making decisions in a context where alternatives are limited to palliative solutions; 4) Accepting to carry, to transmit or to have transmitted a genetic or chromosome disease; 5) Accepting the psychological, familial, personal or social consequences; 6) Managing reproduction based on health criteria; 7) Transferring knowledge to family members.

SNP Selection for Association Studies. *E.L. Goode, D.N. Rider, W. Liu-Mares, X. Wang, J.E. Olson, M. Wiepert, J.P. Kocher, J.R. Cerhan, F.J. Couch, J.M. Cunningham* Mayo Clinic College of Medicine, Rochester, MN.

The availability of high-throughput genotyping platforms enables investigators to create custom arrays of thousands of SNPs. One cost-efficient SNP selection approach relies on the use of publicly-available data from LD characterization efforts. We developed a LD-based SNP selection system using genotype data from the HapMap Consortium (Phase II), Perlegen, SeattleSNPs, and NIEHS EGP (Panel 2). 1,808,564 SNPs within 5 kb of 25,418 Build 35 genes were included from HapMap; 763,543 SNPs within 5 kb of 23,100 genes were included from Perlegen; 29,287 SNPs within 261 genes were included from Seattle SNPs; and 76,862 SNPs within 171 genes were included from NIEHS EGP. We identified tagSNPs using the LDSelect binning algorithm (Carlson et al., 2004) with a minimum European-American MAF = 0.05 and $r^2 = 0.8$ between assayed and unassayed SNPs. Several factors were considered when selecting the best source for each gene (e.g., N chromosomes studied, N SNPs genotyped, and N bins) and selecting tagSNPs within each bin (e.g., assay conversion metrics, putative function, and MAF). Using predefined criteria, we found that 23,593 genes were best covered by HapMap (985,226 SNPs in 301,451 bins), 1,101 by Perlegen, 190 by SeattleSNPs, and 116 by NIEHS EGP; 1,818 genes had no available SNP genotypes. Comparison of information across LD sources suggests that, depending on intron-exon structure, resequencing projects did not always provide the best coverage. Extensions enabling additional comparisons include the use of more ethnic groups and LD projects, linkage with functional SNP databases, and consideration of multiple tagging algorithms. In current studies of 256 candidate genes within the NFkB pathway, we found that 10,104 SNPs in the public LD projects reduced to 3,007 bins (mean = 12 bins per gene, median = 7, range = 1 to 151). Assessment of the transferability of LD from these public projects to our genotyped study participants is currently underway. Although use of public data reduces the expense of SNP discovery and characterization, one must consider whether the chromosomes studied by these projects are representative of those to be genotyped.

Birt-Hogg-Dube: A syndrome the geneticist should know. *C.D. DeLozier[1, 2], T. Treisman[1], Y. Chang[3], D. Tashjian[4], T. McCalmont[5], C.J. Curry[6]* 1) Genetic Medicine, Central California Faculty Medical Group, Fresno, CA; 2) Genetics Department, Kaiser Permanente Fresno, CA; 3) Urology Department, Kaiser Permanente Fresno, CA; 4) Dermatology Associates, Fresno, CA; 5) Dermatopathology, University of California San Francisco, CA; 6) Department of Pediatrics, UCSF-Fresno Medical Education Program Fresno, CA.

In our full-service genetics consultation we see approximately 200 families/year for assessment of a hereditary predisposition to cancer. Most consultations concern breast/ovarian or colorectal cancers, and most gene testing comes back negative. Although textbooks suggests that rare cancer-predisposition syndromes account for less than one percent of familial aggregation of cancer, our experience suggests that syndromic entities may well be under-diagnosed. One such condition is the Birt-Hogg-Dube (BHD) syndrome, due to mutations in the presumed tumor-suppressor gene folliculin (FCN) on chromosome 17p. BHD is best known to dermatologists because of skin lesions with characteristic histology: fibrofolliculomas, trichodiscomas and acrochordons. Renal tumors, specifically oncocytomas and oncocytic hybrid tumors with both malignant and benign lesions, occur in approximately 30% of BHD patients. The gene mutation also predisposes to spontaneous pneumothorax/pulmonic cysts and to benign and malignant tumors of the uterus, ovaries, breast and colon. We describe here the clinical, genetic and histological features of three families with BHD syndrome presenting to the cancer genetics consultation in the same year. All adults over 30 had typical facial and thoracic skin lesions and each family had at least one person with a benign or malignant kidney tumor. Two families had pneumothorax. A fourth proband presented with primary spontaneous pneumothorax, had no other clinical features at the age of 46. We believe that a thorough physical exam and the conscientious histological verification of benign and malignant tumors in individuals attending the cancer genetics clinic will result in improved detection and management of this under-diagnosed cancer predisposition syndrome.

Fetal DNA from maternal plasma: noninvasive prenatal exclusion of Hemoglobin Bart's Hydrops (--^{SEA}/--^{SEA}).

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Aim

Alpha thalassemia carrier(/--)couples risk having fetuses with fatal hemoglobin(Hb)Barts hydrops(--/--). Invasive prenatal testing procedures can cause miscarriages. We hypothesized that HbBarts hydrops can be excluded noninvasively using fetal DNA in maternal plasma and carriers with unaffected fetuses can avoid unnecessary invasive procedures.

Methods

Two microsatellite markers within the common -^{SEA} alpha thalassemia breakpoints were analyzed in 26 families each consisting of maternal plasma, parental and fetal DNA. Parental microsatellite markers discriminate fetal paternally-inherited alleles from maternal alleles in maternal plasma.

Results

Fetal paternally-inherited microsatellite markers were detected in 9 maternal plasma. Presence of 1 fetal paternally-inherited microsatellite marker(n=4), or both(n=5) excluded HbBarts hydrops. Absence of both markers required further confirmation using fetal DNA. Of these, one fetus was confirmed HbBarts hydrops.

Conclusion

HbBarts hydrops was excluded noninvasively with 100% accuracy using fetal DNA in maternal plasma. Of the 25 unaffected cases, 9 were excluded of HbBarts hydrops noninvasively. As such, 36%(9/25) of screened population would avoid unnecessary invasive prenatal testing.

Deletion 7q with FOXP2 involvement transmitted through a familial complex rearrangement. *P.A. Lennon[1], S.W. Cheung[2], D. Peiffer[3], K.L. Gunderson[3], P. Hu[1], A. Patel[2], C.A. Bacino[2]* 1) School of Health Sciences, UT-MD Anderson Cancer Center, Houston, TX; 2) Medical Genetics Laboratories, Baylor College of Medicine, Houston TX; 3) Illumina Incorporated, San Diego, CA.

Children who demonstrate abnormal language development, in the absence of apparent causative factors, are diagnosed with specific language impairment. Previously, fine mapping of microsatellites within in a large, three-generation family, in which half the members had severe specific language impairment, aided the localization of the SPCH1 locus (thought to be responsible for the speech and language deficits within this family) to 7q31 within markers D7S2459 (107.1 MB) and D7S643 (120.5 MB) (Fisher et al., 1998; Lai et al., 2000). Additionally, chromosome rearrangements of 7q31 and mutational analyses have supported the growing evidence that FOXP2, a gene within the SPCH1 locus, is involved with speech and language development. It is unclear whether the AUTS1 (autistic spectrum 1) locus, highly linked to 7q31, overlaps with the SPCH1 locus. Here, we provide the clinical description of a patient with specific language impairment, but not autism, and a deletion of 7q31.1 -7q31.31 (111.4 MB-120MB). His karyotype is 46,XY,der(7)del(7)(q31.1q31.31)ins(10;7)(q24.3;q31.1q31.31)mat. Our patients deletion, which includes FOXP2, adds to the body of evidence which supports the role of FOXP2 in specific language impairment, but does not support the previously reported role of FOXP2 in autism. A reported association between autism and WNT2, another gene which is deleted in our patient, is likewise not supported by the case we present. Our patients apparent delimiting of the proximal boundary of the previously reported SPCH1 locus (Lai et al., 2000) to 111.4 (MB) and our patients presentation with specific language impairment but not autism, when examined in light of Hutchesons et al (2003) reported critical minimal AUTS1 region between 106.9 and 109.7 MB, strongly supports that the SPCH1 and AUTS1 loci are separate, non-overlapping entities.

Global profile of copy number variation in the human genome. *C. Lee[1], H. Aburatani[2], N. Carter[3], M. Hurles[3], K. Jones[4], S. Scherer[5], C. Tyler-Smith[3], Genomic Structural Variation Consortium* 1) Department of Pathology, Brigham and Woman's Hospital, Harvard Medical School, Boston, MA; 2) University of Tokyo, Japan; 3) Wellcome Trust Sanger Institute, Cambridge, UK; 4) Affymetrix Corporation, Santa Clara, USA; 5) The Centre for Applied Genomics, Hospital for Sick Children, Toronto, Canada.

Copy number variation (CNV) within the human genome is of functional importance and yet remains grossly under-ascertained. To redress this imbalance and generate a CNV map of the human genome, we screened all 270 individuals from the four HapMap populations with ancestry in Europe, Africa and East Asia for copy number variation using two complementary technologies: the Affymetrix 500k SNP genotyping platform and comparative genome hybridisation on a spotted microarray containing ~27,000 BAC clones representing the genome tilepath. We rigourously assessed the rate of false positive CNV calls by several independent means, including the validation of over 100 loci by quantitative PCR and FISH. We identified over a thousand CNVs in these four populations, many of which have not previously been identified, and which contain hundreds of genes and non-coding functional sequences. We ascertained the impact of these CNVs on the reliability of SNP genotypes from the International HapMap Project. We also delineated the linkage disequilibrium properties of hundreds of these CNVs, and identified tagging SNPs. Finally, we identified the genomic regions that show the most dramatic variation in copy number between populations, and explored selective explanations for these observations. Our data is also released in public genome browsers for the clinical genetics and basic research community. This represents a comprehensive, genome-wide assessment of CNV and its relationship to haplotypic structure, providing an important resource for the understanding of how genome structure impacts on biological function.

Asian Nomads traces in the mitochondrial gene pool of Slavs. *B.A. Malyarchuk[1], T. Vanecek[2], M.A. Perkova[1], M.V. Derenko[1], M. Sip[3]* 1) Genetics Laboratory, Institute of Biological Problems of the North, Magadan, Russian Federation; 2) Department of Pathology, Medical Faculty Hospital, Charles University, Pilsen, Czech republic; 3) Faculty of Health and Social Studies, University of South Bohemia, Ceske Budejovice, Czech Republic.

Mitochondrial DNA (mtDNA) variability was studied in a sample of 179 individuals representing Czech population from west Bohemia. MtDNA analysis revealed that the majority of Czech mtDNAs belongs to the common West Eurasian mitochondrial haplogroups. However, about 3 per cent of Czech mtDNAs encompass East Eurasian lineages (A, N9a, D4, M*). Comparative analysis of published data has shown that different Slavonic populations contain small but marked amount of East Eurasian mtDNAs (e.g. 1.3 per cent in Eastern Slavs, 1.8 per cent in Western Slavs, and 1.2 per cent in Southern Slavs). It is noteworthy that Baltic populations (Latvians, Lithuanians and Estonians) have avoided a marked influence of maternal lineages of East Eurasian origin (0.3-0.6 per cent). The two East Eurasian mtDNA haplogroups, Z1 and D5, are present in gene pools of North European Finnic populations (Saami, Finns, and Karelians). Unlike them, Slavonic populations in general are characterized by heterogeneous mtDNA structure, defined, in addition to Z1 and D5, by haplogroups A, C, D4, G2a, M*, N9a, F and Y. Therefore, different scenarios of female-mediated East Eurasian genetic influence on Northern and Eastern Europeans should be highlighted: (1) the most ancient, probably originated in the early Holocene, influx of Asian tribes, which brought a few selected East Asian mtDNA haplotypes (like Z and D5) to Fennoscandia (Tambets et al. 2004), and (2) gradual gene flows of historic times occurred mostly in the Middle Ages due to migrations of nomadic peoples (such as the Huns, Avars, Bulgars, Mongols) to Eastern and Central European territories inhabited mainly by Slavonic tribes. We suggest that the presence of East Eurasian mtDNA haplotypes is not original feature of gene pool of the proto-Slavs, but mostly is a consequence of admixture with Central Asian nomadic tribes, who migrated into Central and Eastern Europe in the early middle Ages.

Mutations in the *CEP290/NPHP6* gene, encoding Centrosomal Protein 290/Nephrocystin-6, cause Joubert Syndrome with Nephronophthisis, blindness, and cerebellar defects. *E.A. Otto[1], J.A. Sayer[1], H. Khanna[1], J.F. O'Toole[1], M.A. Kennedy[2], B.V. Fausett[1], M. Attanasio[1], J. Helou[1], D. Williams[3], Y. Liu[4], L. Ma[5], X. Zhu[5], I. Glass[6], A. Swaroop[1], D. Goldman[1], I. Drummond[4], P. Nurnberg[7], A. Swaroop[1], M.R. Leroux[2], F. Hildebrandt[1]* 1) Departments of Pediatrics, Human Genetics, Neuroscience, and Ophthalmology, Univ Michigan, Ann Arbor, MI; 2) Mol. Biology, Simon Fraser Univ, Burnaby, Canada; 3) Pharmacology, UCSD, San Diego, CA; 4) Renal Unit, Harvard Medical School, Boston, MA; 5) Biochemistry, Inst of Biol Sci, Shanghai, China; 6) Dept of Pediatrics, Univ of Washington, Seattle, WA; 7) Center for Genomics, Univ of Cologne, Germany.

Nephronophthisis, an autosomal recessive cystic kidney disease, is the primary genetic cause for chronic renal failure in children. The molecular basis and its association with retinal degeneration and cerebellar vermis aplasia (Joubert syndrome), are poorly understood. Joubert syndrome is a heterogeneous disorder characterized by cerebellar vermis hypoplasia, mental retardation, ataxia, developmental delay, oculomotor apraxia, and irregular breathing pattern. Variable clinical features include retinal dystrophy and renal anomalies (Nephronophthisis). Applying a positional cloning approach, we identified 9 different mutations in CEP290/NPHP6 in 7 families with Joubert syndrome and nephronophthisis and 1 family with nephronophthisis associated with retinitis pigmentosa without cerebellar defects. We demonstrated that CEP290/NPHP6 directly interacts with and modulates the activity of ATF4/CREB2, a transcription factor possibly implicated in cAMP-dependent renal cyst formation. In a cell cycle-dependent manner CEP290/NPHP6 localizes to centrosomes and to the nucleus in renal epithelial cells. In photoreceptor cells the protein is found in the connecting cilium. Abrogation of the function in zebrafish yields convergent extension defects and recapitulates the renal, retinal and cerebellar phenotypes of Joubert syndrome. Our findings help establish the link between centrosome function and transcriptional control in the pathogenesis of cystic kidney disease, retinal degeneration, and central nervous system development.

Genome-wide scan for split-hand/foot malformation with long bone deficiency (SHFLD) (OMIM 119100) in a large multigenerational UAE family shows a novel susceptibility locus on chromosome 1q42.13-1q43. *M. Naveed[1], M.T. Al-Ali[1], S.K. Murthy[1], S. Al-Hajali[1], N. Al-Khaja[1], U. Ratnamala[2], A. Bottani[3], S.E. Antonarakis[3], M. Gaines[4], J. Golla[4], D. Hutchings[4], S.K. Nath[4], U. Radhakrishna[3]* 1) Center for Arab Genomic studies, Dubai, UAE; 2) Green Cross Blood Bank & Genetic Research Centre, Paldi, Ahmedabad, India; 3) Dept. of Genetic Medicine & Development, University of Geneva Medical School, Geneva, Switzerland; 4) Arthritis and Immunology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA.

Split-hand/foot malformation with long bone deficiency (SHFLD) is a very rare severe limb deformity characterized by lack of part or entire tibia with or without split-hand/split-foot deformity. Identification of genetic susceptibility loci for SHFLD has yet remained elusive, due to its rare incidence, variable expression, and uncertain inheritance pattern. This heterogeneous phenotype is usually inherited as an autosomal dominant trait with reduced penetrance although recessive inheritance has been postulated. Genome-wide linkage analysis with the GeneChip Mapping EA 10K Array (Affymetrix) containing ~10,000 SNP markers identified a novel SHFLD locus in a large isolated, non-syndromic, multigenerational, consanguineous UAE national family (UR078) with apparent autosomal dominant inheritance. Multipoint non-parametric analysis indicated significant evidence of linkage for SNP marker rs966302 at chromosome 1q42.3 (NPL = 9.8, P= 0.000065). Multipoint parametric analysis showed a LOD score of 3.20 (= 0) at SNP marker rs950964, supporting linkage in this region. A parametric two-point MLS LOD of 1.95 was detected for SNP marker rs923976 under a dominant mode of inheritance and reduced penetrance. Haplotype analysis with informative crossovers enabled mapping of the SHFLD locus to a region of approximately 18.38cM (8.4Mb) between proximal SNP rs1124110 and distal SNP rs535043. Thus, we have identified a novel genomic region on 1q42.13-1q43 that harbors a high risk variant for SHFLD in this UAE family. To our knowledge, this is the first genome-wide significant linkage results ever reported for SHFLD.

Complex patterns of copy number variation at sites of segmental duplications: an important category of structural variation in the human genome. *H. Kehrer-Sawatzki[1], V. Goidts[1], D.N. Cooper[2], L. Armengol[3], W. Schempp[4], J. Conroy[5], X. Estivill[3], N. Nowak[5], H. Hameister[1]* 1) Human Genetics, University of Ulm, Ulm, Germany; 2) Institute of Medical Genetics, Cardiff University, Heath Park, Cardiff, United Kingdom; 3) Program in Genes and Disease, Center for Genomic Regulation, Barcelona Biomedical Research Park, Barcelona, Spain; 4) Institute of Human Genetics and Anthropology, University of Freiburg, Freiburg, Germany; 5) Department of Cancer Genetics, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, USA.

The structural diversity of the human genome is much higher than previously assumed. To investigate the association between segmental duplications that display constitutive copy number differences (CNDs) between humans and great apes and those which exhibit polymorphic copy number variations (CNVs) between humans, we performed aCGH. Our study documents for the first time that in addition to human-specific gains common to all humans, these duplication clusters also exhibit polymorphic CNVs >40kb. Segmental duplication is obviously an ongoing process during human genome evolution. Importantly, among the CNV-associated genes identified, those involved in transcriptional regulation were found to be significantly overrepresented. Complex patterns of variation were evident at sites of duplications, manifesting as interindividual differentially sized copy number alterations at the same genomic loci. Thus, CNVs associated with segmental duplications do not simply represent insertion/deletion polymorphisms, but rather constitute a wide variety of rearrangements that lead to gene gains and losses with high inter-individual variability. Although the number of CNVs did not differ between Africans and Caucasians/Asians, the average number of variant patterns per locus was significantly lower in Africans. Thus complex variation patterns result from recent genomic rearrangements. The high number of these rearrangements, some of which are probably recurrent, and differences in population size and expansion dynamics, may account for the greater diversity of variation in Caucasians/Asians as compared with Africans.

Evaluating HapMap data transferability in a Shanghai Chinese population. *C. Hu[1], W. Zhang[2], C. Wang[1], R. Zhang[1], Q. Fang[1], J. Lu[1], X. Ma[1], W. Jia[1], International Type 2 Diabetes 1q Consortium* 1) Shanghai Diabetes Institute, Shanghai Clinical Center for Diabetes, Shanghai Jiaotong University Affiliated Sixth People's Hospital, Shanghai, China; 2) Human Genetics Division, University of Southampton, Southampton, UK.

The HapMap project aimed to catalog millions of SNPs in the human genome, in order to facilitate association analysis between phenotypes and disease variants. In this study, we examined the transferability of CHB (Northern Chinese) HapMap data to the Han Chinese in Shanghai (CHS, Southern Chinese). Over 4,500 SNPs in a 21Mb region on 1q21-q25 were genotyped in 80 unrelated Shanghai Chinese subjects as part of the International Type 2 Diabetes 1q Consortium. After removal of SNPs that failed quality control and those not in the HapMap panel, 3,014 SNPs were analyzed. The allele frequencies were highly consistent between the CHB and CHS populations ($r = 0.94$, $P < 0.0001$) whereas they were less consistent between CHS and the other populations ($r = 0.88$ for JPT, 0.46 for CEU and 0.41 for YRI, all $P < 0.0001$). In order to compare linkage disequilibrium between populations, we estimated pairwise r^2 of adjacent SNPs. Higher correlation of r^2 between CHS and CHB ($r = 0.97$, $P < 0.0001$) than that between CHS and the other populations was observed ($r = 0.96$ for JPT, 0.80 for CEU, and 0.64 for YRI, all $P < 0.0001$). SNPs with a minor allele frequency over 0.05 were selected to evaluate the tagging SNP (tSNP) performance in CHS based on the pairwise algorithm of the Tagger program. An r^2 of 0.8 was defined as the threshold for tSNP selection. About 50% of SNPs were selected as tSNPs in the CHB, JPT and CEU populations, and about 75% of SNPs in the YRI population. We found that tSNPs from CHB and JPT were highly informative in the CHS population. Approximately 95% SNPs in CHS were captured by the tSNPs selected from CHB or JPT, whereas less than 90% SNPs were captured by the tSNPs from CEU. Although over 97% SNPs in CHS were captured by the tSNP set from YRI, about 1.5 times more tSNPs were needed. Overall, our study supports the portability of HapMap CHB data to the study of complex diseases in the Southern Chinese population.

Association study in search for genetic backgrounds of myocardial infarction, focused on SNPs in genes encoding molecules that belong to lymphotoxin- signaling cascade. *K. Ozaki[1], H. Sato[2], A. Iida[3], H. Mizuno[2], M. Hori[2], Y. Nakamura[3], T. Tanaka[1]* 1) Laboratory for Cardiovascular Diseases, RIKEN, Tokyo, Japan; 2) Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Suita, Japan; 3) Laboratory for Pharmacogenetics, RIKEN, Tokyo, Japan.

Myocardial infarction (MI) has been a principal cause of death in many countries and inflammation is now considered to play a critical role in its pathogenesis. We have previously found that the functional single nucleotide polymorphism (SNP) in the lymphotoxin- gene (LTA), encoding one of the cytokines produced in an earlier stage of vascular inflammatory processes, conferred susceptibility to MI. To further investigate whether the variants in genes which encode molecules of LTA signaling cascade are also associated with susceptibility to MI, we performed a case-control association study using tag SNPs, selected on the basis of the information in HapMap database as well as JSNP database, for genes of LTA signaling cascade. We found one SNP in one LTA related gene conferring risk of MI (2=21.1, P=0.0000044). This association was replicated with another panel of MI and control subjects. The SNP, located within 5-untranslated region of exon 1 in this gene, enhanced the transcriptional level of the LTA related gene. Moreover, suppression of the gene expression level using siRNA in cultured coronary vascular endothelial cells as well as T lymphocyte cell line reduced activation of NFkB, a central mediator of inflammation, by stabilizing phosphorylated IkB. Thus, the levels of this LTA related gene influence the degree of inflammation, indicating that this SNP is a novel genetic risk factor for MI.

Mutation analysis of genes in the RAS-MAPK pathway in 31 patients with Kabuki syndrome. *H. Kuniba[1,2,10], D. Sato[1,3,10], N. Miwa[1,10], N. Kurotaki[1,10], K. Yoshiura[1,10], T. Kondoh[2], T. Matsumoto[2], H. Tonoki[3], H. Ohashi[4], K. Kurosawa[5], T. Nagai[6,10], Y. Fukushima[7,10], N. Okamoto[8], K. Naritomi[9,10], N. Niikawa[1,10]* 1) Dept Hum Genet, Nagasaki Univ Grad Sch Biomed Sci, Nagasaki, Japan; 2) Dept Pediatr, Nagasaki Univ Hosp, Nagasaki; 3) Dept Pediatr, Hokkaido Univ Grad Sch Med, Sapporo; 4) Div Med Genet, Saitama Child Med Ctr, Iwatsuki; 5) Clin Res Inst, Kanagawa Child Med Ctr, Yokohama; 6) Dept Pediatr, Dokkyo Univ Sch Med Koshigaya Hosp, Koshigaya; 7) Dept Med Genet, Shinshu Univ Sch Med, Matsumoto; 8) Dept Planning and Res, Osaka Med Ctr Res Inst for Maternal Child Health, Osaka; 9) Dept Med Genet, Univ Ryukyus Sch Med, Nishihara, Japan; 10) SORST, JST, Kawaguchi, Japan.

Kabuki (Niikawa-Kuroki) syndrome (KS) is a multiple congenital anomaly (MCA)-mental retardation (MR) syndrome characterized by long palpebral fissures with eversion of the lower eyelids, skeletal anomalies, fingertip pads, short stature, and occasional immune abnormalities. Molecular cytogenetic approaches including genome CGH have failed to reveal the etiology. Recently, germline mutations in genes of the RAS-MAPK pathway were shown to be causes of MCA/MR syndromes, i.e., *PTPN11* in Noonan syndrome; *HRAS* in Costello syndrome; *KRAS*, *BRAF*, *MEK1* and *MEK2* in Cardio-facio-cutaneous syndrome. Because of some similar facial features, we hypothesized that KS may be associated with germline mutations in the RAS-MAPK pathway.

Ten selected candidate genes (*RAF1, ARAF, BRAF, ERAS, NRAS, HRAS, KRAS2, GRB2, PTPN11* and *SOS1*) in the pathway were screened for mutations. DNA was extracted from 31 KS patients (15 females, 16 males), and their entire coding regions and splice junctions were sequenced. Consequently, we did not detect mutations in any of the genes and any patients analyzed. Although our results do not totally rule out their role in KS, candidate gene approaches should be tried, since there has been no clue to identify the putative gene causative for KS, especially those involved in signal transduction in an immune system.

Comparison of the mapping coverage provided by model-free and model-based tagging SNP definitions. *M. Nothnagel[1], A. Wollstein[2], M. Krawczak[1]* 1) Institute for Medicical Informatics and Statistics, University of Kiel, Kiel, Germany; 2) Humboldt University, Institute of Informatics, Berlin, Germany.

Genome-wide association studies are regarded as a major tool for fine-mapping the genetic causes of common diseases. Exploiting information redundancy due to associations between single nucleotide polymorphism (SNP) markers potentially reduces the efforts in terms of time and cost for those studies. Initial methods sought to define haplotype blocks and therein SNPs that distinguish between block haplotypes but this approach is unreliable due to the questionable validity of the block concept. Block-free and model-free methods that consider pairwise linkage disequilibrium (LD) to define tagging SNPs are now a standard tool in association studies. A block-free but model-based method has recently been suggested which additionally takes marker information content and marker distance into account for the selection of tagging SNPs. Using the International HapMap data, we assess the ability of both approaches to provide sufficient levels of LD for mapping hidden causal genetic variation in association studies on a chromosomal scale. We demonstrate that both approaches have similar mapping coverage in high-density marker sets but capture partially different sets of genetic variants. We also show that a considerable loss of information results from use of tagging SNPs with either method compared to the use of all available markers.

Genetic testing in autism: How much is enough? *G.E. Herman, A. Sommer, B. Enrile, M. Pastore, S. Fitzgerald, J. Atkin, K.L. McBride* Children's Research Institute and Dept. of Pediatrics, The Ohio State University, Columbus, OH.

79 unrelated patients referred with a provisional diagnosis of an autism spectrum disorder (ASD) were evaluated between 1/105 - 3/706 in our developmental and genetics clinics. 8 patients were excluded because they did not meet criteria for diagnosis. The remaining 71 patients included 57 males and 14 females (ratio 4.1:1) with an age range of 19 months - 15 years. Macrocephaly (HC95%;) was present in 18 (25%). 28 had a formal psychological evaluation. 50 met DSMIV criteria for autism, with the rest being Asperger or PDD-NOS. There were 5 nuclear and 5 extended families with more than a single individual affected with an ASD, 5 extended families with significant psychiatric disease and 1 with both, by history (23% total). The diagnostic yield of testing was 11% (8/71). Two children had visible chromosome abnormalities - one with 47XYY and one with several small marker chromosomes. 2 patients (ages 2 and 4, respectively) with classic autism and significant macrocephaly had different de novo heterozygous mutations in PTEN. One male with autism was noted to have a submicroscopic duplication, detected by DNA CHIP, but not regular chromosome analysis. The rearrangement was noted in an interstitial region on chromosome 1 that is not associated with a defined genetic syndrome. One parent carried the identical genomic rearrangement. Thus, the relevance of the finding to the etiology of autism in the child remains unclear. Finally, 3 females had Rett syndrome, with DNA sequencing of the MECP2 gene confirming the diagnosis in each. Interestingly, metabolic screening (plasma amino acids, urine organic acids, total homosyteine, plasma/urine guanidinoacetate, sterol profiles, uric acid) produced no positive testing results, nor did Fragile X DNA testing. PTEN gene sequencing should definitely be performed in any autistic or delayed child with significant macrocephaly. Further, careful pedigree analysis is likely to reveal a higher frequency of families with psychiatric disease and/or extended realtives with an ASD. Metabolic screening may not be warranted without more specific indications or additional findings.

Neoplasms in Myotonic Dystrophy. *C. Mueller[1], R.T. Moxley[2], J. Hilbert[2], M.H. Greene[1]* 1) Clinical Genetics Branch, DCEG, NCI, NIH, Bethesda, MD; 2) Department of Neurology, Department of Neurology, University of Rochester Medical Center, Rochester, NY.

Myotonic dystrophy type 1 (DM1) is the most common form of muscular dystrophy in adults, affecting about 1 in 8,000 individuals. In addition to muscular weakness, atrophy and myotonia, there are a myriad of clinical features including cardiac conduction defects, respiratory insufficiency, cataracts, diabetes, reduced immunoglobulins, frontal balding, testicular atrophy, and varying degrees of mental impairment. DM1 is the result of an unstable trinucleotide (CTG) expansion in the 3 untranslated region of the *dystrophia myotonic-protein kinase (DMPK)* gene, located on chromosome 19q13.3. In reflecting on our clinical experience, we have wondered whether there might be an increased incidence of tumors in patients with DM1. There have been multiple case reports of DM1 and pilomatrixoma, a benign calcifying cutaneous tumor thought to be derived from hair matrix cells. We present a comprehensive literature review of reported pilomatrixomas and various other neoplasms that have been observed in patients with DM1. We also present our initial analysis of tumors reported by DM1 patients enrolled in the NIH sponsored National Registry of Myotonic Dystrophy and Facioscapulohumeral Muscular Dystrophy Patients and Family Members. Pilomatrixomas emerged as the strongest candidate for a genuine molecular relationship to DM1, which gained support from the occurrence of pilomatrixomas in patients with Rubinstein-Taybi syndrome and Gardner syndrome, both of which are associated with the development of various neoplasms. The other most common tumor types implicated, based on our findings include thymoma, adenomas of the parotid, pituitary, parathyroid, pancreas and thyroid glands, as well as multiple basal cell carcinomas. This constellation of tumors also led us to our hypothesis that dysregulation of the Wnt/-catenin signaling pathway may be the molecular basis for this relationship, as it has been implicated in the development of pilomatrixomas in the disorders under consideration, in sporadic pilomatrixomas, and in a number of the other neoplasms that have been reported in DM1 patients.

Comprehensive genetic analysis of relevant four genes in 49 patients with Marfan syndrome or Marfan related phenotypes. *N. Matsumoto[1,2], H. Sakai[1], A. Nishimura[1], T. Mizuguchi[1]* 1) Dept Human Genetics, Yokohama City Univ Grad Sch Med, Yokohama, Japan; 2) Solution-Oriented Research for Science and Technology, JST, Kawaguchi, Japan.

In order to evaluate the contribution of FBN1, FBN2, TGFBR1, and TGFBR2 mutations to the Marfan syndrome (MFS) phenotype, the four genes were analyzed by direct sequencing in 49 patients with MFS or suspected MFS as a cohort study. A total of 27 FBN1 mutations (22 novel) in 27 patients (55%, 27/49), one novel TGFBR1 mutation in one (2%, 1/49) and two recurrent TGFBR2 mutations in two (4%, 2/49) were identified. No FBN2mutation was found. Three patients with either TGFBR1 or TGFBR2 abnormality did not fulfill the Ghent criteria, but expressed some overlapping features of MFS and Loeys-Dietz syndrome (LDS). In the remaining 19 patients, either of the genes did not show any abnormalities. This study indicated that FBN1 mutations were predominant in MFS but TGFBRs defects may account for approximate 5-10% of patients with the syndrome.

Mosaic status of lymphocytes in Klinefelter syndrome (KFS). *M. Tanwar[1], R. Prabu[1], R. Kumar[2], N. Gupta[2], K. Kucheria[1], R. Dada[1]* 1) Anatomy, AIIMS, N Delhi, India; 2) Urology Department, AIIMS, N Delhi.

KFS is the commonest genetic cause of Infertility. These cases present with hypogonadism and are generally believed to be azoospermic. Recently few studies have shown that Klinefelter cases have 46, XY cell line and in such cases there may be isolated foci of spermatogenesis. Such cases have better prognosis on assisted reproductive techniques (ART) [If it is followed by preimplantation genetic diagnosis]. So we tried to look for low-level mosaicism in ten infertile Klinefelter patients who did not show features of hypogonadism to identify for presence of normal cell line which could not be identifyied on analyzing twenty metaphases. In these cases we analyzed 150 well spread G banded metaphases in each case and found that two cases showed 4.5% 46, XY cell line.This had not been identified on analyzing 2o metaphase spreads. Klinefelter syndrome shows a variation in phenotype and thus diagnosis is often delayed or patients remain undiagnosed. Early diagnosis by karyotyping allows initiation of testosterone substitution therapy to avoid symptoms of androgen deficiency. This may allow future fertility to be preserved for young Klinefelter patients. The main problem with conventional karyotyping is that routinely 20 metaphases are analyzed and counted, this may miss low-grade mosaicism. Such cases may have a normal 46, XY cell line and may have foci of spermatogenesis, which could be used for TESA in ART. Therefore analyzing a large number of metaphases in such cases with no features of hypogonadism may detect low percentage of 46, XY cell line and such cases carry a good prognosis on ART. Recent techniques like FISH (florescence in situ hybridization) are rapid and sensitive and can help in analyzing a large number of metaphases and interphases cells in short time and can detect cryptic and low level mosacism, which may have been missed cytogenetically. In conclusion, infertile Klinefelter patients with no features of are likely to have low-grade mosaicism with 46,XY normal cell line may have successive sperm retrivel even if such cases are azoospermic.

Insertion/Deletion Polymorphisms in Rajasthan Tribal Populations and Rajputs. *S. Kamboj[1], R. Dada[1], S. Bhardwaj[2], K. Kucheria[1]* 1) Anatomy, AIIMS, N Delhi, India; 2) Dept of Zoology, Banswara, Rajathan.

In recent times, polymorphic DNA markers are widely used to study the genomic diversity of Indian populations as most are selectively neutral, more ubiquitous and have higher heterozygosities than polymorphic protein and enzyme markers. As new alleles are not generated at Alu insertion/deletion loci, and as there is no identified selection pressure on these loci, these loci have gained importance in the study of genetic structures of human populations. In the present study six human - specific insertion/deletion polymorphisms were studied in five endogamous tribal populations, namely Minas, Garasiyas, Damariyas, Sahariyas and Bhils of Banswara and Rajputs of Rajasthan. DNA samples from 222 unrelated individuals were analysed. Of these polymorphic markers five are Alu insertion/deletion markers (Alu PV 92, Alu FX III B, Alu D1, Alu APO, Alu ACE and Alu-CD4), while the sixth marker (mt NUC) pertains to a mitochondrial DNA segment, 540 bp in length, which got inserted into human nuclear genome. The results of this study show that all loci are polymorphic in all three populations. Most of the loci showed high levels of heterozygosity in all three populations. Genetic data allow researchers to determine the relatedness of different racial and ethnic groups and to arrange them in an evolutionary or phylogenetic tree. Special statistical packages will be used for statistical analysis of the data.

A comparison of linear and logistic regression-based methods for disease gene identification. *E.D. Kelly* Hitachi Dublin Laboratory, Hitachi Europe Limited, Dublin 2, Ireland.

Simple tests which seek to identify associations between drug response, disease status and individual genetic polymorphisms have been in use for some time. Recently the focus has shifted to considering extended haplotypes, rather than single loci. The attraction of these more complex methods is in the subsequent increased power of the tests, coupled with the belief that the underlying nature of common diseases and drug responses may only be discovered by considering more complex interactions. Regression-based methods have proved effective and powerful tools for the identification of such associations. Fundamental to the regression-based process is the selection of a suitable model. Clinical data commonly feature a binary response, which mathematically lends itself to the logistic regression model. Logistic regression modelling, however, can pose insurmountable computational problems given certain data, and thus linear models have also been investigated as an alternative. We have developed both logistic and linear regression models which are capable of handling long-chain haplotypes and several non-genetic covariates simultaneously. Here we present the results of a comparison between the two models for a variety of data sets. For each data set and each regression model we perform a number of tests. Initially we test the overall null hypothesis that no independent variable (haplotype or non-genetic covariate) is associated with the response. If the resultant P-value proves significant, then each individual independent variable is tested in turn. For the overall P-value we see strong matching between the two sets of results. For the individual covariates the matching is less marked, though qualitatively similar. This suggests that, given the computational difficulties of increased burden and possible insolubility inherent in logistic regression, linear regression may be the preferred initial option for both quantitative and binary response data. If the overall P-value proves significant, it may then be appropriate to consider, for the binary response, the logistic model for the analysis of the individual independent variables.

Identification of distinct QTLs linked to length or weight variability in humans. *S. Heath*[1], *D. Fradin*[2], *J. Lepercq*[3], *M. Lathrop*[4], *P. Bougnères*[5] 1) Dept Epidem & Biostatistics, Centre National de Génotypage, Evry, France; 2) INSERM U561, Paris, France; 3) EA University Paris V and Department of Gynecology Obstetrics, Paris, France; 4) Centre National de Génotypage, Evry, France; 5) INSERM U561 and Department of Pediatric Endocrinology, Paris, France.

Birth length, as well as birth weight, follows a continuous distribution in a given human population, with macrosomia and smallness for gestational age at the two edges of this distribution. Fetal growth is a complex, dynamic, multifactorial process controlled by maternal, placental and fetal factors including interactions between the fetus and intrauterine environment. To approach the genetic factors implicated in the normal variation of birth length and of birth weight, we conducted a genome-wide approach of these two quantitative traits in 220 French pedigrees (412 sib-pairs) using a variance components method. While birth length and weight showed a powerful phenotypic interaction ($r=0.76$, $p<0.0001$), their genetic variability appeared dependent on distinct QTLs. Birth length was linked to two suggestive QTLs on chromosome 2, which encompassed 6 and 11 consecutive markers on 2q and 2p respectively. The first peak spans ~10cM, with a MLS of 2.69 ($p=2.10^{-4}$). The second peak spans ~12cM, with a MLS of 3.57 ($p=2.10^{-5}$). Ten peaks with LOD scores between 1.0 and 2.2 were seen on chromosomes 7q36, 8p23-p21, 10q21, 13q11 and 17q25. Birth weight variability was linked to a QTL on 7q35 (LOD=3.1, $p=8.10^{-5}$). Several other regions showed peaks with a nominal LOD score for birth weight: twelve peaks with LOD scores between 1.0 and 2.2 were found on chromosome 1p36-p35, 1q42-1q44, 2qter, 6q22-q24, 7p21-p11, 8p22, 10p12-p11, 13q31-q33, 14q11, 15q21, 17q24 and 20p13. These QTLs provide a first step towards the identification of the genomic variants involved in the variability of fetal growth in humans. Because of the risk of false positivity, our results should however be considered preliminary until they are replicated in other studies.

Loss of Sprouty gene function leads to dental anomalies by modulating FGF signaling. *O. Klein[1], G. Minowada[2], R. Peterkova[4], H. Lesot[5], G. Balooch[3], J. Jernvall[6], G. Martin[2]* 1) Pediatrics; 2) Anatomy; 3) Biomaterials, Univ California, San Francisco, San Francisco, CA; 4) Institute of Experimental Medicine, Prague, Czech Republic; 5) INSERM, Strasbourg, France; 6) Institute of Biotechnology, University of Helsinki, Finland.

There is growing evidence that activating mutations in the FGF signaling pathway cause a variety of disorders, such as Apert, Noonan, Costello, and cardiofaciocutaneous syndromes. Dental and craniofacial anomalies are central features of several of these conditions. We have modeled gain-of-function of FGF signaling in mice by inactivating members of the Sprouty family. This gene family encodes intracellular proteins that antagonize FGF signaling downstream of the receptor. Our studies in mice show that two Sprouty family member, *Spry2* and *Spry4*, are essential for the formation of the normal number of teeth. In mice, a toothless gap known as a diastema is present between the incisor and molar regions. During embryogenesis, tooth primordia form in the diastema but cease developing and undergo apoptosis. In both *Spry2* and *Spry4* null embryos, a diastema bud develops into a supernumerary tooth just anterior to the first molar, and these two genes function in different tissues: *Spry2* is predominantly expressed in the epithelium and *Spry4* in the mesenchyme. The formation of supernumerary teeth in *Spry2* null mice can be prevented by reducing the dosage of FGF signaling pathway components, indicating that *Spry2* normally reduces the sensitivity of the diastema bud epithelium to FGF signaling. We propose that Sprouty genes modulate two discrete parts of an epithelial-mesenchymal FGF signaling loop in diastema tooth development. Additionally, we demonstrate that when *Spry2* and *Spry4* are inactivated together, dramatic effects on incisor development are observed. These include ectopic deposition of enamel as a result of abnormalities in regulation of ameloblast stem cells. Thus, Sprouty genes are critical regulators of mammalian tooth development. Given the importance of gain-of-function of FGF signaling in human syndromes, it is possible that loss-of-function of FGF antagonists such as Sprouty contribute to human disorders as well.

Constitutional *BCR* gene deletion; a possible new microdeletion syndrome. *F.M. Mikhail[1], M. Descartes[1], A. Piotrowski[1,2], R. Andersson[3], T. Diaz de Stahl[2], J. Komorowski[3], C. Bruder[1,2], J.P. Dumanski[1,2], A.J. Carroll[1]* 1) Dept. of Genetics, Univ. of Alabama at Birmingham, USA; 2) Dept. of Genetics and Pathology, Uppsala Univ., Sweden; 3) Linnaeus Centre for Bioinformatics, Uppsala Univ., Sweden.

We report a 15-year old boy with history of learning and behavior problems. He was diagnosed with ADHD. Developmental milestones were within normal limits, but performance on the VMI test yielded an age equivalent of 8.4 years. Physical examination showed normal growth with slight facial dysmorphism, including short and narrow forehead, flared eyebrows, up-slanting palpebral fissures, cupped ears with small lobules, short philtrum and short neck. HRB chromosome analysis revealed a normal male karyotype. FISH analysis using the subtelomere probe panel showed normal subtelomeric regions, but TelVysion mixture no. 3 containing the 22q subtelomere probe showed an interstitial deletion of the LSI *BCR* control probe (22q11.2). These results were confirmed using the LSI *BCR/ABL* single fusion translocation probe set. In order to determine if the DiGeorge critical region was included in the deletion, we used the *Tuple1* probe (22q11.2), which showed a normal hybridization signal on both chromosomes 22. Using a 32K BAC array CGH chip, we were able to narrow the proximal and distal breakpoints of the deletion. This interstitial microdeletion was estimated to be 1.4-1.7 Mb in size and to span at least ten genes. Both parents proved to be normal cytogenetically and by array CGH. The patients final karyotype was: 46,XY.ish del(22)(q11.2q11.2)(*BCR*-)dn. This microdeletion region is flanked by LCRs containing several modules with a high degree of homology, and therefore could be implicated in its origin. Interestingly, eight patients with the same interstitial *BCR* deletion have been reported in the literature. Common features reported included developmental delay and hypotonia. Our current case as well as the previously reported cases may represent a new microdeletion syndrome on chromosome 22. Further characterization of the microdeletion boundaries as well as comparison of the phenotype of these patients is currently in progress.

Loss of Heterozygosity in Normal and Pre-neoplastic Lung Tissue from High-Risk Patients. *K.L. Meadows[1], D.M. Ducharme[1], C. Markunas[1], R.J. Slebos[2], M.P. Rivera[3], G.P. Flake[1], P. Stockton[1], J.A. Taylor[1,4]* 1) LMC, NIEHS, RTP, NC; 2) Vanderbilt University, Nashville, TN; 3) UNC Hospitals, Chapel Hill, NC; 4) Epidemiology, NIEHS, RTP, NC.

Lung cancer is the leading cause of all cancer-related deaths. To date, there are no effective, early screening tools to identify individuals at high-risk for developing lung cancer. Loss of heterozygosity (LOH) is a common genetic alteration involved in tumorigenesis. In relation to lung cancer, most LOH analyses have examined only cancer cells. As a result, the timing of early LOH events and the possible correlation of these molecular changes to environmental exposure and neoplastic transformation in the lung have not been well characterized. We established a prospective study to assess LOH in individuals lesions that were serially biopsied over time in a group of people at high-risk for developing lung cancer. During each bronchoscopy, lung tissue biopsies were obtained at any areas of abnormality and four standard sites to obtain normal tissue. Follow-up bronchoscopies were scheduled according to the severity of the histology report. In total, 47 patients enrolled in the study, 106 bronchoscopies were performed and over 400 biopsies collected. We have initiated a comprehensive, genome-wide LOH analysis using the Affymetrix 100K SNP chip to determine if there are specific molecular LOH profiles that may be used to: 1) identify those lesions most likely to histologically progress, 2) identify patients at the highest risk of lung cancer and 3) identify patients who have the highest level of chromosome damage following environmental exposure. Preliminary evidence suggests the presence of LOH in the respiratory epithelium of high-risk individuals without lung cancer. The present study provides a rare resource for the detection of early LOH patterns in individuals at high-risk for developing lung cancer. Accordingly, if histological progression and exposure correlate with specific LOH profiles, then LOH may potentially serve as an effective, early screening tool to identify and monitor those persons at highest risk for this disease.

Gene-gene interaction methods for family-based case-control data. *D.B. Hancock, E.R. Martin, Y.J. Li, W.K. Scott*
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Gene-gene interactions are often assessed in familial case-control data, wherein parent ascertainment is difficult for late-onset diseases. Conditional logistic regression (CLR) and generalized estimating equations (GEE) both test for interactions in families with missing parents but adjust for relatives correlations differently to maintain nominal significance. To compare these methods, we used the SIMulation of Linkage and Association program to create datasets of 700 singleton (affected proband), 200 nuclear multiplex (affected sibpair), and 100 extended multiplex (affected cousin pair) pedigrees with sibship sizes of 3 and with no grandparental/parental genotype data. We created 2 disease loci (D_1,D_2) with relative risks (RRs) for main genetic effects of 3 or 1.5 and joint effects of 10 or 3, respectively. We tested 4 scenarios with 5 biallelic markers: linkage but no association (M_1), linkage and association [M_2 in perfect linkage disequilibrium (LD) with D_1 and M_5 in perfect LD with D_2], association but no linkage (M_2), and no linkage or association (M_4). Interactions between M_5 and each M_1-M_4 marker were tested. We built interaction models in SAS program packages and estimated type I error, power, and average odds ratios (ORs) for CLR and GEE across 1000 replicates. The simulations reflect case-control data, so ORs were used to estimate RRs. Both methods were valid tests of association in the presence of linkage. GEE revealed inflated type I error as a test of linkage in the presence of association (23% for RR=10) but offered more power to detect interaction (100% v. 79% for RR=10, 53% v. 33% for RR=3). GEE also offered more power in datasets of reduced size, imperfect LD, and singleton pedigrees only. With large interaction, CLR produced inflated ORs for interaction (11.9-17.4), while GEE produced underestimated ORs (2.0-4.7). With modest interaction, CLR provided more accurate ORs (2.4-2.8); GEE again underestimated effects (1.3-1.8). These data show that GEE achieves more power to detect gene-gene interactions in families with no parents but appears less robust to cases of association but no linkage and underestimates risk as compared to CLR.

A novel mutation at N-terminal of SMN Tudor domain inhibits its interaction with target proteins. *H. Nishio[1], M.J. Lee[1], T. Kotani[1], R. Sutomo[1], A.H. Sadewa[1], T.H. Sasongko[1], Gunadi[1], M. Matsuo[2]* 1) Department of Public Health, Kobe University Graduate School of Medicine, Kobe-City, Hyogo, Japan; 2) Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe-City, Hyogo, Japan.

More than 95% of patients with spinal muscular atrophy (SMA) are homozygous for deletion of the SMN1 gene. However, a small number of SMA patients bear one SMN1 copy with a subtle mutation within the gene. Such intragenic mutations may be helpful, not only in confirming the diagnosis, but also in elucidating the structural and functional domains of the survival motor neuron (SMN) protein. In this study, we describe a novel mutation identified in two Japanese patients who were clinically diagnosed as having SMA. Combination of denaturing high performance chromatography and sequence analysis using genomic DNA and cDNA showed that they harbored a novel intragenic point mutation in SMN1 exon 3, 275G>C, leading to tryptophan-to-serine substitution at amino acid 92 (W92S) at the N-terminal of the SMN Tudor domain. To test whether the W92S mutation destroys the function of SMN, we studied the in vitro interaction of the Tudor domain with SmB protein and fibrillarin. The mutated Tudor domain showed reduced interaction with these proteins, suggesting that the W92S mutation was responsible for the development of the disease. In conclusion, we report that a mutation at the N-terminal of the Tudor domain affects the interaction of SMN with the target proteins.

Robertsonian translocation and Recurrent ICSI failure. *M. Jena[1], R. Kumar[2], N. Gupta[2], R. Prabu[1], K. Kucheria[1], R. Dada[1]* 1) Anatomy, AIIMS, N Delhi, India; 2) Urology,AIIMS, N Delhi, India.

Chromosomal anomalies have been postulated to be one of the principal genetic factors in reproductive failure. Robertsonian translocation is the most common structural rearrangement of human chromosomes occurring in 1% infertile men. Assisted reproductive techniques have served as a boon for infertile males with severe oligozoospermia. However in the present study we found that of 184 infertile men, 4 men had robertsonian translocation between D/G group. The most common treanslocation was t (13;14) whixch was seen in three cases while the fourth case had t (13;13). All these men were oligozoospermic and had recurrent ART/ICSI failure. The wives of these were cytogenetically normal and had no gynaecological or obstretic abnormality. It has been reported that chromosomal rearrangements, particularly robertsonian translocation, could disturb meiotic disjunction of chromosomes not involved in the rearrangements resulting in a predisposition towards trisomy. During meiosis, the robertsonian translocation chromosomes & the two normal homologue synapse as a trivalent. Alternate segregation produces normal or balanced gametes, while adjacent segregation produces two disomic & two nullisomic gametes. These aneuploid gametes result in difficulty in fertilization, poor blastocyst development and implantation failure. This results in severe emotional , physical and financial stress to the couples.These results highlight the need for cytogenetic investigation in the infertile men who opting for ART, as this will help in providing most adapted therapeutics and counseling to such men.

Identification of a unique Alu-based polymorphic locus and its use in human population studies. *D. Kass, N. Jamison, M. Mayberry, E. Tecle* Department of Biology, Eastern Michigan Univ, Ypsilanti, MI.

Alu elements comprise a retrotransposon family of short interspersed DNA elements (SINEs) found in primate genomes. Recent integrations of Alu elements within the human genome have generated presence/absence variants useful as DNA markers in human population studies as well as in forensic and paternity analyses. Besides the ease of use, this type of marker is unique because the absence of the Alu element represents the ancestral form. We have identified a novel Alu-based polymorphic locus that consists of four alleles in which we can predict their evolutionary order. Additionally, we have developed a simple and rapid assay for genotyping individuals for this locus. Thus far, we have analyzed DNA samples comprising four different ethnic groups with at least seventeen samples per group. We have observed variability that was not originally found by simply analyzing the presence/absence forms of this marker. Among the results, all four alleles were common in the African American population, whereas one allele was not found in the Asian population and a different allele was absent in both the European Caucasians and South Americans. The distinctive features of this marker in conjunction with the use of a simple assay system, as well as the initial findings, afford this marker as a unique tool in the study of both global and regional analyses of human populations.

RET proto-oncogene genotyping using unlabeled probes, the masking technique and amplicon high-resolution melting analysis. *R.L. Margraf[1], R. Mao[1,2], W.E. Highsmith[3], L.M. Holtegaard[3], C.T. Wittwer[1,2]* 1) ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT; 2) Department of Pathology, University of Utah Medical School, Salt Lake City, UT; 3) Molecular Genetics Laboratory, Mayo Clinic, Rochester, MN.

Single base pair mutations in the *RET* proto-oncogene can cause multiple endocrine neoplasia type 2 syndromes. The conventional approach for genotyping *RET* mutations is sequencing the exons. A closed-tube *RET* proto-oncogene genotyping assay using a saturating DNA dye, unlabeled probes, and amplicon high-resolution melting analysis was developed. The method required two sequential PCR stages, a primary and secondary assay. The primary assay analyzed *RET* exons 10, 11, 13, 14 and 16 with a total of seven reactions using eight unlabeled probes. The primary assay genotyped wild type exons, a common exon 13 polymorphism and an exon 16 mutation, while other *RET* sequence variation was detected. The primary unlabeled probe data limited the possible genotypes for the detected *RET* sequence variation, which permitted genotyping in a secondary assay with only two to five reactions. Six probes were designed with the masking technique and masked selected sequence variations to allow unambiguous analysis of other mutations elsewhere under the probe. After this two-stage *RET* assay, less than 0.2% of exons tested would require sequencing for genotype. A blinded study generated from 5 wild type and 29 available *RET* sequence variation samples was 100% concordant with sequencing. Amplicon high-resolution melting analysis with unlabeled probes and the masking technique is a fast, accurate method for genotyping the >50 *RET* sequence variations.

Heredity of endothelin secretion: Human twin studies reveal the influence of polymorphism at the chromogranin A locus, a novel determinant of endothelial function. *E.O. Lillie, M. Mahata, S. Khandrika, F. Rao, R.A. Bundey, G. Wen, L. Taupenot, B.K. Rana, D.W. Smith, S.K. Mahata, M.G. Ziegler, N.J. Schork, D.T. O'Connor* University of California San Diego, La Jolla, CA.

Background. Endothelial dysfunction predisposes to vascular injury in association with hypertension. Endothelin (ET-1) is a potent vasoactive peptide that is synthesized and released by the vascular endothelium and is a marker of endothelial function. Chromogranin A (CHGA) regulates the storage and release of catecholamines, and may also have direct actions on the microvasculature. CHGA is a candidate gene for intermediate phenotypes that contribute to hypertension, and shows a pattern of SNP variations that alter the expression and function of this gene, both in vivo and in vitro. **Methods and Results.** In a study of twins (N=239 pairs), plasma ET-1 was 58+/-5% ($p<0.0001$) heritable. Plasma ET-1 was both correlated and associated with chromogranin fragment levels, and the two were influenced by shared genetic determination (rhoG=0.318+/-0.105,p=0.0032). We therefore hypothesized that variation in the CHGA gene may influence ET-1 secretion. Carriers of the CHGA promoter -988 G, -462 A, and -89 A alleles showed significantly higher mean plasma ET-1 than their major allele homozygote counterparts (p=0.02, 0.006, 0.03 respectively). Analysis of a linkage disequilibrium block that spans these 3 SNPs showed a significant association between the GATACA haplotype and plasma ET-1 (p=0.0075). In cultured human umbilical vein endothelial cells (HUVEC), CHGA caused dose-dependent secretion of ET-1. **Conclusions.** These results suggest that common, heritable variation in expression of the human CHGA gene influences endothelial ET-1 secretion in vivo, explained by a CHGA stimulus/ET-1 secretion coupling in endothelial cells in vitro. The findings document a previously unsuspected interaction between the sympathochromaffin system and the endothelium, and suggest novel genetic and cell biological approaches to the prediction, diagnosis, and mechanism of endothelial dysfunction in human disease.

Biological evidence of skeletal dysplasia in ancient Egypt. *C. Kozma* Department of Pediatrics , Georgetown University Hospital, Washington , DC.

The ancient Egyptian civilization originated along the banks of the Nile River spanning three thousand years before the Christian era. Through the monuments and records left by ancient Egyptians, we are well informed about many aspects of their culture including the existence of skeletal dysplasia and in particular achondroplasia. The artistic documentation of dwarfs is plentiful including hundreds of objects. Dwarfs gained a sacred status and two key gods, Bes and Ptah, were dwarfs. The hot and dry climate allowed for excellent preservation of bodies and as a result, Egypt has several partial and complete skeletons of dwarfs. Some skeletal remains are catalogued and published in the medical and archeological literature and some are known from excavators account. There was a burial area near the great Pyramids for high-ranking dwarfs. A skeleton of a female dwarf from the Old Kingdom (2686-2190 BCE) was found with a babys remains in situ. It is believed that she died during delivery. The Badarian skeleton, which dates back to 4500 BCE, is an example of short limbed dwarfism possibly representing an epiphyseal disorder. The remains of dwarfs from ancient Egypt are also present in British museums. They are in the Natural History Museum in London, in Cambridge University, and in the Institute of Archeology. The British Museum has a complete skeleton of a child who was affected with osteogenesis imperfecta. Furthermore, the Natural History Museum has a partial skeleton with osteogenesis imperfecta and a skeleton possibly affected with mucopolysaccaridosis. The ancient Egyptians followed a strict moral conduct as documented in their Wisdom Teaching. Amenemope, a wise man who lived in 1391-1354 BCE said: Beware of stealing from a miserable man, and of raging against the cripple. Do not stretch out your hand to touch an old man, Nor snip at the words of an elder. He moreover recommended respect and tolerance for individuals with disabilities: Do not jeer at a blind man nor tease a dwarf, Neither interfere with the condition of a cripple. Do not taunt a man who is in the hand of God, Nor scowl at him if he errs.

Expression Analysis of Mouse Atherosclerotic Aorta Revealed Significant Dysregulation of Genes in the Calcium Signaling Pathway. *S.K. Mak[1], B. Teng[2], L.C. Shimmin[1], J.E. Hixson[1]* 1) HMG,UT-Houston,Health Sciences Center, Houston, TX; 2) IMM,UT-Houston, Health Sciences Center, Houston, TX.

Atherosclerosis susceptibility results from the interaction of several genetic and environmental risk factors. Our lab generated a LDLR-/-/Apobec1-/-atherosclerotic mouse model(LDb)which had elevated plasma LDL cholesterol profile as in human disease. Aortic lesions of LDb mice at 2-and 8-month old fed on a chow diet had a 0.20% and 20.6% lesion, respectively and a 0.20% and 29.7% when fed on a high fat diet. Histological analysis showed that these lesions were predominantly clustered in the aortic arch where disturbed shear stress(DSS)was implicated. In this study we use a global gene expression approach to identify regulatory pathways that are significantly impacted by the interaction of age,diet,DSS and strain. Mice(n=32)were fed either chow or high fat diet and sacrificed at age 2-and 8-months. RNA was isolated from aortic DSS and non-DSS regions. Using 64 murine Affymetrix chips with 45,000 genes, we profiled differentially expressed genes that have p-value of 0.01,fold change 1.5, FDR 0.05, and analyzed the data by the t-test and ANOVA. Significant genes were subjected to pathway analysis by Pathway Express to assess the significance of differentially regulated pathways in atherosclerosis. This Pathway analysis identified calcium signaling as the leading pathway in which a significant proportion of the genes are differentially regulated in atherosclerosis. Out of 180 genes in the KEGG calcium signaling pathway, we identified 55 genes that are differentially regulated. The expression fold changes were confirmed by real time PCR. In addition,8-month old LDb aortic sinus stained with Alazarin Red indicated positive calcified lesions. Functional annotation further revealed that many of these genes have been implicated in cardiovascular disease. Our study demonstrates that calcium signaling pathway are significantly altered in atherosclerosis. However, further biological and clinical study of calcium signaling genes is needed to assess its role in cardiovascular disease. Clinically, this pathway may serve as an important target for future therapeutic intervention.

Analysis of Segmental DNA Changes in Cancer using GSP-Array7700. *Y. Murayama[1], S. Ozawa[2], S. Asakawa[1], Y. Saikawa[2], H. Hasegawa[2], H. Jinno[2], K. Aiura[2], A. Takayanagi[1], M. Maekawa[3], M. Kitajima[2], N. Shimizu[1]* 1) Dept. Mol. Biol., 144-8 Ogura Saiwai-ku, Kawasaki, Kanagawa, Japan; 2) Keio University School of Medicine, Department of Surgery, Tokyo, Japan; 3) GSP Lab. Inc., Japan.

BAC microarray-based comparative genome hybridization (CGH) provides a highly efficient method to detect deletion or amplification of specific DNA segments in the genomes at individual chromosome basis. We constructed human BAC library (Keio BAC library) in 1994 and since then we have utilized it for the genomic DNA sequencing of human genome. To develop a high resolution BAC microarray, we read the end-sequences of 10,000 BAC clones, mapped them on the human chromosomes using the updated genome sequence data (Build35), and selected 7,718 BAC clones. Then, these BAC-DNAs were spotted on a glass microscope slide in triplicate (GSP-Array7700) so that the resolution of the GSP-Array7700 is about a BAC probe/400kb throughout the human genome. We employed the GSP-Array7700 to detect segmental DNA copy number changes in cancer cells and tissues including epidermoid carcinoma A431 cells, TE-series esophagus cancers cells, and esophagus cancer tissues from patients. We in fact detected copy number changes of various DNA segments, some of which corresponded to oncogenes such as EGFR and CCND1 and suppressor genes such as FAT3. Further analysis of these data would provide new information on the cancer related marker genes.

Homozygosity mapping in the Old Order Amish identifies a region of Chromosome 3 as linked to obesity related traits. *P.F. McArdle[1], J.R. O'Connell[1], A.R. Shuldiner[1,2], B.D. Mitchell[1], M. Abney[3]* 1) School of Medicine, University of Maryland, Baltimore, MD; 2) Geriatrics Research and Education Clinical Center, VA Hospital Medical Center, Baltimore MD; 3) Departments of Human Genetics and Statistics, University of Chicago, Chicago, IL.

We performed genome wide homozygote by descent (HBD) linkage scans of several obesity related traits in an Old Order Amish population from Lancaster, PA (n = 743 subjects). Our analyses relied on a non-zero probability of having parents share at least one common ancestor in this young founder population. The conditional probability of being HBD was based upon complete pedigree information and an individuals multi-locus genotype and was included as a fixed effect in a linear variance component model. We detected consistent linkage to chromosome 3q26 for several obesity related traits including BMI, total cholesterol, LDL, and leptin. Our highest peak in the region was to total cholesterol at 196cM (LOD = 2.2). There was little or no evidence for linkage within this region to the same traits in our standard variance components linkage analysis, although we and others have previously observed linkage of this region with plasma adiponectin levels. Among the relevant candidate genes in this region are APM1, which encodes the gene for adiponectin, and PSARL, variants in which have been shown to be associated with type 2 diabetes and the metabolic syndrome. These analyses suggest that HBD mapping in relevant populations may be a useful technique for mapping complex disease genes. Further investigation is required to determine if variants in the adiponectin gene or other genes are responsible for the HBD linkage to obesity related traits.

Linkage analysis using GeneChip Microarray maps a possible locus for Crohn's disease to chromosome 3p. *N. Magal[1], R. Rahav[1], R. Shapiro[3], T. Shohat[2], M. Shohat[1,2]* 1) Department of Medical Genetics, Rabin Medical Center and Felsenstein Medical Research Center, Petah Tikva, Israel; 2) Sackler Faculty of Medicine, Tel Aviv University, Israel; 3) Schneider Childrens Medical Center, Institute of Gastroenterology, Hepatology and Nutrition, Petah Tikva, Israel.

We report a large Israeli family in which six out of 12 children have severe Crohn's disease. A dense genomewide linkage search of the family was undertaken using Affimetrix GeneChip Human Mapping 10K array. Initial analysis was performed on DNA from the six affected family members and their parents. The results showed several regions with the same haplotype in all the patients in more than six SNP's in a row. All these regions were excluded except for one locus on chromosome 3p, which was identified in previously published association studies as a candidate locus for Inflammatory Bowel Disease. This interval, which was found to contain the same SNPs profile in all the patients, is 24Mb long and contains 123 genes. Further haplotype analysis using polymorphic markers within this region in affected and unaffected family members enabled us to confirm that all the patients shared the same two alleles, while four out of the six unaffected siblings had a different haplotype. A maximum lod score of 1.8 (theta = 0.00) was found at marker D3S1263. Other known candidate loci for Inflammatory Bowel Disease were analyzed and excluded according to their SNP's profile. The TGF-bR2 (transforming growth factor, beta receptor II) gene, located in this interval, was sequenced but no mutation was detected. This study identified a possible new locus responsible for Crohn's disease located on chromosome 3p.

Biochemical and molecular analysis of 14 patients with thiamine-responsive pyruvate dehydrogenase deficiency.

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We report biochemical and molecular analysis of 14 patients with thiamine-responsive pyruvate dehydrogenase complex (PDHC) deficiency. PDHC activity was assayed using two different concentrations of thiamine pyrophosphate (TPP) in cultured lymphoblastoid cells or cultured fibroblasts from 14 patients. These 14 patients displayed very low PDHC activity in the presence of a low ($1x10^{-4}$ mM) TPP concentration. These PDHC activities increased at a high (0.4 mM) TPP concentration. Especially, PDHC activity of seven patients increased within normal range. Therefore, PDHC deficiency in 14 patients was due to a decreased affinity of PDHC for TPP. Treatment with a high dose of thiamine resulted in a reduction in lactate and clinical improvement in these patients, suggesting that these 14 patients have a thiamine-responsive PDHC deficiency and a high dose of thiamine is very effective for patients with this type of PDHC deficiency. The DNA sequence of these patients X-linked E1 subunit revealed a point mutation in all 14 patients including 7 patients in exon 3, one patient in exon 4, one patient in exon 5, two patients in exon 7, and three patients in exon 8 . Seven patients (V71A, R88C, G96S, C101F, S152P, L216F, and L260Q) of them were novel. Two mutations (L216F and R263G) were found in the genomic DNA of the patients mother, and the PDHC activity of these two mothers was decreased in the presence of a low TPP concentration. Half of 14 patients with thiamine-responsive PDHC deficiency were caused by mutations in exon 3 of E1 subunit gene. Therefore, exon 3 in the E1 subunit gene appears to be important in thiamine-responsive PDHC deficiency.

Development and evaluation of a novel single nucleotide polymorphism panel to detect hidden population substructure in individuals of European ancestry. *K.K. Nicodemus[1,2], Y. Yao[2], I. Giegling[3], D. Rujescu[3], N. Stefanos[4], C. Stefanis[4], D.R. Weinberger[1]* 1) GCAP, CBDB/NIMH/NIH, Bethesda, MD; 2) Epidemiology, Johns Hopkins SPH, Baltimore, MD; 3) Psychiatry, Ludwig Maximilians University, Munich, Germany; 4) University Mental Health Research Institute, Athens, Greece.

Several recent disease association studies have demonstrated population stratification (PS) in European-based populations. When genetically distinct subpopulations are unknowingly differentially sampled in cases and controls and the disease rate varies between the subpopulations, false positive association signals may be spuriously inflated and true association may be masked or reversed. Statistical methods have been proposed to detect PS using randomly selected genetic markers. Markers chosen to be ancestry informative may be more effective to detect PS. We selected SNPs likely to be informative in persons of European ancestry using the following SNP sets: ancestry informative SNPs, SNPs under selection in European populations (e.g., LCT), skin pigmentation SNPs, forensic identification SNPs, SNPs reported to be most informative for population ancestry and SNPs with > 10% difference in allele frequency in European samples (ALFRED) for a total of 141 SNPs. Study populations included: western European (German), southern European (Greek), European American (EA), HapMap CEPH trios and the NIMHGI EA schizophrenia families (N=3,538). A Bayesian method and a latent-class method were used to detect PS. Preliminary results suggest the German and EA samples were similar based on skin pigmentation SNPs; however, the NIMHGI family sample showed a higher proportion of ancestral skin pigmentation alleles than other European samples. We are currently examining all subsets of SNPs and evaluating the entire panel for informativeness of PS in European-based populations. In addition, we present a novel method (Genomic Matching) to control PS: cases are matched to controls who share a high proportion of genotypes from our SNP panel before performing association tests. Simulation studies are being conducted to assess how the proposed method performs versus current structured association methods to control for confounding due to PS.

Non-invasive genotyping fetal Kell blood group (KEL1) using cell-free fetal DNA in maternal plasma by MALDI-TOF mass spectrometry. *Y. Li[1], K. Finning[2], G. Daniels[2], W. Holzgreve[1], S. Hahn[1]* 1) Department of Research, University of Basel, Basel, Switzerland; 2) International Blood Group Reference Laboratory, National Blood Service, Bristol, UK.

Alloimmunization against the fetal Kell (KEL1) blood group antigen is gaining in importance and is the second most important cause of haemolytic disease of the newborn (HDN). The KEL*1 gene differs from the highly prevalent KEL*2 allele by a C-to-T base substitution in exon 6, thereby encoding threonine instead of methionine at residue 193. Currently, prenatal diagnosis of KEL1 involves invasive procedures, such as amniocentesis and chorionic villus sampling, which present a risk for both fetus and mother. The discovery of fetal cell-free DNA (cf-DNA) in maternal plasma provides the possibility for non-invasive prenatal diagnosis. However, the detection of fetal gene point mutations is difficult when using conventional PCR-based methods, due to the overwhelming presence of maternal cf- DNA sequences. In this context, it has recently been shown that the MALDI-TOF mass spectrometry-based single allele base extension reaction (SABER) assay may permit the detection of paternally inherited fetal SNPs from cf-DNA in maternal plasma. In this study, we examined KEL*1 from KEL:-1,2 pregnant women using the MALDI-TOF MS-based SABER assay. Thirty maternal plasma samples at risk for KEL1-alloimmunization were taken at 14-35 (mean: 22.8) weeks of gestation. The results were confirmed by serological tests on cord blood or PCR typing on amniocyte-derived fetal DNA. We detected the fetal KEL*1 allele in 11 of the 13 KEL1-positive samples (85% sensitivity). In total, the presence or absence of the paternal KEL*1 allele could be correctly determined in 93% of cases (28/30). Therefore, the MALDI-TOF mass spectrometry-based SABER assay may be useful for the detection of the fetal KEL1 status, but needs to be improved for clinical application. More precise methods, such as size fractionation of cf-DNA approach, might help improve the detection.

Slow acetylator genotype at the N-acetyltransferase 2 gene is a key predictor for adverse events by an anti-pneumocystis drug co-trimoxazole in patients with systemic lupus erythematosus. *N. Kamatani[1], M. Soejima[1], T. Sugiura[1], M. Kawamoto[1], Y. Katsumata[1], K. Takagi[1], A. Nakajima[1], T. Mitamura[2], A. Mimori[3], M. Hara[1], Y. Kawaguchi[1]* 1) Div Genomic Med, Tokyo Women's Med Univ, Tokyo, Japan; 2) Div Inernal Med, JR Tokyo General Hospital, Tokyo, Japan; 3) Div Internal Med, Internatinal Medical Center of Japan, Tokyo, Japan.

Objective. For the individual optimization of the co-trimoxazole (anti-pneumocystis drug) therapy in the patients with systemic lupus erythematosus (SLE), we investigated the genomic polymorphisms in the gene coding for N-acetyltransferase 2 (NAT2). Methods. 51 patients with SLE to whom prophylactic dose of co-trimoxazole had been administered were enrolled in a cohort study, and 54 patients including additional 3 patients who had suffered from severe adverse events by TMP - SMX were analyzed as a case-control study. Haplotypes were estimated using the PENHAPLO program by the EM-based maximum likelihood method to see whether the individuals possess NAT2*4 (wild-type) haplotype that renders the subjects the fast acetylator phenotype. Results. Adverse events occurred in 18 of the 51 SLE patients (35.3%) in the cohort study. Genotyping analysis followed by the haplotype inference of the NAT2 gene indicated that the numbers of fast (with NAT2*4 haplotype) and slow (without NAT2*4 haplotype) acetylator diplotype configurations (combinations of haplotypes) were 44 and 7 patients, respectively. Five (71.4%) out of 7 patients with slow acetylator diplotype configuration experienced adverse events, and the frequency was significantly higher than that in the patients (13/44, 29.5%) carrying fast acetylator diplotype configuration (P = 0.045, RR = 2.42, 95% CI = 1.26-4.65). In the case control study, the frequency of slow acetylator diplotype configuration was significantly higher in patients with severe adverse events (3/5, 60%) than in patients (6/49, 12.2%) without severe adverse events (P = 0.028, OR = 10.7, 95% CI = 1.4-78.0). Conclusions. The NAT2 slow acetylator diplotype configuration as inferred by the EM algorithm is an important predictor of adverse events by co-trimoxazole in the patients with SLE.

A Japanese case of Oto-palato-digital syndrome type II: an apparent lack of phenotype-genotype correlation. *T. Kondoh[1], N. Okamoto[2], N. Norimatsu[1], H. Moriuchi[1]* 1) Dept Pediatrics, Nagasaki City, Nagasaki Univ Sch Medicine, Nagasaki, Japan; 2) Dept Planning and Research, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan.

Oto-palato-digital (OPD) syndrome is characterized by hearing impairment, cleft palate, deformities of extremities and characteristic facies including frontal bossing, marked micrognathia, hypertelorism and downslant palpebral fissures. OPD syndrome is classified into two types from the aspect of clinical severity, OPD I (mild form) and OPD II (severe form). Filamin A gene (FLNA) is the causal gene of OPD I and OPD II as well as Melnic-Needles syndrome and frontometaphyseal dysplasia; however, it is not clear why mutation of the same gene results in different clinical entities. We here report a 12-year-old Japanese boy with OPD II. He was born as the first child of nonconsanguineous healthy parents at 41 weeks and two days of gestation with weight and length of 3,068 g (-0.3 SD) and 50.0 cm (+0.3 SD), respectively. He had various anomalies at his birth, bilateral cataracts, bilateral glaucoma, bilateral severe hearing impairment, congenital heart defect, umbilical herniation, bowling extremities and constrictions of various joints. These clinical features and the whole body X-ray findings were compatible of OPD II. He was also complicated with respiratory failure due to deformed chest and adenoid hyperplasia, urinary tract stenosis, apnea attack and poor weight gain. His mental development cannot be assessed exactly because of skeletal anomalies, hearing loss, and near total blindness. His karyotype was normal, 46,XY. FLNA analysis by ordinary PCR-direct sequencing method showed a C586T (R196W) missense mutation in exon 3. Another patient who also had the R196W mutation was reported previously; however, his clinical phenotype was OPD I (mild form). In addition, a patient with a C586G (R196G) mutation was previously reported as OPD II. Thus, phenotype-genotype correlation of OPD is not clear in those patients. Further clinical and genetic studies are needed to clarify the relationship between phenotype and genotype, or to identify other factor(s) that influence the clinical features.

Nodular hidradenocarcinoma: molecular cytogenetic analysis of a sweat gland tumor in a young male. *B. Horst[1], S. Volpert[2], Ch. Lee[1], HJ. Schulze[3], J. Atzpodien[4], J. Tchinda[5]* 1) Department of Pathology, Columbia University Medical Center, New York, NY; 2) Institut für Humangenetik, Universitätsklinikum Münster, Germany; 3) Fachklinik Hornheide, Department of Dermatology, Münster, Germany; 4) Fachklinik Hornheide, Department of Medicine Oncology, Münster, Germany; 5) Brigham and Womens Hospital, Harvard Medical School, Boston, U.S.A.

Hidradenocarcinomas are among the least common adnexal tumors of uncertain origin. They range from locally recurring, low-grade well differentiated tumors to highly aggressive tumors with potential for local destruction and distant metastasis to lymph nodes, bone and lungs. Data on cytogenetic analysis of these tumors are very limited. Here, we report the case of a 29-year-old man presenting with a nodular hidradenocarcinoma of the toe and filia to the lung. We used array comparative genomic hybridization (aCGH) on paraffin embedded tissue to characterize the tumor. An approximately 39.2 Mb large deletion of the long arm of chromosome 14 was detected as the sole DNA copy number change related to the tumor. The deletion of cell cycle and RNA transcription regulators in this genomic area may have been contributory to tumor formation in this patient. The patient is doing well with no sign of recurrence two years and five months after surgical resection of the primary tumor and lung metastasis.

Hemophilia A: molecular defects and mutation detection rate in severe, mild and moderate affected patients. *J. Horst[1], A. Markoff[2], R. Eisert[4], C. Wermes[4], H. Pollmann[5], A. Todorova[6], U. Nowak-Göttl[3], N. Bogdanova[1]* 1) Chairman, Inst Human Genetics, Univ Muenster, Muenster, Germany; 2) Institut für Medizinische Biochemie, Münster, Germany; 3) Pädiatrische Hämatologie und Onkologie, Münster, Germany; 4) Pädiatrische Hämatologie und Onkologie, Hannover, Germany; 5) Hämophilie Zentrum, Münster, Germany; 6) Laboratory of Molecular Pathology, Sofia, Bulgaria.

Hemophilia A (OMIM 306700) is the most frequent X-linked bleeding disorder affecting 1 to 2 per 10 000 males worldwide. Recently we showed that the mutation detection rate in severely affected male patients is virtually 100% when testing for the common intron 22- / intron 1- inversions and big deletions, followed by genomic sequencing of the F8 gene. We also showed that protein truncating molecular defects are prevalent in those patients [Bogdanova, Markoff et al., 2005, Hum Mutat 26(3):249-54]. Here we report on the spectrum of mutations and their distribution throughout the F8 protein in 136 moderately (n=24) or mildly (n=112) affected patients with hemophilia A. The performed sequencing analysis revealed a molecular defect in 121 (89%) of the patients, whereas 15 (11%) had no mutation in the coding region of the F8 gene, in the exon/intron borders or in the promoter region. All negative patients were mildly affected with exception of one patient with FVIII:C 2-3%. Thirty six of the mutations identified are novel. The vast majority of the detected mutations were missense mutations (n=104). Two molecular changes in the promoter region of the factor VIII gene were detected in two patients with mild hemophilia A. To our knowledge this is the first report on promoter mutations in the F8 gene. Our data show that, in contrast to severe hemophilia A, the analysis on the genomic level fails to detect the molecular defect in about 4% of the moderately and in 12.5% of the mildly affected patients. We postulate that in a part of these patients the reduction of the F8 activity is due to defects in other steps of the coagulation cascade.

Cole-Carpenter as a severe skeletal dysplasia. *G. Nishimura[1], M. Takeuchi[2], M. Nakayama[2], G. Knopfle[3], A. Superti-Furga[4], B. Zabel[4], S. Unger[5]* 1) Department of Radiology, Tokyo Metropolitan Kiyose Children's Hospital, Tokyo, Japan; 2) Department of Pathology, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan; 3) Institute of Pathology, University Hospital of Bonn, Bonn, Germany; 4) Centre for Pediatrics and Adolescent Medicine, University of Freiburg, Freiburg, Germany; 5) Institute for Human Genetics, University of Freiburg, Freiburg, Germany.

In 1987, Cole and Carpenter reported 2 unrelated children with a previously unrecognized phenotype whose main findings were bone fragility and craniosynostosis. The authors called this disorder a newly recognized type of osteogenesis imperfecta. However, since type 1 collagen analysis was normal, this condition has subsequently been labeled the Cole-Carpenter syndrome (MIM112240). Since that first report, only a few cases have been described and only one which had clinical/radiographic findings closely matching the original patients (Amor et al., 2000). However, unlike the first 2 patients, this third patient was detected prenatally and was abnormal at birth with osteopenia, bowing of the femurs, and a single rib fracture. We report a further 3 sporadic cases who had in utero bowing of the limbs and were suspected of having osteogenesis imperfecta. After delivery, they were found to have pronounced proptosis and midface hypoplasia suggesting craniosynostosis. Post-mortem radiographic examination revealed a strikingly consistent picture of generalized osteopenia, mid-shaft femoral fractures without bone thickening, bowing of femurs, tibias, and fibulas, as well as beaded ribs. Of note, the clavicles were also affected (short, thick, and misshapen) and the sacrosciatic notches were abnormally wide. This case series confirms that (1) the Cole-Carpenter bone fragility-craniosynostosis syndrome is a distinct entity with radiographic findings that allow differentiation from osteogenesis imperfecta; (2) while the two original cases were ascertained in infancy, severe cases can be detected prenatally. Since fetuses with bone fragility tend to be diagnosed as OI, the incidence of Cole-Carpenter syndrome may have been underestimated so far.

Multi Information for Measuring Linkage Disequilibrium among Multiple Loci and its Applications. *L. Luo, M. Xiong* Biostatistics, University of Texas - Houston, Houston, TX.

Measure of linkage disequilibrium (LD) between two loci is a fundamental quantity in genetics and plays an essential role in population genetics, statistical genetics and genetic epidemiology. The traditional LD measures, such as , although they have been applied successfully to genetic studies of complex diseases, they have serious limitations in providing overall information about LD among multiple loci due to the pair-wise feature of the traditional LD measures. Very few generally accepted measures of LD among multiple loci exist in the literature. To develop simple, but efficient measure of LD at multiple loci is urgently needed. In this report, we present a novel concept of multi information that is the extension of mutual information between two variables to measure LD among multiple loci. We demonstrate that multi information on multiple markers is a simple function of pair-wise as well as high order LD among the multiple markers. We also develop conditional multi information and formula for recursively computing multi information. To facilitate the application of multi information in population genetics and association studies of diseases, we investigate their properties and derive his asymptotical distribution. The mean and variance of multi information as well as the relationships with the parameters in the population genetics are also studied. To evaluate the performance of the multi information for measuring LD among multiple loci, we apply it to a HapMap data set. Our computational results from the real data coincide with the theoretical results and demonstrate its widely potential application as a powerful tool in evolution of populations and association studies of diseases.

An analysis of the *GRIK2* Modifier effect in Huntingtons disease. *S. Kishikawa[1], W. Zeng[1], T. Gillis[1], L. Djoussé[2], R.H. Myers[2], M.E. MacDonald[1], J.F. Gusella[1]* 1) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA; 2) Department of Neurology, Boston University School of Medicine, Boston, MA.

Huntingtons disease (HD) is an autosomal dominant late-onset neurodegenerative disorder caused by an expanded polyglutamine tract in the huntingtin protein. Human genotype-phenotype studies show that while age at neurological onset is primarily determined by polyglutamine length, it is also altered by genetic modifiers. *GRIK2*, which encodes the GluR6 subunit of kainate receptor, is currently the only genetic modifier of HD that has been specifically identified and confirmed in multiple independent studies. Our previous study revealed that affected individuals who have the relatively rare 16 TAA repeat allele in *GRIK2* 3 UTR show an average 5.5 years younger onset age compared with those with other TAA repeat alleles. To identify the mechanism of the genetic modifier effect of *GRIK2*, we checked the sequences of all *GRIK2* exons and flanking regions in four individuals, three with 16 alleles and one with a more extreme 17 TAA repeat allele, all with much earlier age at onset than expected from the length of their HD CAG mutation. Sequencing of all exons showed no coding sequence or boundary changes in the HD samples. Haplotype analysis using microsatellites and SNPs in *GRIK2* suggested no common ancestral origin for the similar TAA repeat alleles, suggesting that the TAA allele itself, rather than a linked polymorphism, is responsible for the modifier effect, possibly through a regulatory effect at the level of *GRIK2* RNA. We have confirmed expression levels of 6 alternative splice variants of *GRIK2* in regions of normal human brain by RT-PCR. Two of the these represent the major isoforms found in human brain and their relative proportions did not vary across brain region, including striatum. We next plan to test the effect of different TAA alleles on the proportion of the *GRIK2* splice and editing forms present in normal and HD brain.

Effects of Raspberries on cAMP-responsive element modulator (CREM) Expression in Rat Testes. *B.H. Lee, H. K. Lee, W.M. Yang, W-N. Kim, D. R. Kim, W. Park, S.K. Park* Prescriptionology, Kyung Hee Univ. College of Oriental Medicine, Seoul, Korea.

Rubi Fructus (RF), the dried unripe fruit of raspberries is used to manage impotence, spermatorrhea, enuresis, asthma, and allergic diseases; it has also been used as a stomachic and tonic in Korean medicine. Rats were treated RF for 56 days consecutively. Animals were sacrificed and the testes were removed. Total RNA was extracted from rat testes using Trizol method. Then we carried out Western blotting assay using anti-CREM antibody. CREM mRNA levels were significantly increased in testes from the rats treated with RF (p 0.05). The relative expression of CREM mRNA in the RF treated group was 189% than that in the normal group. There was significantly enhanced expression of CREM-immunoreactive bands in the RF treated group compared to the vehicle treated group (p 0.05). The relative expression of CREM in the RF group was 112% than that in the normal group. This result confirmed mRNA data. In conclusion, RF has an enhancing effect of CREM expression in rat testes both at mRNA and protein level. These results suggest that RF may have an effect on proliferation and differentiation of germ cells closely related with CREM gene expression.

The Fruit of *Cornus officinalis* Enhances Expression of cAMP-responsive element modulator (CREM) in Rat Testes. *W-N. Kim, J.H. Lee, W.M. Yang, B.H. Lee, D.R. Kim, W. Park, S.K. Park* Prescriptionology, Kyung Hee Univ. College of Oriental Medicine, Seoul, Korea.

Corni Fructus (CF), the fruit of *Cornus officinalis* has long been used as a tonic according to Korean medicine, it nourishes the liver and kidneys. Although many studies have examined how CF affects antioxidative damage and spermatogenesis in vitro, the relationship between CF and its effects on male reproductive malfunction associated with CREM gene expression and spermatogenesis in vivo has not yet been elucidated. Rats were treated CF for 56 days consecutively. Animals were sacrificed and the testes were removed. Total RNA was extracted from rat testes using Trizol method. Then we carried out Western blotting assay using anti-CREM antibody. CREM mRNA levels were significantly increased in testes from the rats treated with CF (p 0.05). The relative expression of CREM mRNA in the CF treated group was 153% than that in the normal group. There was significantly enhanced expression of CREM-immunoreactive bands in the CF treated group compared to the vehicle treated group (p 0.05). The relative expression of CREM in the CF group was 132% than that in the normal group. In conclusion, CF has an enhancing effect of CREM expression in rat testes both at mRNA and protein level. These findings suggest that CF may have a role of improving male infertility related with the sperm alteration and proliferation or differentiation of germ cells closely related with CREM gene expression.

Familial ATP7B Gene Analysis for Wilson Disease. *G. Kaur[1], S. Kumar[2], B.R. Thapa[3], R. Prasad[2]* 1) Dept Physiology, Goverment Medical College, Chandigarh, UT, India; 2) Dept Biochemistry, PGIMER, Chandigarh, UT; 3) Pediatric Gastroenterology, PGIMER, Chandigarh, UT.

Background: A number of PCR based techniques like SSCP, ARMS-PCR, seminested PCR and dinucleotide-repeat markers analysis have been used in the diagnosis of asymptomatic Wilson disease patients and carrier status of Wilson disease families. In present study, we explore the utility of mutation analysis with combination of RFLP in genetic diagnosis of Wilson disease. Aim: The study was planned to provide molecular diagnostic tool for diagnosis of asymptomatic Wilson disease patients as well as assessment of carrier status of Wilson disease families. Subjects and methods: We have analyzed four Wilson disease families in which parents and siblings showed no clinical manifestations and biochemical abnormalities. The parents of Wilson disease patients were not consanguineous and without any family history of Wilson disease. Mutations in ATP7B were characterized using SSCP and DNA sequencing. Further, RFLP was developed for analysis of characterized mutations in ATP7B from Wilson disease patients, their parents and siblings. Results: We have characterized three mutations A3767G, A1003T and I1102T by using SSCP and DNA sequencing in wilson disease patients. These mutations created/deleted restriction site for AccII, Bsh 1236I and EcoRI restriction enzymes respectively. Despite of no clinical manifestations and without any significant alteration in biochemical investigations in thirteen familys members, we have diagnosed 8 carriers and 1 asymptomatic wilson disease patients by restriction digestion analysis. Conclusion: Our report demonstrates that mutation analysis in combination with RFLP is useful for diagnosis of asymptomatic wilson disease patients as well as for elucidation of the carrier status among the patients family members. It is noteworthy here that this combinational methodology provide a positive diagnosis in the siblings/parents where biochemical parameters are ambiguous.

CFTR gene study in African children with cystic fibrosis phenotype: identification of a novel A204T missense mutation and frequencies of common polymorphisms. *L. MUTESA[1], C. VERHAEGHE[1], K. SEGERS[1], J.F. VANBELLINGHEN[1], L. NGENDAHAYO[2], C. OURY[1], L. KOULISCHER[1], V. BOURS[1]* 1) Department of Human Genetics,CHU Sart-Tilman, Center for Biomedical Integrative Genoproteomics (CBIG) University of Liège, Belgium; 2) Department of Anatomy Pathology, CHU-Butare, National University of Rwanda, Butare-Rwandaÿ.

Cystic fibrosis (CF) is one of the most common autosomal recessive disease in Caucasian with an incidence of 1 in 2500 births. However, very little is known about CF in Black population from Africa where this disease has been believed to be extremely rare. Nevertheless, CF is still under-diagnosed in this population. Indeed, clinical features of this disease are often similar to that of other frequent diseases in Africa such as malnutrition, tuberculosis, chronic pulmonary infections and HIV/AIDS. Moreover, molecular analyses, required to confirm the diagnosis, often remain inaccessible in many African countries. We have investigated 60 unrelated Rwandan children patients with CF clinical features. We applied a gene scanning approach using DHPLC and MLPA systems to analyse all exons and flanking intron sequences of the CFTR gene, in order to characterise CF mutations, sequence variations and gross genomic rearrangements. Four different CF-mutations, including one previously undescribed missense mutation (A204T in exon 6a), and 9 polymorphisms were identified. The CFTR-A204T mutation was studied by immunoblotting and pulse-chase in transiently transfected HeLa cells. The results revealed a lower level of CFTR mature protein relative to CFTR-wild type. The frequencies and association of coding single-nucleotide polymorphisms (c.2694T>G, c.4521G>A and c.1540A>G) were compared with control group and previous data. All these results are of interest in designing an appropriate strategy for genetic testing and counselling of CF patients in Africa.

snp.plotter: An R based SNP/haplotype association p-value and linkage disequilibrium plotting package. *A. Luna[1], D.R. Weinberger[1], K.K. Nicodemus[1,2]* 1) GCAP/CBDB, NIH/NIMH, Bethesda, MD; 2) Epidemiology, Johns Hopkins SPH, Baltimore, MD.

snp.plotter is a free and easy-to-use R package which produces publishable-quality plots of p-values using single SNP and/or haplotype data. Main features of the package include options to display a linkage disequilibrium (LD) plot below the p-value plot using either the r2 or D' LD metric with a user-specified LD heatmap color scheme, setting the X-axis to equal spacing or to use the physical SNP map, and specification of plot labels, colors and symbols for desplaying p-values. A major strength of the package is that it can plot multiple datasets simultaneously. Plots can be created using global and/or individual haplotype p-values along with single SNP p-values. R is a free software environment for statistical computing and graphics available for most platforms. The proposed package provides a simple way to convey both association and LD information in a single appealing graphic for candidate gene or whole genome association studies and requires virtually no knowledge of the R programming language - snp.plotter requires only the option for input data file names. In addition, snp.plotter can be used as a point-and-click web-based version at http://cbdb.nimh.nih.gov/~kristin/snp.plotter. The proposed package should facilitate the creation of complex graphics for genomewide or candidate gene association studies.

Preimplantation Genetic Screening for aneuploidy rates in couples undergoing donor egg in vitro fertilization cycles. *W.G. Kearns[1], R. Pen[1], A. Benner[1], K. Richter[2], P. Browne[2]* 1) Shady Grove Ctr for Preimplantation Genetics, Rockville, MD; 2) Shady Grove Fertility RSC, Rockville, MD.

PGD is used to decrease miscarriages, prevent the birth of aneuploid offspring and to potentially increase the implantation rate of preimplantation embryos. All information regarding rates of aneuploidy among human preimplantation embryos comes from PGD of embryos of people with impaired fertility. In this population, aneuploidy screening is suggested for women with recurrent pregnancy loss, those with the transfer of good quality embryos without achieving a viable pregnancy or women of advanced maternal age. Typically women using donor egg IVF have the highest success rates due to the selection of egg donors in a low risk population; less than 34 years of age with normal Day 3 FSH and estradiol levels. Therefore, PGD for aneuploidy screening is not a common recommendation for this patient group. However, embryonic aneuploidy rates occurring within the general human population remain unknown. Therefore, we determined aneuploidy rates in couples undergoing donor egg IVF cycles. 32 couples underwent donor egg IVF-PGD. Laser-assisted embryo biopsy was performed on day-3 and PGD was done on 440 cleaving embryos from 32 initiated cycles. The mean donor age was 26.5 yrs (21-31). Multi-color fluorescence in situ hybridization (FISH) was used to determine aneuploidy for chromosomes 13, 14, 15, 16, 17, 18, 21, 22, X and Y. Clinical outcomes were determined. Pregnancy was defined by ultrasound identification of an intrauterine gestational sac. All 32 women had an embryo transfer. 5% (22/440) of the embryos were not diagnosed due to poor embryo quality. 50% (209/418) of the embryos were abnormal for at least 1 of the 10 chromosomes tested. The clinical pregnancy rate was 66% (21/32) per patient and per embryo transfer. There were no miscarriages, misdiagnosis, or mosaic embryos. These data 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 PGD. The miscarriage rate was 0% which could be due to the PGD testing or alternatively due to the small sample size.

Concurrent deleterious germline mutations in both *BRCA1* and *BRCA2* in a large series of patients. *M. Martin, C. Frye, L.A. Burbidge, A.M. Deffenbaugh, D. Pruss, B.E. Ward* Myriad Genetic Laboratories, Salt Lake City, UT.

Infrequent case reports of individuals with germline mutations in both the *BRCA1* and *BRCA2* genes exist but there has been no systematic analysis of prevalence in a large cohort of patients. The aim of this study was to determine the prevalence and review available personal and family history of individuals with two deleterious germline mutations. The presence of double heterozygotes (DH) was detected in patients analyzed by comprehensive sequencing of *BRCA1* and *BRCA2* (bidirectional sequencing of all exons, splice site junctions of both genes, plus detection of five common large rearrangements in a large proportion of patients) and in Ashkenazi Jewish (AJ) patients who underwent analysis of the three founder mutations (*BRCA1* 185delAG, 5385insC and *BRCA2* 6174delT). Of 76,825 individuals tested by full sequence analysis, 9541 patients were found to have one deleterious mutation and 26 were found to be DH (6 of whom were AJ). Of 19,673 individuals tested for only the founder mutations, 3455 patients were found to have one deleterious mutation and 33 were identified as DH. As expected, the increased incidence of mutations in the AJ population led to an increased frequency of double *BRCA1* and *BRCA2* heterozygotes. Within the total population of DH, 42 of the probands reported a personal history of an HBOC related cancer, including 76% with invasive breast cancer, 7% ovarian cancer and, 14% reported both breast and ovarian cancer, and 2% with DCIS. 15 of the probands reported only familial cancer history and no personal history of HBOC related cancer, and 2 specified only familial history and did not specify personal history. The reported age of diagnosis of breast cancer ranged from 22-63 years, with a mean age of 40.1. The reported age range for diagnosis of ovarian cancer was 41-88 with a mean age of 56. The clinical presentation of personal and family history does not differ significantly from individuals that carry one mutation in either the *BRCA1* or *BRCA2* gene suggesting that the conferred risk for cancer in DH is on the same order of magnitude as that of single mutation carriers.

A practical method of genome screening for population-specific selective sweeps: candidate regions contributing to inter-population phenotypic divergences. *R. Kimura[1, 2], A. Fujimoto[1], K. Tokunaga[1], J. Ohashi[1]* 1) Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; 2) JSPS research fellow.

Phenotypic divergences among modern human populations have been produced as genetic adaptation to local environments. To identify genes involved in population-specific phenotypes, therefore, it is significant to search for signs of recent positive selection in the human genome. Although detection of elongated linkage disequilibrium (LD) has been a powerful tool in the field of evolutionary genetics, such current methods are not applicable to identify already fixed loci. Here, we report a new LD-based method of screening for population-specific selective sweeps including already fixed regions. The present method depending on the ratio of observed homozygosity for the haplotypes between populations enables us to measure the inter-population differences in the extent of nucleotide diversity, genetic differentiation, and LD. Another advantageous point of our method is that it does not require haplotype phasing of SNPs that should be determined from family data or estimated from population data with a possibly inaccurate, time-consuming computation. We analyzed the genome-wide SNP typing data form the International HapMap project (Phase I 16c.1) and performed a genome-imitating simulation under neutrality using a well-fitting demographic model. The results suggest that rapid fixations of new advantageous mutations frequently occurred after the "Out of Africa" probably due to environmental changes and to population bottlenecks, while in the African population, advantageous alleles fixed slowly, if any, after generation of the polymorphisms. The present study, detecting the genes already reported to be under strong selection such as *ABCC11* in the Asians, *SLC24A5* and *MATP* in the Europeans, and *FY* in the Africans, provides other candidate regions of population-specific selective sweeps in the human genome that must contribute to a variety of phenotypes among human populations.

Molecular Diagnosis of Norrie Disease. *G. Modabber, M. Houshmand, M.H. Sanati* Medical Genetic, National Institue of Genetic Engineering and Bioth, Tehran, Iran.

G.modabber , M. Hoshmand, M.H.Sanati Iran,National Institue for Genetic Engineering and Biotechnology Norrie disease Norrie disease is a rare X-inked recessive (Xp11.4) condition characterized by congenital blindness in males due to degenerative and proliferative processes in the neuroretina . Retinal dysgenesis occurs early during embryogenesis and is often associated with microphthalmia . The most prominent feature at birth is an intra-ocular mass which can be misdiagnosed for retinoblastoma and ultimately leads to shrinkage of the eye globe . The main histopathological findings are rosettes of immature retinal cells embedded in a vascular connective tissue of hyperplastic primary vitreous . Norrie disease is a rare disorder, its exact incidence is unknown. It is not associated with any specific racial or ethnic group.Mutations in the NDP cause Norrie disease. The NDP gene produces a protein called Norrin , which is believed to be crucial to normal development of the eye , brain , inner ear , nasal epithelium and other body systems. In affected males, mutations in the NDP gene are associated with a spectrum of retinal findings ranging from Norrie disease (ND) to X-linked familial exudative vitreoretinopathy (FEVR), including some cases of persistent hyperplastic primary vitreous (PHPV), Coats disease, and advanced retinopathy of prematurity (ROP).These phenotypes appear to be a continuum of retinal findings with considerable overlap. References: 1. Norrie disease gene sequence variants in an ethnically diverse population with retinopathy of prematurity ,Molecular Vision 2005; 11:501-508 2. Norrie, 1927;Warburg,1966; Warburg,1975.

Sequencing Analysis of two Iranian patients with Allgrove Syndrome. *L. Komeilian[1], Sh. Salehpour[2], H. Tonekaboni[2], M. Houshmand[1]* 1) Medical Genetic, National Institue of Genetic Engineering and Bioth,Komeilian,BS.Houshmand,PhD Tehran, Iran; 2) Mofid Pediatrics Hospital,Salehpour,MD Tonekaboni,MD.

L.Komeilian(1),Sh.Salehpour(2),H.Tonekaboni(2),M.Houshmand(1) 1: National Institute of genetics Engineering and Biotechnology 2: Pediatrics Hospital of Mofid Allgrove syndrom: The triple A syndrome is a rare autosomal recessive disorder (gene locus is on chromosome 12q13, contains 16 exons and code for a 546 amino acid protein which called ALADIN). characterized by adrenal insufficiency,achalasia and alacrima. Material and methods: Patient No1: An 8.5 years old boy, a product of consanguineous marriage with, Alacrima, Achalasia, Short acting ACTH stimulating test showed primary Adrenal insufficiency. A low serum cortisol level, History of an acute encephalopathy, mild ataxia. Generalized skin hyper pigmentation, Epigastric pain, A sensory autonomic neuropathy. Patient No 2 An 2.5 years of old boy, product of nonconsanguineous marriage with , History of poor weight gain, Generalized skin hyper pigmentation, Alacrima, Achalasia ,Dysphagia, Lethargy and Seizure, Hyponatremia(Serum Na=112 meq/lit), Hyper Kalemia(Serum K=5.7 meq/lit), Hypoglycemia(BS=52 mg/dl), Hhypotention. Sensory autonomic neuropathy. Xerophtalmia, Keratosis. DNA Analyzing: PCR and Sequencing methods were used to analyze 12 out of 16 exons in AAAS . The exons :4,5,6,7,8,9,10,11,12,13,14,15. Result: G7329Cmutation was found in IVS7-1 of AAAS gene in both patients. G13504A mutation was found in intron 14 of AAAS gene in patient 2. Conclusion: More investigations need to show pathogeneticity of these mutations.

Acentric supernumerary marker chromosome characterized as inv dup(3)(qter-q26.1::q26.1-qter) in a child with several dysmorphic features. *S.K. Murthy[1], A.K. Malhotra[1], P.S. Jacob[1], S. Naveed[1], E.E.M. Al-Rowaished[1], S. Mani[1], S. Padariyakam[1], R. Pramathan[2], S. Lyndell[2], R. Nath[2], A. Redha[3], M.T. Al-Ali[1], L. Al-Gazali[2]* 1) Department of Genetics, Al Wasl Hospital,DOHMS, Dubai, United Arab Emirates; 2) Department of Pediatrics, Faculty of Medicine and Health Sciences and Al Ain Hospital, UAE; 3) Department of Pathology, Dubai Hospital, DOHMS, Dubai, UAE.

Pure tetrasomy 3q26-qter is an extremely rare observation. We report here our molecular cytogenetic results from an one month old child presenting with several dysmorphic features including depressed nasal bridge, low set ears, micrognathia, prominent hairy forehead, tail like sacrococcygeal appendage, hypoplasia of corpus callosum and streak pigmentation on the lower aspect of the forearm. Initial peripheral blood karyotyping and all-telomere FISH screening showed normal results. Chromosome analysis from fibroblasts obtained from a skin biopsy showed the presence of a marker chromosome in 98% of the cells analyzed. The marker chromosome was C-band and NOR negative. Oligo array-CGH studies showed an amplification of 3q26.1-qter region, confirming the origin of the marker chromosome. Further characterization of the marker by subtelomere FISH studies revealed it to be an inversion duplication of 3q26.1-qter. Only three cases of similar acentric supernumerary marker characterized as inv dup(3q) have been reported so far. Tissue specific mosiacim, a possible activation of neocentromere at 3q26 and genotype-phenotype correlation due to tertrasomy 3q26-qter in our patient is discussed.

The New Zealand Maori Population as a Candidate for Admixture Gene Mapping. *R.A. Lea[1,2], D. Hall[1,3], G.K. Chambers[3], L.R. Griffiths[2]* 1) Institute of Environmental Science and Research Ltd, Wellington, New Zealand; 2) Genomics Research Centre, Griffith University, Queensland, Australia; 3) School of Biological Sciences, Victoria University of Wellington, New Zealand.

The Maori population of New Zealand (NZ) represents the final link in a long chain of island-hopping voyages stretching across the South Pacific - The last of the great human migrations. This indigenous population originated from restricted groups of founding ancestors (~1000 years ago) and underwent rapid growth followed by more recent genetic admixture with British colonisers over the past 200 years (8 generations). Compared to Caucasians the modern Maori population (~15% of NZ) exhibits markedly increased rates of genetically-influenced diseases such as lung cancer, diabetes and gout. It is plausible that these disease differences are partly due to higher frequencies of susceptibility genes in this indigenous group and/or genetic interaction arising from intermarriage with Europeans (admixture). Population-based linkage disequilibrium (LD) studies that incorporate genetic admixture (ie. admixture LD mapping) can be a powerful approach for identifying disease susceptibility genes. Before such studies can be properly designed it is important to understand the genetic structure of the target population. We have conducted statistical analyses of genetic data for the Maori population to show that a) long-range admixture LD (~6Mb) exists and b) the proportion of European genes in Maori is ~40%. We have also found correlations between Maori ancestral variation and clinically important phenotypes such as nicotine metabolic rate and BMI suggesting ancestry-specific genes may modulate variation in these traits. Our genetic research has established valuable baseline genetic information for this unique Polynesian population and, coupled with the marked disease disparities compared to Europeans, suggests that the Maori population is an excellent candidate for admixture gene mapping studies.

An open-label study of long-term miglustat use in adult patients with Type 1 Gaucher disease. *R. Heitner[1], M. Hrebicek[2], D. Elstein[3], C.E.M. Hollack[4], A. Zimran[3]* 1) Department of Paediatrics, University of the Witwatersrand, Johannesburg, South Africa; 2) Institute of Inherited Metabolic Disorders, Prague, Czech Republic; 3) Shaare Zedek Medical Centre, Jerusalem, Israel; 4) Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands.

The objective of this study was to assess the long-term safety and efficacy of oral miglustat (Zavesca) in patients (pts) with Type 1 Gaucher disease (GD1) who had completed one of the prior miglustat pivotal trials. In this Phase IIIb open-label, multinational study, miglustat efficacy was evaluated by assessing liver and spleen volumes, haemoglobin level, platelet count and chitotriosidase activity. Miglustat safety was also investigated. This analysis took into account individual baseline conditions upon entry into the pivotal studies. For organ volume and chitotriosidase activity, statistical significance of change from baseline was assessed using a paired t-test. Twenty-three individuals (74% female) were recruited from four national Gaucher referral clinics. The mean age SD was 39.39.8 years. A total of 14 pts completed the 24 month assessments. Nine pts were withdrawn. Reasons for withdrawal were: loss of follow-up (1 pt), compliance issues (1 pt), switch to commercial drug (2 pts), pt request (pregnancy planning; 2 pts), diarrhoea (1 pt), increased liver volume (1 pt), and a serious adverse event (SAE) (cerebral haemorrhage; 1 pt). Total miglustat exposure including treatment during previous clinical trials ranged from 48 to 72 months. The continuous improvement observed for organ volumes and blood parameters in the pivotal phase (up to 3 years) was maintained in the additional follow up. Chitotriosidase activity declined continuously over time. There were no new cases of peripheral neuropathy or abnormal cognitive function. This study confirms long-term efficacy of miglustat on all standard GD parameters with an additional 2 years of data. The safety profile was also consistent with previous pivotal clinical trials. With a miglustat exposure of up to 6 years, this study represents the longest follow-up of a cohort of GD1 pts in a clinical trial setting.

Novel KRIT1 mutations mediating Cerebral Cavernous Malformations. *N. Limaye[1], N. Revencu[1,2], L. Boon[1,2], M. Vikkula[1], The CCM Study Group* 1) Laboratory of Human Molecular Genetics, Christian de Duve Institute of Cellular Pathology, Université catholique de Louvain, Brussels, Belgium; 2) Cliniques St Luc, Université catholique de Louvain, Brussels, Belgium.

Cerebral Cavernous Malformation (CCM; OMIM 116860) is characterized by lesions of densely clustered, enlarged capillary-like sinusoids lined with an endothelial layer, with no intervening brain parenchyma. They occur in the brain, spinal cord, retina, and as hyperkeratotic cutaneous capillary-venous malformations (HCCVMs) in the skin, and can sometimes cause headaches, seizures, neurological deficits and cerebral hemorrhages. Truncating mutations in three different genes: KRIT1 (CCM1), MGC4607 (malcavernin, CCM2), and PDCD10 (CCM3), have so far been found to cause the familial form of the disease. While the precise functions of the CCM genes remain to be elucidated, KRIT1 has been shown to be critical for arterial morphogenesis and identity in a knockout mouse model. In humans, the KRIT1-containing 7q locus is estimated to account for about 40% of CCM-affected kindreds, with mutations in the gene causing the malformation with a fairly high clinical penetrance of about 62-88%. In the current study, we screened all 16 coding exons of the KRIT1 gene, along with flanking intronic sequence, by dHPLC followed by sequencing, to identify mutations in 40 affected families. We identified 17 different mutations, 11 of which are novel, in 18 families. Five of these new mutations are insertions, three are deletions, two are intronic nucleotide substitutions, and one a nonsense substitution. All of these cause frame-shifts and/or premature termination of the coding sequence. All of the identified mutations co-segregate with the CCM phenotype within the families, with a total of thirty-one affected and four non-penetrant mutation carriers identified. In keeping with earlier reports, KRIT1 mutations account for 45% of the affected families in our study, and invariably cause loss-of-function of the protein. (http://www.icp.ucl.ac.be/vikkula) (vikkula@bchm.ucl.ac.be).

Epistatic Effect Between 11q22 and 17p11 for Attention-Deficit/Hyperactivity Disorder in the Paisa Genetic Isolate. *M. Jain[1,2], F.X. Castellanos[3], D. Pineda[4], F. Lopera[4], J. Palacio[4], K. Berg[1], J. Bailey-Wilson[1], M. Muenke[1], M. Arcos-Burgos[1]* 1) NHGRI/Medical Genetics Branch, NIH, Bethesda, MD; 2) HHMI-NIH Research Scholar, Chevy Chase, MD; 3) NYU, Child Study Center, New York, NY; 4) University of Antioquia, Colombia.

Attention-deficit/hyperactivity disorder (ADHD, [MIM 143465]) is the most common behavioral disorder of childhood. Segregation and epidemiological analyses both suggest genetic factors play a role in the pathogenesis of ADHD. Eighteen multigenerational extended families from the Paisa genetic isolate demonstrate linkage to four regions: 4q13.2, 5q33.3, 11q22 and 17p11. Regions on 5q, 11q and 17p replicate regions found in other populations and suggest that putative causative genes in these regions will have effects across populations. In an attempt to unravel genetic complexities underlying ADHD previous work has confirmed the presence of genetic heterogeneity between families and pleiotropy between ADHD, disruptive behaviors and substance abuse. We examined the possibility of interactive effects between the linked loci in 134 nuclear families from the Paisa genetic isolate using SNPs at a 200kb resolution in these four regions. We used Coxs nonparametric linkage correlation method where linkage in families to a region is evaluated based on the condition that the families are linked to a reference region. Conditioning on families linked to chromosome 17p11 led to a significant increase in the nonparametric LOD on 11q22 from 0.55 to 3.88. Conditioning on families linked to 4q13.2 increased the nonparametric LOD on 11q22 from 0.55 to 3.24. Thorough parametric analysis using the method implemented in TLINKAGE did not disclose an interactive effect, again pointing to the increased power to detect interaction using nonparametric methods compared to parametric ones. These results suggest that candidate genes on 4q13.2 and 17p11 interact with a locus on 11q22 to confer susceptibility for ADHD. Using ADHD as a disease model we have now demonstrated heterogeneity, pleiotropy and epistasis to be important for this seemingly complex trait.

Association between PDE4D genotype/phenotype and young ischemic stroke. *Y.C. Liao[1], H.F. Lin[2], C.W. Liou[3], E. Hsi[1], S.H. Juo[1,4,5]* 1) Graduate Institute of Medical Genetics, Kaohsiung Medical University, Kaohsiung, Taiwan; 2) Department of Neurology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 3) Department of Neurology, Chang Gung Memorial Hospital, Kaohsiung, Taiwan; 4) Department of Clinical Research, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 5) Department of Medical Research, Mackay Memorial Hospital, Taipei, Taiwan.

Background: The phosphodiesterase 4D (PDE4D) gene was shown as a susceptibility gene to ischemic stroke in the Icelandic population. Previous studies primarily focused on late-onset stroke. We tested for the association between PDE4D and young ischemic stroke in a Taiwan population.

Methods: Stroke patients with age between 15 and 45 years were recruited from five medical centers. The present study included 156 patients, 130 young (45 years) and 187 old controls (46 years). We selected four SNPs which have been implicated to be associated with stroke in some previous studies: SNP45 (rs12188950), SNP56 (rs702553), SNP83 (rs966221), and SNP87 (rs2910829). Genotype and haplotype effects were evaluated.

Results: The genotypes in cases and controls were in Hardy-Weinberg equilibrium. SNP45 was monomorphic in our population. For SNP56, allele T was relatively common in cases than in controls. After adjusting for age and sex, the OR for the TT genotype of SNP56 was 2.45 (the nominal p = 0.017 and the permutated p = 0.028) between the cases and young controls. Using old participants as control, the statistical results did not reach the significant level, but showed a similar trend. There was no statistical significance for SNP83. The frequency of CT genotype at SNP87 was significantly lower in old controls (OR=1.99, the nominal p = 0.014 and the permutated p = 0.035). We further investigated the haplotypes comprising SNP56 and SNP87. The AC haplotype of SNP56-SNP87 was negatively associated (p=0.006) with young stroke.

Conclusion: our study suggests that the PDE4D gene may play a role in young stroke in the Taiwanese population.

RNA interference-mediated allele-specific silencing rescues sialic acid levels in the dominant disorder sialuria. *E. Klootwijk, P.J. Savelkoul, C. Ciccone, D. Krasnewich, W.A. Gahl, M. Huizing* MGB, NHGRI, NIH, Bethesda, USA.

Sialuria is a rare autosomal dominant disorder caused by a missense mutation in the allosteric site of the rate-limiting enzyme of sialic acid biosynthesis, UDP-GlcNAc 2-epimerase/ManNAc kinase, encoded by the *GNE* gene. This results in loss of feedback inhibition of GNE by CMP-sialic acid, and overproduction of sialic acid. Sialuria patients manifest variable signs and symptoms, including mild hepatomegaly and developmental delay. Since dominantly inherited disease alleles are attractive therapeutic targets for allele-specific silencing mediated by RNA interference (RNAi), we employed this method in fibroblasts of sialuria patients. Small interfering RNA (siRNA) was designed to specifically target a sialuria *GNE* missense mutation (c.787G>T, R263L). This siRNA was transfected into patients fibroblasts and the extent of silencing was assessed after 48 hours. After silencing, allele-specific real-time PCR analysis demonstrated that expression of the mutant *GNE* transcript was decreased by 71 3 (SD) % (*n*=3). Furthermore, HPLC analysis of fibroblast extracts showed that sialic acid levels decreased 59 15% (*n*=3) after silencing. Finally, GNE enzymatic activity measurements showed a 41 6 % (*n*=3) recovery of feedback inhibition by CMP-sialic acid. These results demonstrate that RNAi can correct the underlying metabolic defect *in vitro* in a human inborn error of metabolism. We plan to extend this RNAi study to a mouse model of sialuria that we are generating. Allele-specific RNAi therapeutics in sialuria provides an example for correcting dominant-negative mechanisms through elimination of specific mutant transcripts.

High resolution detection of copy number changes and LOH using the Affymetrix GeneChip 500K Mapping Array on formalin-fixed, paraffin-embedded tumor tissue. *S. Jacobs[1], E.R. Thompson[2, 3], Y. Nannya[4], Y. Go[4], R. Pillai[1], S. Ogawa[5], D.K. Bailey[1], I.G. Campbell[2, 3]* 1) Genomics Collaborations, Affymetrix, Inc, Santa Clara, CA; 2) VBCRC Cancer Genetics Laboratory, Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia; 3) Department of Pathology, University of Melbourne, Parkville, Australia; 4) Departments of Hematology/Oncology, University of Tokyo, Tokyo, Japan; 5) Department of Regeneration Medicine for Hematopoiesis, University of Tokyo, Tokyo, Japan.

In this study, we demonstrate the application of the Affymetrix GeneChip Mapping 500K arrays for an integrated analysis of genotype, loss of heterozygosity (LOH), and copy number using DNA derived from formalin-fixed, paraffin-embedded (FFPE) tissues. FFPE material tends to yield degraded DNA and is thus sub-optimal for use in many downstream applications. The Mapping 500K arrays can be used for genotype and copy number analysis of >500K SNPs, employing a technology that includes PCR amplification of SNPs on fragment sizes from 100bp to 1143bp. We found that a pre-qualifying PCR test adequately predicted the performance of FFPE DNA on the arrays. Average call rates for the FFPE samples were reduced compared to nonFFPE samples, but a closer examination of SNP by SNP performance revealed that genotyping success was dependent on amplicon size and therefore easily predicted and corrected for. Average concordance rates between FFPE and fresh frozen samples from the same tumor surpassed >98% on the smaller fragment sizes, despite some genetic differences due to tumor heterogeneity. Importantly, FFPE and fresh frozen tumor samples provided the same copy number and LOH profiles, indicating the feasibility of a complete analysis of FFPE-derived samples using this platform. In conclusion, we have shown that the Mapping 500K Array can be applied to FFPE-derived samples for genotype, copy number, and LOH analysis, thereby potentially allowing thousands of archival samples to be studied using this integrated system.

Congenital Diaphragmatic Hernia associated with Duplication of 11q23-qter. *M. Klaassens[1,2], D.A. Scott[3], B. Lee[3,4], D. Tibboel[2], A. de Klein[1]* 1) Clinical Genetics, Erasmus MC, Rotterdam, the Netherlands; 2) Pediatric Surgery, Erasmus MC, Rotterdam, the Netherlands; 3) Molecular and Human Genetics, Baylor College of Medicine, Houston (TX), USA; 4) Howard Hughes Medical Institute, Chevy Chase (MD), USA.

Little is known about the etiology of Congenital Diaphragmatic Hernia (CDH), a relatively common birth defect with a significant mortality, but there is increasing evidence for a strong genetic contribution. Both numerical and structural chromosomal abnormalities have been described in patients with CDH. Partial trisomy 11q and partial trisomy 22 associated with the common t(11;22) has been reported in several cases of CDH. It has been assumed that the diaphragmatic defect seen in these individuals was due primarily to duplication of material from chromosome 22q11. Here we describe a family with a t(11;12) in which one of two brothers with partial trisomy 11q has a left sided posterolateral CDH. This is the second case of CDH in partial trisomy 11q due to an unbalanced translocation other than t(11;22). Using array-based comparative genomic hybridization and fluorescent in situ hybridization we mapped the breakpoints in both brothers and their mother who is a balanced translocation carrier. Our results suggest that duplication of one or more genes on an ~19 Mb region of 11q23.3-qter predispose for the development of a diaphragmatic hernia. These effects may be the primary cause of CDH in individuals with a t(11;22) or may be additive to effects from the duplication of chromosome 22 material. We also conclude that the partial trisomy 11q syndrome has a variable phenotype and that CDH should be added to the spectrum of anomalies that can be present in this syndrome.

A male with a small duplication at 17p11.2 (Smith-Magenis region) characterized by FISH and microarray CGH.

J. Jung[1], *J. Xu*[2] 1) Medical Genetics Program of Southwestern Ontario; 2) Cytogenetics, London Health Sciences Centre and University of Western Ontario, Canada.

Our patient presented with an early history of moderately severe developmental delays, behavioral difficulties, failure to thrive and recurrent respiratory tract infections. He exhibited mild dysmorphic features which included bilateral epicanthal folds, periorbital fullness, anteverted nares, smooth philtrum, relatively thin upper lip, a high arched palate and mild fifth finger clinodactly. Initial chromosomal analysis and Fragile-X studies were reportedly normal. Later features noted included hypernasality of speech and a prominent nose. At 20 years of age he had a weight of 112 pounds (5th percentile), height 64 inches (<3rd percentile) and head circumference 53.5 cm (5th percentile). He had relatively large hands measuring 19.5 cm (97th percentile). Radio-ulner synostosis was associated with limited range of movement. His ears measured 6.3 cm (50th-75th percentile). He remains developmentally delayed but has good verbal skills coupled with a friendly and outgoing personality. General physical health is good. A repeat chromosomal G-banding analysis at 600 band level revealed a small interstitial duplication at 17p11.2. Metaphase FISH using a probe for Smith-Magenis syndrome (SMS, Vysis) region confirmed this duplication. Microarray CGH analysis (Signature Genomics Laboratories, LLC) of 969 clones for 304 loci on 41 chromosome arms identified a single copy gain for 3 BAC clones (RP11-524F11, RP11-1149K20, RP11-958E14) covering the RAI1 gene at 17p11.2. This duplication is further confirmed by interphase FISH using RP11-1149K20. This duplication is the reciprocal recombination product of the deletion found in SMS. This is a de novo rearrangement as the parents have a normal karyotypes. More cases with cytogenomic characterizations (e.g. microarray) are needed for the molecular karyotype/phenotype correlation.

Congenitally adducted thumbs and cognitive impairment in a mother and her two sons. *S. Nikkel* Dept Genetics, CHEO, Ottawa, ON, Canada.

A unique family is reported consisting of a mother and her two sons who have the same clinical features, with an otherwise negative family history. All three have mental retardation. The upper limbs are quite unique. The thumbs congenitally were in a fixed adducted position. All three have had surgery to obtain some release. There appears to be both bone and soft tissue malformation. There is limited supination and pronation at the wrists. The eldest son has camptodactyly of the fourth and fifth fingers of the right hand. Their voices are unusual in that they have a high-pitched quality. They have similar facial features with synophrys and pinched nasal tip, however these features are seen in other family members who do not have the limb differences and are cognitively normal. Growth is unremarkable. Karyotype and subtelomere analysis was normal. Neuroimaging was likewise unremarkable. Adducted thumbs are seen in a number of syndromes where there is arthrogryposis, spasticity and/or intracranial anomalies. This family does not appear to fit into any of these groupings. It is suspected that the mother has a new dominant mutation that she has passed on to her two children. An X-linked condition is also possible, although the mother is as severely as affected as her boys.

A new mechanism for genomic rearrangements causing genomic disorders. *J.A. Lee*[1], *J.R. Lupski*[1,2,3] 1) Dept. Molecular & Human Gen, Baylor College of Medicine, Houston, TX; 2) Dept. Pediatrics, Baylor College of Medicine, Houston, TX; 3) Texas Children's Hospital, Houston, TX.

Genomic disorders are a group of human genetic diseases caused by DNA rearrangements that result in the gain, loss, or disruption of a gene or genes for which dosage is critical. These disorders are characterized by similar genomic features in the rearrangement-susceptible regions, but represent a wide spectrum of unrelated clinical entities. Thus far, the prevailing mechanism for rearrangements causing genomic disorders is non-allelic homologous recombination (NAHR) between region-specific low-copy repeats (LCRs) for recurrent events that have breakpoints which cluster and for some non-recurrent alterations; NAHR can use closely-related repetitive (eg. *Alu*) sequences as recombination substrates. Non-homologous end joining (NHEJ) has also been implicated as a recombination mechanism for numerous non-recurrent rearrangements. In the context of the genomic disorder Pelizaeus-Merzbacher disease (PMD), an X-linked recessive dysmyelinating disorder caused most frequently by non-recurrent duplication including the dosage-sensitive *PLP1* gene, but also by deletion and point mutations, our data suggest an alternative mechanism involving errors of replication as opposed to recombination. We have analyzed breakpoint junctional sequences in PMD patients with different-sized (~200 kb - 7 Mb) genomic duplications and deletions, and found evidence for sequence at the breakpoint coming from different genomic locations. The most parsimonious explanation involves a stalled DNA replication fork that switches strand templates and reinitiates DNA synthesis at a nearby but distinct genomic location. Our model of replication Fork Stalling and Template Switching (FoSTeS) may help to explain some of the more complex duplication and deletion rearrangements associated with PMD that have been reported in the literature, and potentially other non-recurrent complex rearrangements as well.

Evaluation of Sequencing Analysis Software for Complex Disease Studies. *C. Liu* Dept Psychiatry, Knapp Res Ctr, Univ Chicago, Chicago, IL.

In the study of complex disease genetics, resequencing may be required for the discovery of polymorphic markers, and it is especially needed for study of rare variants. A multiple-rare-variants common disease model normally requires resequencing of hundreds of case and control individuals. In order to identify all the rare variants and to obtain genotype data of all the subjects for statistical analyses, sequencing analysis software must be adapted to this larger size samples and data output needs. We propose that the key needs for the sequencing analysis software in rare variants model studies are: 1) use of annotated reference sequences; 2) low false negative and false positive calls; 3) informative SNP and genotype reports that are ready for statistical analysis; 4) friendly interface for manual review of SNPs and genotypes; 5) correlation of identified variants with SNPs in dbSNP; 6) functional annotation of discovered variants; 7) speed and capacity to handle large amount of sequencing data; 8) Database to manage sequence data and analysis results. Using sequencing data from a 2 Kb region in 360 individuals, we evaluated the major current resequencing analysis software packages including: Mutation Surveyor 2.2 from SoftGenetics, Sequencher 4.6 from GeneCodes, SeqScape 2.5 from Appliedbiosystems, SeqMan 7.0 from DNASTAR, NovoSNP, SNPDetector, and Phred/Phrap/Polyphred/consed suite. Key parameters were compared among the software. Phred/phrap score-based Unix/Linux academic-free software outperforms commercial software in many aspects. However, the Unix environment might be a significant hurdle for many desktop computer users. By and large, all the existing software requires further development to work more efficiently for a complex disease rare variant project. For example, Polyphred cannot use annotation information from reference sequences while SeqScape is good at using annotation input. Function of checking of variants against dbSNP data is not available. High false positive rate seems to be a more serious problem than a false negative rate in our evaluation. We propose that future software development needs to address the eight needs listed above.

G protein-coupled receptor GPR143 mutation causes X-linked recessive inheritance congenital idiopathic nystagmus. *J.Y. Liu[1], X. Ren[1], X. Yang[2], T. Guo[2], Q. Yao[1], L. Li[3], L. Wang[4], X. Dai[1], Z. Cai[1], Z. Tang[1], M. Liu[1], Q.K. Wang[1,3]* 1) Human Genome Research Ctr, Huazhong Univ Sci & Tech, Wuhan, Hubei,China; 2) Development of Proof-Testing, Renmin Hospital of Tanghe, Tanghe, Henan, China; 3) Department of Molecular Cardiology, Lerner Research Institute, and Center for Cardiovascular Genetics, The Cleveland Clinic Foundation, Cleveland, OH, USA; 4) Eye Center, Peking University, Beijing, China.

Abstract: Objectives:Congenital idiopathic nystagmus (CIN) is characterized by involuntary, rhythmical, repeated oscillations of one or both eyes without other diseases. No specific gene has been identified for CIN. We studied a five-generation Chinese family with nystagmus as the sole phenotype to map and identify a disease-causing gene for CIN. Methods: Linkage analysis was used to identify the chromosomal location of the disease gene and direct DNA sequence analysis was used for mutation detection. Results: The family showed X-linked recessive inheritance. Using the family, a new genetic locus for CIN was mapped to an approximately 10.6 Mb region flanked by DXS996 and DXS7593 on Xp22 with a peak multipoint LOD score of 8.89. Analysis of 21 candidate genes in the region revealed a novel S89F mutation in the second transmembrane domain of GPR143, a G protein-coupled receptor. All male patients in the family carried the mutation, and the female carriers were heterozygous for the mutation. The S89F mutation was not identified in 200 normal controls. Interpretation: Our results identify a new genetic locus for congenital idiopathic nystagmus on chromosome Xp22 and indicate that mutations of GPR143 can be a cause of disease in families with nystagmus as the sole neurological manifestation. Key Words: GPR143 mutation; congenital idiopathic nystagmus; X-linked recessive inheritance.

A female patient with Aicardi syndrome and Duplication of Williams Syndrome Critical region(7q11.23). *P. Jayakar[1], J. Teppenberg[2], I. Gadi[2], M. Duchowny[1], J. Aicardi[3]* 1) Miami Childrens Hospital,Miami, FL; 2) Laboratory Corporation of America,Research Triangle Park, NC; 3) University College of London,London.

Aicardi syndrome (AS) is an X- linked dominant condition characterized by infantile spasms, callosal agenesis and chorioretinal 'lacunae'.The outcome of AS is severe, with a high mortality,considerable morbidity and usually a poor developmental outcome.A locus at Xp22.3 has been suggested but not confirmed.We present a 15 month-old non dysmorphic female who started having infantile spasms at age 3months. She is severely delayed, microcephalic and visually inattentive. EEG was consistent with infantile spasms. MRI brain showed closed lip schizencephaly on the left,operculum polymicrogyria on the right and agenesis of corpus callosum. Ophthalmology evaluation showed numerous round retinal lacunae consistent with Aicardi syndrome.Chromosomal analyses and metabolic newborn screen were normal.Comparative Genomic hybridization (CGH) performed, using a 434 BAC constitutional Spectral Genomics array, revealed a gain of DNA in 9contiguous BACs (clone RP5-1177A1,RP5-1127A24,RP11-622P13,RP11-17A14,RP4-439N19,CTB-51J22,CTB-52H6,CTA-27oD13,RP4-665P5) corresponding to a duplication of proximal 7q-arr cgh 7q11.23(RP5-1177A1-----> RP4-665P5)x3.The BAC duplication was confirmed by conventional FISH using a Williams syndrome critical region probe (WSCR,Vysis Inc.) for ELN, LIMK at 7q11.23.The FISH result showed 2 contiguous signals in the metaphase analysis and 3 signals on interphase analysis.Parental chromosomes were normal.The reported clinical phenotype of duplication of 7q11.23 is severe expressive speech delay and minor facial dysmorphic features. To our knowledge this is the first case of Aicardi syndrome phenotype associated with duplication of WSCR(7q11.23) genes. Additionally, the schizencephaly and polymicrogyria are atypical for either syndrome.CGH is a powerful molecular cytogenetic diagnostic tool, which may help define new genomic mechanisms of disease.With the molecular heterogeneity of AS we propose that all patients with atypical AS phenotype should undergo genetic evaluation and CGH testing.

Genetic Variations in the TORC2 Gene Are Not Associated with Type 2 Diabetes in Japanese. *P. Keshavarz, H. Inoue, M. Itakura* Division of genetic information, Institute for Genome research, The University of Tokushima, Tokushima, Japan.

Type 2 diabetes (T2D) often exhibits fasting hyperglycemia due to elevated hepatic gluconeogenesis. The transducer of regulated cAMP responsive element-binding protein 2 (TORC2; MIM 608972) plays a central role in transcriptional regulation of genes encoding gluconeogenic enzymes such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase). Interestingly, the human TORC2 gene is located on chromosome 1q21, an important candidate locus for T2D. We therefore hypothesized that genetic variation(s) in the TORC2 gene might affect the risk of developing T2D. To test this, we first screened all the TORC2 coding exons for single nucleotide polymorphisms (SNP) in 32 Japanese T2D patients. We identified seven SNPs, including one common (Met147Val) and three rare (Arg379Cys, Ala423Gly and Arg482Trp) non-synonymous SNPs, and a SNP in the 3' untranslated region. In addition, we selected an additional seven validated SNPs within and near the gene. Subsequently, these SNPs were genotyped in a large Japanese population comprising 911 unrelated patients with T2D and 876 control subjects. Single-locus tests of association between either SNP allele frequencies or SNP genotype frequencies and case-control status revealed no significant associations. The linkage disequilibrium (LD) analysis revealed a LD block (Lewontin's coefficient $|D'|>0.99$), covering the entire TORC2 gene region and spanning >22 kb in full length. Three common haplotypes were identified, accounting for >99% of chromosomes, and there were no statistically significant association between these haplotypes and T2D. These findings suggest that the SNPs in the TORC2 gene are unlikely to have major effects on susceptibility to T2D in Japanese.

Meiotic segregation and location of chromosome breakpoints in rare Robertsonian translocations: sperm studies and molecular cytogenetic studies in 7 cases of rare rearrangements. *K. Moradkhani[1, 2], J. Puechberty[2], S. Bhatt[1], G. Lefort[2], P. Sarda[2], S. Hamamah[3], F. Pellestor[1, 3]* 1) Cytogenetics Human Gametes, Inst Human Genetics, CNRS UPR 1142, Montpellier, France; 2) Department of Cytogenetics, CHU Arnaud de Villeneuve, Montpellier, France; 3) Department of Reproductive Biology B, CHU Arnaud de Villeneuve, Montpellier, France.

The mechanisms underlying the occurrence and the meiotic behaviour of rare Robertsonian translocations are poorly understood. To better elucidate the formation and the transmission of these rare chromosomal rearrangements, we have studied the sperm segregations and the breakpoint locations of 7 rare Robertsonian translocations. FISH analyses using LSP and WCP probes for chromosomes 13, 14, 15, 22, were performed on spermatozoa from the translocation carriers. The location of breakpoints was performed on chromosome preparations from the 7 patients. Different sets of centromeric-specific DNA probes were used to accurately identify breakpoint locations in acrocentric chromosomes. In all translocations, a high incidence of alternate segregation producing normal or balanced sperm was observed (78.5% to 93.2%) Significant variations in rates of imbalances resulting from the adjacent segregation mode were also noted (6.7% to 21.4%). Identification and comparison of breakpoints in the 7 translocations revealed a high degree of variability in location of breakpoints. The majority of breakpoints were located in the proximal short arms of the chromosome involved. This study shows that rare Robertsonian translocations display also a high predominance of alternate meiotic segregation over adjacent mode. However, significant variations in rates of imbalances exist in sperm, which could reflect variations in the formation and the meiotic behaviour of rare Robertsonian translocations. The high diversity of breakpoint locations in rare translocations might result in discernible variations in the production of unbalanced gametes. This could also be one of the keys to the variable alterations of spermatogenesis observed in Robertsonian translocation carriers. This study was supported by a reseach project PHRC (N7732) from the C.H.U. of Montpellier.

Generation of novel mouse models for Down syndrome using a human artificial chromosome (HAC) vector system. *J. Kudoh[1], K. Miyamoto[1], N. Suzuki[2], K. Sakai[1], S. Asakawa[1], M. Ikeno[2], T. Okazaki[2], N. Shimizu[1]* 1) Dept. Mol. Biol., Keio Univ. Sch. Med., Shinjuku-ku, Tokyo, Japan; 2) Inst. Compr. Med. Sci., Fujita Health Univ., Toyoake, Aichi, Japan.

Down syndrome (DS), or trisomy 21, is caused by the inheritance of three instead of two copies of human chromosome 21 (HC21). Individuals with DS show characteristic faces, mental retardation, congenital heart defect, increasing risk of leukemia in postnatal periods and early onset type of Alzheimer disease in adulthood. It is difficult to identify critical gene(s) for each phenotype because the number of partial trisomy 21 is limited and the phenotype is highly variable even with full trisomy 21. Therefore mouse models have been used to study phenotype-genotype correlations in DS. HC21 is homologous to three different mouse chromosomes (MMU): The 28 Mb region of HC21 is homologous to MMU16 (23.2 Mb), 1.6 Mb to MMU17 (1.1 Mb), and 3 Mb to MMU10 (2.3 Mb). The best studied mouse model Ts65Dn with partial trisomy MMU16 shows deficits in learning and memory, and display a variety of phenotypes that are seen in DS. However, the heart defect characteristic of DS has not been observed in Ts65Dn mice. So, we focus on genes in regions homologous to MMU17 and MMU10 to generate novel mouse models for DS. BAC clones containing the entire target gene(s) were selected and transferred into mouse embryonic stem (ES) cells using a human artificial chromosome (HAC) as a vector. Using these HAC containing ES cells, we are now producing mice harboring a HAC that carries human chromosome 21 gene(s). These novel mouse model will be useful to identify a gene(s) responsible for a phenotype(s) of DS and analyze gene-dosage effects at the levels of transcriptome, proteome and metabolome.

Array-based comparative genomic hybridization identifies high frequency of cryptic chromosomal rearrangements in patients with syndromic autism spectrum disorders. *ML. Jacquemont[1], S. Leclerq[1], D. Sanlaville[1], R. Redon[2], V. Malan[1], O. Raoul[1], V. Cormier-Daire[1], S. Lyonnet[1], J. Amiel[1], M. Le Merrer[1], D. Héron[3], MC. de Blois[1], M. Prieur[1], M. Vekemans[1], NP. Carter[2], A. Munnich[1], L. Colleaux[1], A. Philippe[1]* 1) Inserm U 781 & Departement de Genetique, Hopital Necker-Enfants Malades, Paris, France; 2) The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK; 3) Département de Génétique, Hôpital de la Pitié-Salpétrière, Paris, France.

Autism Spectrum Disorders (ASD) refer to a broader group of neurobiological conditions, Pervasive Developmental Disorders (PDD). They are characterized by a symptomatic triad associating qualitative alteration in social interactions, defect in communication abilities and repetitive and stereotyped interests and activities. Prevalence of ASD ranges from 1 to 3/1000 individuals. Despite several arguments for a strong genetic contribution, molecular basis of a majority of cases remain unexplained. About 5% of autistic patients have a chromosome abnormality visible with cytogenetic methods. The most frequent are 15q11-q13 duplication, 2q37 and 22q13.3 deletions. Many other chromosomal imbalances have been described. However most of them remain undetectable using routine karyotype analysis, thus impeding diagnosis and genetic counselling. We investigated 59 patients presenting with syndromic ASD using a DNA microarray constructed from large insert clones spaced at approximately 1 Mb intervals across the genome. Sixteen clinically relevant rearrangements were identified in 15 patients (25.4 %): 8 deletions and 7 duplications. Altered segments ranged in size from 200kb to 16 Mb (1 to 19 clones). No recurrent abnormality was identified. These results clearly demonstrate that array-CGH should be considered as an essential aspect of the genetic analysis of patients with syndromic ASD. Moreover, besides their importance for diagnosis and genetic counselling, they may allow the delineation of new contiguous gene syndromes associated with ASD. Finally, the detailed molecular analysis of the rearranged regions may open the way for the identification of new autistic spectrum disorders genes.

Providers knowledge of genetics: A survey of 5,915 individuals and families with genetic conditions. *E.K. Harvey*[1], *C.E. Fogel*[2], *K.D. Christensen*[3], *S.F. Terry*[3], *J.D. McInerney*[1] 1) NCHPEG, Lutherville, MD; 2) Gallaudet University, Washington, DC; 3) Genetic Alliance, Washington, DC.

Individuals affected by genetic conditions are increasingly seeking genetics-related information from their primary care providers rather than a geneticist. **Objective:** To analyze individuals and families experiences as patients of a variety non-genetics-trained healthcare providers. **Design:** Staff at the Genetic Alliance (GA) and National Coalition for Health Professional Education in Genetics worked with a University of Maryland graduate student in genetic counseling to design a web-based survey for patients and families with genetic conditions. We recruited study participants from genetic advocacy organizations that are members of the GA. A total of 5,915 respondents completed the survey between December 2004 and August 2005. **Outcome Measures:** Respondents assessments of their providers knowledge of the genetic condition in the respondents family; respondents self-assessed knowledge of genetics; respondents sources of genetics information; and factors contributing to positive and negative experiences with providers. **Results:** The distribution of poor knowledge scores assigned by respondents to 25 provider types was: minimum 16% (hematologist/oncologist), maximum 62% (emergency physician), median 31.2%, average 31.7%. The most frequently consulted provider type, family practice/primary care, received a poor knowledge score from 39% of respondents. Sixty-four percent of total respondents reported receiving no genetics-education materials from their providers. Respondents who saw a genetics specialist rated their own knowledge of the relevant genetics significantly higher than those who did not. Most respondents lacked confidence in their providers knowledge of the genetics related to the conditions in the respondents families, and most reported receiving no genetics-education materials from their providers. **Conclusion:** Educational institutions and professional societies should consider increasing and improving educational offerings in genetics to meet the needs of the increasing number of patients who require genetics-related services.

OST3 mutation in non-syndromic mental retardation expands the spectrum of Congenital Disorders of Glycosylation? *F. Molinari[1], S. Romano[1], W. Morelle[2], P. de Lonlay[1], A. Munnich[1], L. Colleaux[1]* 1) INSERM U781, Hopital Necker-Enfants Malades, Paris, France; 2) Unité Mixte de Recherche CNRS/USTL 8576, Université des Sciences et Technologies de Lille 1, Lille, France.

Congenital disorders of glycosylation (CDG) is a group of inherited disorders that affect glycoprotein biosynthesis. Eighteen different CDG types have been described so far and they are characterized by a central nervous system dysfunction and multi-organ involvment. Group I CDG results in the failure of assembly or transfer of the N-glycan chain while group II CDG is defined as defects in the processing of the protein-bound glycan. Recent studies have underlined the extreme clinical variability of CDG syndromes. Here, we report on a non syndromic mental retardation (MR) in two sibs born to first cousin French parents. Homozygosity mapping in the two mentally retarded sibs and the two healthy children led to the identification of a unique homozygous region of 8 Mb on 8p23.1-p22. This interval encompasses the gene *TUSC3/OST3* (*Ost3 S.cerevisae* homologue) encoding a protein involved in the oligosaccharyltransferase complex which catalyses the transfer of an oligosaccharide chain on nascent proteins, the key step of the N-Glycosylation. Sequencing the *OST3* gene identified a one base-pair insertion in exon 6, 787_788insC. The mutation co-segregated with the disease and resulted in a premature stop codon 37 codons downstream of the coding sequence (N263fsX300). Extensive work-up (Caryotype analysis, liver function, clotting factors, brain MRI, EEG) was unremarkable with normal isoelectric focusing and Western blotting assay of serum N-glycoproteins. Recent studies of glycoprotein fucosylation and polysialic acid (PSA) modification of neuronal cell adhesion molecules have shown the critical role of glycoproteins in synaptic plasticity (in particular on their glycan structures). However, our results provide the first demonstration that a defect in N-Glycosylation can result in non syndromic MR providing therefore new insights in the understanding of the pathophysiological bases of MR.

Peters' Plus syndrome is a congenital disorder of glycosylation caused by mutations in *B3GALTL*. *S.A.J. Lesnik Oberstein[1], M. Kriek[1], S.J. White[1], M.E. Kalf[1], K. Szuhai[2], J.T. den Dunnen[1], M.H. Breuning[1], R.C.M. Hennekam[3,4]* 1) Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands; 2) Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, The Netherlands; 3) Clinical and Molecular Genetics Unit, Institute of Child Health, London, United Kingdom; 4) Department of Pediatrics, Academic Medical Center, Amsterdam, The Netherlands.

The purpose of this study was to identify the genetic basis of Peters Plus syndrome, an autosomal recessive disorder characterized by anterior eye chamber defects, a disproportionate short stature, developmental delay, characteristic craniofacial features and cleft lip/palate.

To detect potential micro-rearrangements affecting the disease locus, we performed genome-wide 1 Mb resolution array-CGH (comparative genomic hybridization) on DNA of patients clinically diagnosed with Peters Plus syndrome. In two brothers, adjacent BAC clones were found to be present in a single copy, representing an approximately 1.5 Mb interstitial deletion on chromosome 13 (q12.3q13.1), spanning six genes. As none was an obvious candidate gene, we sequenced all exons and flanking sequences of these genes in one of the affected brothers. A point mutation (c.1020+1G>A) was detected in the beta 1,3-galactosyltransferase-like gene (HGNC symbol *B3GALTL*), within the donor splice site of exon 8. We subsequently performed targeted sequencing analysis for the presence of the c.1020+1G>A mutation in an additional 18 Peters Plus patients from 15 families and detected a homozygous c.1020+1G>A mutation in 16 of 18 patients. In the remaining two patients (siblings), a single c.1020+1G>A mutation was present. Upon sequencing the remainder of the gene we detected a second point mutation in intron 5 (c.437+5G>A). The deleterious effect of both mutations on normal splicing was confirmed by reverse-transcriptase PCR (RT-PCR) on patient material.

Peters Plus syndrome is hereby shown to be a monogenic disorder of glycosylation caused by mutations in *B3GALTL*.

Confirmation of Protective Haplotypes Spanning the CFH Gene in Age-related Macular Degeneration. *J.L. Haines[1], K. Spencer[1], L.M. Olson[1], M.A. Hauser[2], S. Schmidt[2], W.K. Scott[2], P. Gallins[2], N. Schnetz-Boutaud[1], A. Agarwal[3], E.A. Postel[4], M.A. Pericak-Vance[2]* 1) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN; 2) Center for Human Genetics and Department of Medicine, Duke University Medical Center, Durham, NC; 3) Vanderbilt Eye Institute, Vanderbilt University Medical Center, Nashville, TN; 4) Duke University Eye Center and Department of Ophthalmology, Duke University Medical Center, Durham, NC.

Age-related macular degeneration is a devastating disorder, which may result in legal blindness and adversely affects the quality of life for the nearly 2 million Americans suffering from the advanced forms of the disease. In addition to the well-known risk imparted by carrying the Y402H variant in the complement factor H (CFH) gene on chromosome 1, recent evidence for the existence of protective haplotypes spanning CFH has been reported (Hageman et al. 2005, Okamoto et al. 2006). Age-adjusted score statistics provided support for two of these protective haplotypes in our large dataset of 584 sporadic cases and 248 controls ($p<0.01$ and $p=0.069$). We also observed protective effects of these haplotypes in a completely independent family-based dataset with 127 multiplex families($p=0.021$ and $p=0.018$). Most interestingly, we found a marginally significant interaction between one of the protective haplotypes and ever/never smoking status, adding to the growing body of evidence that smoking is an important environmental covariate for AMD, which needs to be considered in genetic studies.

Co-chaperones of the glucocorticoid receptor and depression during pregnancy. *E.R. Katz[1,2], T.C. Deveau[2], D.J. Newport[2], Z.N. Stowe[2], J.F. Cubells[1,2], E.B. Binder[2]* 1) Department of Human Genetics, Emory University, Atlanta, GA; 2) Department of Psychiatry and Behavioral Sciences, Emory University, Atlanta, GA.

Depression during pregnancy is a major womens health concern. Pregnancy induces dramatic changes in the function of the hypothalamic-pituitary-adrenal axis, leading to elevated maternal cortisol levels. Several mechanisms may limit the activity of cortisol during pregnancy, one of them being increased glucocorticoid receptor (GR)-resistance, which is observed in approximately 80% of pregnant women. The absence of this adaptive response to increasing levels of cortisol may lead to a hypercortisolemic state, which may contribute to depression during pregnancy. One potential mechanism altering GR sensitivity in pregnancy is changes in the expression profile of GR co-chaperones, which have been shown to regulate GR signaling. To test the hypothesis that co-chaperone proteins are regulated throughout the peripartum period and that altered regulation of these proteins may contribute to depression, we investigated the peripheral-blood monocyte expression profiles of 12 co-chaperones of the GR in 8 women at 6 time points (pre-conception, 12, 24 and 36 weeks gestation and 4 and 12 weeks postpartum) and 8 depressed and 8 non-depressed women at 24 weeks gestation. During gestation, we observed a significant up-regulation of FKBP5 and NCOA1 expression, while FKBP4 and STUB1 were significantly down-regulated. Additionally, a significant up-regulation of GR expression itself was observed. The GR and its co-chaperone transcripts normalized by 12 weeks postpartum compared to preconception levels, except for PPIA, which exhibited significant up-regulation at both 4 and 12 weeks postpartum. Expression levels of FKBP5 and BAG1 were negatively correlated with depression scores at 24 weeks gestation. These data suggest that in peripartum depression, a mal-adaptive regulation of chaperone expression could oppose the pregnancy associated GR-resistance. The down-regulation of FKBP5 and BAG1 in women with peripartum depression would lead to less GR resistance, increasing the biologic activity of cortisol which may contribute to depression in pregnancy.

Making Policy for Our Children: The Advisory Committee on Heritable Disorders in Newborns and Children.

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The **Advisory Committee on Heritable Disorders and Genetic Diseases in Newborns and Children** was created by Congress to advise and guide the Secretary of the federal Department of Health and Human Services regarding the most appropriate application of childhood and universal newborn screening tests, technologies, policies, guidelines and programs for effectively reducing morbidity and mortality in newborns and children having or at risk for heritable disorders. This Committee began its work in 2003 and provides technical information to the Secretary HHS in order that the Secretary might develop policies and priorities to enhance the ability of State and local health agencies to provide for newborn and child screening, counseling and health services. The Committee also provides advice on a specific grant program delineated in its authorizing legislation. Coordination and operational services are provided by the Associate Administrator, MCHB, HRSA, with direction and guidance from the Assistant Secretary for Health.

The 15 members of the Committee are appointed by the Secretary and provide special expertise in the field of heritable disorders or in providing screening, counseling, testing or specialty services for newborns and children at risk for heritable disorders. The Committee also includes members of the public having special expertise about or concern with heritable disorders.

The early meetings of the Committee focused on the American College of Medical Genetics report which recommends an expanded and uniform newborn screening panel. The Committee accepted this report and unanimously recommended the report to the Secretary for his consideration and action. Currently the Committee is finalizing its process for nominating, evaluating and recommending screening tests for newborns and children for specific genetic disorders.

This presentation will outline that recommendation process and its implications for screening newborns and children. We will also address other relevant Committee activities.

Interaction of Hermansky Pudlak Proteins Within Biogenesis of Lysosome-related Organelle Complex-2. *P.K. Held, W. Westbroek, M. Ayub, H. Dorward, A. Helip-Wooley, M. Huizing, W.A. Gahl* MGB, SHBG, NHGRI/NIH, Bethesda, MD.

Hermansky Pudlak syndrome (HPS) is a rare, genetically heterogeneous autosomal recessive disease characterized by oculocutaneous albinism, prolonged bleeding, and occasional colitis or pulmonary fibrosis. The proteins encoded by HPS genes play a role in the biogenesis of lysosome-related organelles, but their precise biological functions remain elusive. Different HPS proteins interact with each other in BLOCs, i.e., Biogenesis of Lysosome-related Organelle Complexes. Specifically, human HPS3, HPS5, and HPS6 proteins interact in BLOC-2, but the mode of interaction has not been identified. It has been suggested that HPS5 and HPS6 directly interact, while HPS5 and HPS3 engage in an indirect interaction. In previous studies, we unsuccessfully attempted to confirm the direct interaction of human HPS5 and HPS6 by yeast two-hybrid screening; we speculate that yeast lack the machinery necessary for posttranslational modifications to enable interaction of the human proteins. Consequently, we employed a mammalian interaction system. Using the MAPPIT (MAmmalian Protein-Protein Interaction Trap) system, we demonstrated a strong, direct interaction between the HPS6 protein and the longer of two splice forms of HPS5. Immunoprecipitation studies in cultured human embryonic kidney cells confirmed the direct interaction of HPS6 with the long form of HPS5, which has a putative WD40 domain at its N-terminal site. This domain is interrupted in the short form of HPS5, and it may function as the platform for a protein-protein interaction with HPS6 and, perhaps, other proteins. Validating the mode of interaction between HPS proteins is critical to understanding how the BLOCs are formed and whether additional proteins exist within the different complexes. There are many patients who are clinically, but not genetically, diagnosed with HPS, suggesting that additional interacting proteins exist within the known BLOCs. We plan to use the MAPPIT system to identify these additional HPS proteins, with a goal of identifying the basic defect in HPS patients who do not have subtypes 1-8.

Natural history of aging in Cornelia de Lange syndrome. *A.D. Kline[1], P. Sponseller[2], C. Pichard[2], M. Grados[3], D. Tuchman[4], H. Levy[5], A. Kimball[1], N. Blagowidow[1]* 1) Harvey Institute Human Genetics, Greater Baltimore Medical Center, Baltimore, MD; 2) Department of Orthopaedics, Johns Hopkins University School of Medicine; 3) Department of Psychiatry, Johns Hopkins University School of Medicine; 4) Pediatric Gastroenterology, Sinai Hospital of Baltimore; 5) Department of Internal Medicine, Johns Hopkins University School of Medicine.

Findings of 44 patients with Cornelia de Lange syndrome (CdLS), seen in a multidisciplinary clinic for adolescents and adults, have allowed the following observations about the natural history. The patients ranged from 13 to 37 years, with 48% older than 17 years, and demonstrated a sex ratio of 1:1. Although most patients remain small, 15% develop obesity with age, most commonly in the truncal region. Gastroesophageal reflux persists or worsens, and there are early long-term sequelae, including development of Barrett esophagus in the teens and 20s. Over 35% have chronic sinusitis, of whom one-third have nasal polyps. Blepharitis improves with age, and both cataracts and detached retina have been noted. Osteopenia and osteoporosis have been observed, with occasional fractures. One third have leg length discrepancy. Low levels of vitamin D, estrogen and testosterone have been measured. Most females have delayed menarche and irregular menses, but normal gynecologic exams and pap smears. Although the phenotype is somewhat variable, a distinct pattern of facial changes with aging is evident. Premature gray hair is frequent; two patients have had cutis verticis gyrata. Behavioral issues and specific psychiatric diagnoses, including self-injury, anxiety, attention deficit disorder, autistic features, depression and obsessive-compulsive behavior, may arise or worsen with aging. These findings present evidence for accelerated aging in CdLS. Of the 45% who underwent mutation analysis, only a third demonstrated a detectable mutation in NIPBL or SMC1L1, and no genotype-phenotype correlations have been able to be made. Specific clinical management for adults with CdLS can be recommended.

Initial Analysis of Linkage Disequilibrium in the Mexican Mestizo Population. *A. Hidalgo-Miranda, I. Silva-Zolezzi, J. Estrada, E. Barrientos, S. March, L. del Bosque-Plata, O. Perez-Gonzalez, E. Balam-Ortiz, A. Contreras, A. Inchaustegui, C. Davila, L. Orozco, G. Jimenez-Sanchez* National Institute of Genomic Medicine, Mexico.

Mexico develops a national platform in genomic medicine to improve health of the Mexican population. A key component of this effort is the production of a haplotype map to improve the design of association studies for common diseases. The Mexican population has a unique genetic origin, more than 80% of the population is considered Mestizo, resulting from the admixture of any of 62 ethnic groups with the Spaniards in the last 500 years. We collected 1,200 blood samples from distant states of Mexico. From these, we analyzed 104 samples from Sonora (n=20), Yucatan (n=17), Guerrero (n=21), Zacatecas (n=19), Veracruz (n=18) and Guanajuato (n=9) using the 100K Affymetrix SNP array. Our goal was to determine minor allele frequency (MAF) distribution and estimate linkage disequilibrium (LD) decay with genomic distance, measured as the fraction of markers with highly correlated alleles using r^2 and D' (r^2>0.8, D'>0.8) in the Mexican population. We compared our results with the Affymetrix 100K SNP array data from the International HapMap Project. We included samples with a SNP call rate 95% using the Dynamic Model (DM) algorithm. Additional analysis with the Bayesian Robust Linear Model with Mahalanobis distance classifier (BRLMM) increased our SNP call rate to 97% Allele frequency distribution in these samples showed a similar pattern to that of other populations when MAF 15% In contrast, the percentage of markers in the 0-5% bin is among the lowest when compared to a similar analysis performed by Bonnen et al, 2006 on the European, Asian, African and Kosrean populations. Our results show similar LD decay as compared to the non-African populations included in the International HapMap Project. MAF distribution in this sample suggests a higher genetic heterogeneity in Mexican Mestizos compared to other populations. To increase the resolution of this analysis, 100 samples from each of these states are being analyzed using the Affymetrix 500K SNP array.

Values in conflict: U.S. public attitudes on embryonic stem cell research. *K.L. Hudson, D.J. Kaufman, R. Faden, J. Scott* Genetics & Public Policy Center, Berman Bioethics Institute, Johns Hopkins Univ, Washington, DC.

Purpose: The policy debate surrounding embryonic stem cell (ESC) research has been animated and divided by many moral issues. To assess the publics attitudes about ESC research, related moral issues, and the effect of using either the term *somatic cell nuclear transfer (SCNT)* or *cloning*, we surveyed 2,122 Americans.

Results: A majority of the sample (67%), which cut across most demographic groups, approved of ESC research using embryos remaining after IVF. A majority (59%) supported a federal policy for ESC research more permissive than the current policy while 22% favored retention of the current U.S. policy and 16% would ban ESC research. Attitudes towards ESC research were not explained solely by views on the moral status of embryos. For example, fully one third of those giving a week-old embryo in a Petri dish maximum moral status nonetheless approved of ESC research and supported relaxing federal ESC research policy. Similarly, 17% of those affording the embryo no/low moral status disapproved of ESC research.

The sample was divided and half were asked questions about *SCNT* and the other half about *cloning*. Both terms were defined identically. When the process of creating embryos for ESC research was called *cloning*, 29% approved of its use, while 46% approved when *SCNT* was used ($p<0.0001$). Approval for using *SCNT* to make a child was twice as high as approval for using *cloning* (24% vs. 10%, $p=0.004$).

Conclusion: Wide support for ESC research in the US cuts across political, religious and socioeconomic lines. Unlike the polarized views expressed in the policy debate, for many Americans, opinions on ESC do not mirror their attitudes about the moral status of the human embryo. Even though defined identically, use of the term SCNT or cloning resulted in vastly different stated opinions. SCNT was associated with much higher levels of support for both ESC research and reproductive purposes.

Counseling Implications in Familial 3q29 Microdeletion Syndrome. *S. Jamal[1], XL. Huang[1], HFL. Mark[1,2], JM. Milunsky[1,3,4]* 1) Center for Human Genetics, Boston University School of Medicine; 2) Dept. of Pathology, BUSM; 3) Dept. of Pediatrics, BUSM; 4) Dept. of Genetics and Genomics, BUSM.

3q29 microdeletion syndrome [MIM 609425] is a newly identified condition that was first described in 2001 in a 2 month old with moderate mental retardation, facial dysmorphism, horseshoe kidney, and hypospadias. Six other patients have been identified as having this syndrome with all determinable cases being reported as isolated/*de novo*. We present the first known familial case of two siblings with 3q29 microdeletion syndrome. The proband, aged 4 years 10 months, presented with mild mental retardation, significant speech delay, and atypical behaviors. Her growth parameters are between the 25-50th %ile. She is noted to have dysmorphic features including: a long and narrow face, broad nasal bridge, upslanting palpebral fissures, deep philtrum, micrognathia, pectus excavatum, and ligamentous laxity. She also has a right ear pit, posteriorly rotated small ears, history of recurrent otitis media, and bilateral conductive hearing loss. Renal ultrasound was normal. The probands brother, aged 2 years, presented with global developmental delay, significant speech delay, and possible autistic spectrum disorder. His growth parameters are at the 50th %ile. He is noted to have dysmorphic features including: a long and narrow face, broad nasal bridge, long philtrum, carp-shaped mouth, micrognathia, and pes planus. He also has a left ear tag, borderline low set ears, and possible bilateral conductive hearing loss. High resolution chromosome and subtelomere FISH analysis on blood samples of both sibs revealed a deletion of 3q29: 46,XX,del(3)(q29).ish(pcp3q-,wcp3+) and 46,XY,del(3)(q29).ish(pcp3q-,wcp3+), respectively. Maternal high resolution chromosome and subtelomere FISH analysis were normal. A paternal blood sample was not available for analysis. We describe the first known case of familial 3q29 microdeletion syndrome. Describing these two affected siblings will further elucidate the clinical phenotype and significance associated with 3q29 microdeletion syndrome and have significant implications for genetic counseling.

Implications for Clinical Management in Incomplete WAGR(O) Syndrome with an Atypical 11p13 Deletion. *XL. Huang[1], S. Jamal[1], HFL. Mark[1,2], TA. Maher[1], JM. Milunsky[1,3,4]* 1) Center for Human Genetics, Boston University School of Medicine; 2) Dept. of Pathology, BUSM; 3) Dept. of Pediatrics, BUSM; 4) Dept. of Genetics and Genomics, BUSM.

WAGR syndrome is a rare, yet well described contiguous gene disorder typically characterized by Wilms tumor, Aniridia, Genitourinary abnormalities, and Mental Retardation [MIM 194072]. A diagnosis requires the presence of aniridia and at least one other major acronymous criterion in conjunction with a constitutional chromosomal deletion at 11p13, the location of the PAX6 and WT1 genes. Deficiencies in the PAX6 gene result in ocular, central nervous system, and pancreatic abnormalities. Abnormalities in the WT1 gene result in an increased risk for Wilms tumor, nephropathy, and genitourinary abnormalities. We present a 19 year old female with multiple ocular abnormalities including: bilateral aniridia, corneal opacities, optic nerve hypoplasia, microcornea, ptosis, and a history of strabismus and nystagmus, as well as microcephaly, bilateral SNHL, short stature, patent ductus arteriosus repair, marked obesity, dextroscoliosis, mental retardation, and behavioral problems. Several urinalyses and renal ultrasounds were normal. High resolution chromosomal analysis revealed a deletion between 11p11.2-12 or 11p13-14. BAC-FISH analysis revealed an interstitial chromosomal deletion: 46,XX,del(11)(p13p14.3). This 9.8Mb deletion encompasses *at least* the PAX6 gene promoter and distal genes. The WT1 gene, although typically deleted in patients with WAGR syndrome, was present in our patient. Consequently, these findings are compatible with the absence of genitourinary abnormalities and no evidence for an increased risk of developing Wilms tumor in our patient. We present a patient with an atypical 11p13 deletion with clinical features consistent with WAGR(O) syndrome. Elucidating the particular region of 11p that is absent in patients with WAGR(O) syndrome has significant clinical implications since individuals with the WT1 gene present do not require Wilms tumor surveillance. This patient provides further elucidation of WAGR(O) syndrome and the value of FISH determination of the genotype.

Association Analysis of vitamin D binding protein (DBP) Gene Polymorphisms with Variations of Obesity Related Traits in Caucasian Nuclear Families. *H. Jiang[1,3*], D.H. Xiong[2,3*], Y.F. Guo[1], F. Yang[1,3], Y. Chen[1,3], F. Zhang[3,4], H.W. Deng[1,3,4], *The first two authors contributed equally to this work.* 1) Laboratory of Molecular and Statistical Genetics, Hunan Normal University, Changsha, Hunan, P R China; 2) Departments of Orthopedic Surgery and Basic Medical Sciences, University of Missouri - Kansas City, Kansas City, MO,USA; 3) Osteoporosis Research Center and Department of Biomedical Sciences, Creighton University, Omaha, NE, USA; 4) The Key Laboratory of Biomedical Information Engineering of Ministry of Education and Institute of Molecular Genetics, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an, P R China.

Vitamin D binding protein (DBP) gene is well known for its function on glucose and vitamin D metabolism in human populations. Previous studies suggested that the in vivo DBP level may play a role in the etiology of obesity. However, few studies explored the contribution of DBP gene to the variance of obesity phenotypes. In the present study, we investigate the relationship of DBP polymorphisms and obesity in Caucasian nuclear families. We genotyped fourteen SNPs (Single Nucleotide Polymorphisms) located in and around the DBP gene in 1873 Caucasian subjects from 405 nuclear families. Three obesity-related quantitative phenotypes were investigated, including body mass index (BMI), fat mass and percentage of fat mass (PFM). Single SNPs and haplotypes (three blocks) were tested by family based association using the FBAT software. SNP2 (rs17467825) and its corresponding haplotype GAA (frequency 0.270) in block1 showed strongest associations with PFM (P=0.0011 and 0.0023, respectively). In multivariate test significant association was also observed for SNP2 with fat_mass & BMI & PFM (P=0.0098). Subsequent sex-stratified analyses demonstrated nominal association for SNP2 and haplotype GAA with PFM in the female subgroup. Polymorphisms of DBP gene were significantly association with human PFM, especially in female, suggesting the importance of DBP gene in the pathogenesis of human obesity.

Dysregulation of sodium channel 4 subunit by expanded polyglutamine. *F. Oyama, H. Miyazaki, K. Okamura, Y. Machida, M. Kurosawa, T. Sakurai, N. Nukina* Lab Structural Neuropathology, RIKEN Brain Science Inst, Saitama, Japan.

Sodium channel 4 (4) is an auxiliary subunit of the voltage gated-sodium channels. We have identified 4 as an EST that was significantly downregulated in the striatum of HD model mice and found that reduction in 4 started at a presymptomatic stage of the HD model mice. In contrast, spinal cord neurons, which generate only negligible levels of expanded polyQ aggregates, maintained normal levels of 4 expression even at the symptomatic stage. Expanded polyQ with NLS expression suppressed the promoter activity of 4 gene in PC12 cells. Forskolin, an activator of the cAMP/PKA pathway, did not affect 4 promoter activity, indicating that 4 is not cAMP-responsive gene. These findings strongly suggest that sodium channel 4 subunit is a novel molecule, which is an upstream non-cAMP-responsive gene in HD pathogenesis.

PharmGKB - Accentuating the Knowledge in the Knowledge Bases. *T. Klein, C. Thorn, T. Boussard, M. Carrillo, J. Hebert, A. Hodge, M. Woon, J. Gallerani, M. Gong, W. Gor, D. Holbert, M. Kiuchi, F. Liu, A. MacBride, T.C. Truong, T. Zhou, R. Altman* Dept Genetics, Stanford Univ, Stanford, CA 94305-5120.

The mission of the Pharmacogenetics and Pharmacogenomics Knowledge Base (PharmGKB; http://www.pharmgkb.org) is to catalyze pharmacogenetic and pharmacogenomic research. The PharmGKB collects, organizes, and disseminates data and knowledge about pharmacogenetics and pharmacogenomics. It is focused on supporting hypothesis generation.

In order to deliver optimal health care, scientists must understand the mechanisms of human drug response. The relevant literature is extensive, but does not efficiently support hypothesis generation, particularly when considering large data sets. Scientists therefore require data resources that 1) provide links to successful experimental approaches, 2) provide a starting point for studying analogous systems, 3) set standards of evidence in the field, 4) allow data to be pooled for increased power, and 5) store public data and knowledge in an archival manner. The PharmGKB is designed to provide these resources.

The PharmGKB team has created an infrastructure composed of data, curated knowledge, software, and hardware. The PharmGKB is expected to attract important public data sets relating to the variation in drug response, as it becomes the major repository for pathways of drug action and metabolism. We are helping to define standards for phenotype data exchange. Because PharmGKB maintains data associated with single individuals, it must protect the confidentiality and privacy of study subjects.

The PharmGKB is part of the NIH/NIGMS Pharmacogenetics Research Network (U01GM61374).

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Assessment of allele-specific gene silencing by siRNA duplexes and short hairpin RNAs with wild and mutant-type reporter alleles. *Y. Ohnishi[1,2], M. Yoshida[2], Y. Tamura[2], K. Tokunaga[1], H. Kimura[2], H. Hohjoh[2]* 1) Dept Human Genetics, Univ Tokyo, Tokyo, Japan; 2) National Institute of Neurosience, NCNP, Tokyo, Japan.

Allele-specific gene silencing by RNA interference (RNAi) is therapeutically useful for specifically suppressing the expression of alleles associated with disease. To realize such allele-specific RNAi (ASP-RNAi), the design and assessment of small interfering RNA (siRNA) duplexes conferring ASP-RNAi is vital, but is also difficult. We have developed an easy evaluation system, which depends on heterozygous reporter plasmids encoding the *Photinus* and *Renilla luciferase* genes, for siRNA duplexes conferring ASP-RNAi. The system could allow assessment, if designed siRNA duplexes have the potential for specifically inhibiting the expression of target alleles without suppressing the expression of other alleles. Using the system with the *Swedish APP* variant related to familial Alzheimers disease as a model, we examined effects of various synthetic siRNA duplexes and also of their structural modifications on ASP-RNAi. Based on the results, we further constructed shRNA expression vectors for a long lasting ASP-RNAi. The results, however, indicated that the effect of shRNA on ASP-RNAi was different from those of synthetic siRNA duplexes; this may be caused by possibly mixed siRNA duplexes derived from shRNA by various Dicer digestion. To improve ASP-RNAi with shRNA, we introduced base substitutions in siRNA sequences so that the resultant siRNAs could strongly discriminate mutant allele from wild type one. From a series of screening of siRNAs carrying various substitutions by using the system described above, we found competent siRNA duplexes conferring a strong ASP-RNAi. Based on the observations, construction of new shRNA expression vectors is now in progress. In this meeting, we would like to discuss base substitutions in siRNA sequence, by which ASP-RNAi could be enhanced, and the system with reporter alleles to assess ASP-RNAi.

Regulation of a sense/antisense transcript within TAF1/DYT3 that contains a sequence change (C>T) exclusively found in patients with X-linked dystonia-parkinsonism. *U. Müller, T. Herzfeld, J.A. Kress, D. Nolte* Institute of Human Genetics, Justus-Liebig University, Giessen, Germany.

X-linked dystonia parkinsonism (XDP) is associated with disease-specific sequence changes (DSCs) within TAF1/DYT3. TAF1/DYT3 encodes multiple transcripts including four major variants (var.1-var.4) relevant to XDP (Nolte et al., 2003). Var.1 and var.2 include exons of TAF1 (TATA-box binding protein-associated factor 1) in addition to exons not previously recognized as parts of the TAF1 gene (referred to as exons 1-5). Var. 3 and var.4 are encoded by exons 2-4 and exons 3-4, respectively. Exon 4 is utilized by all four major transcript variants and includes a C>T exchange (DSC3) in patients exclusively. We performed functional analyses of exons 2-4 encoding transcript variant 3. In silico analysis of the 5'region of exon 2 revealed several sites for human transcription factors including Sp1, HIF-1, MEF-2, Ikaros, and c-Ets-2. We cloned fragments upstream of exon 2 of various sizes (1644bp, 639bp, 253bp, 137bp) in pGL3/luciferase. Expression studies in U87 and NT2/D1 cells demonstrated that the two larger but not the smaller fragments activated luciferase and thus contain the functional promoter (referred to as 5'var.3 promoter). Coexpression of Ikaros isoform 2 in cells containing either the 1644bp or the 639bp fragment resulted in promoter suppression. Additional functional analyses including HIF-1 and MEF-2c did not affect promoter activity. The 3'end of exon 4 contains a LTR promoter with strong activity in antisense direction. This promoter is a member of the HERV9 family and is included in a 521 bp fragment that is functional in the luciferase assay. We are currently exploring whether the LTR promoter regulates other promoters of the TAF1/DYT3 transcript system, such as the 5' var.3 promoter, and whether DSC3 affects this potential regulation. Nolte D, Niemann S, Müller U: Specific sequence changes in multiple transcript system DYT3 are associated with X-linked dystonia parkinsonism. Proc. Natl. Acad. Sci (USA) 100: 10347-10352 (2003).

ENCODE Multiple Species Sequencing Data elucidate the early history of Mammals and allow the detection of evolutionary characteristics in Hominoid lineage. *S. Nikolaev[1], J. Montoya-Borgos[2], E.H. Margulies[3], NISC Comparative Sequencing Program[3,4], J. Rougemont[5], B. Nyffeler[5], S.E. Antonarakis[1]* 1) Genetic Medicine and Development, University of Geneva, Switzerland; 2) Animal Biology, University of Geneva. Switzerland; 3) Genome Technology Branch, National Human Genome Research Institute, NIH, Bethesda, 20892, USA; 4) NIH Intramural Sequencing Center, National Human Genome Research Institute, NIH, Bethesda, 20892, USA; 5) Vital-It team, Swiss Institute of Bioinformatics, Lausanne. Switzerland.

Understanding the early evolution of placental mammals remains the last challenging issue in mammalian phylogeny. Here we address this question by using the sequence data of the ENCODE consortium which include orthologous regions of genomes in 18 species belonging to all main mammalian lineages. Phylogenetic reconstructions based on an unprecedented amount of Coding Sequences (CDS) taken from 218 genes resulted in a highly supported tree placing the root of Eutheria between Afrotheria and Exafroplacentalia (Boreoutheria and Xenarthra). This topology was validated by the phylogenetic analysis of a new class of genomic phylogenetic markers, conserved non-coding sequences (CNC). Applying the Shimodaira-Hasegawa test on the CDS dataset resulted in the rejection of the Atlantogenata theory (Xenarthra grouping with Afrotheria), while this test rejected the second alternative scenario, the Epitheria theory (Xenarthra at the base), when performed on the CNC dataset. Thus the two datasets contain complementary phylogenetic signal, and only taken together settle the debate about the position of the eutherian root. Using phylogenetic reconstructions based on CNCs, non-synonymous (dN) and silent (dS) substitutions from CDS, we show that, within mammals, primates display a slowdown of evolutionary rates in all three genomic features. Hominids and particularly humans show the most severe slowdown, which is consistent with the longer generation time. Despite the observed general slowdown, hominoids and particularly humans have undergone a relaxation of purifying selection acting on constrained elements, which is consistent with the smaller effective population size found in these lineages.

Malignant Hyperthermia Susceptibility: Diagnostic procedure and novel RYR1 mutations. *S. Levano, M. Singer, S. Treves, A. Urwyler, Th. Girard* Departments of Anesthesia and Research, University Hospital, Basel, Switzerland.

Malignant hyperthermia (MH) is a dominantly inherited pharmacogenetic disorder of skeletal muscle whose diagnosis is established by the in-vitro contracture test (IVCT). This method is invasive, not applicable to young children, handicapped and elderly persons leading many researchers to refine and develop new diagnostic procedures. Since the introduction of new EMHG guidelines in 2001 implementing genetic testing in those families where causative mutations in the ryanodine receptor type 1 (RYR1) have been confirmed, we have found that 40 % of the Swiss MH families harbour 8 of 23 causative RYR1 mutations. More importantly, in Switzerland, in 2001 more patients were diagnosed MH susceptible (MHS) by molecular genetic methods than the IVCT (29 vs 14, respectively): the V2168M RYR1 mutation was the most frequent causative mutation and has been found in 16 Swiss families. Since in humans MH is a heterogenetic disease, there is a need to find new RYR1 causative mutations in order to include them in a genetic based-MH diagnostic assay. In the present report we screened the whole RYR1 cDNA in 36 samples and describe the genotype and phenotype correlation of 4 novel potentially causative mutations. The nucleotide substitutions alter the encoded amino acids that are highly conserved throughout evolution as well as in the different human RYR isoforms. We found good concordance between the segregation of the novel mutations among three generations and the MH phenotypes based on the IVCT. The R2336H RYR1 substitution was detected in 9 out of 36 families in 26 out of 30 individuals who were diagnosed as MHS. The other 4 MHS individuals were excluded from this analysis because of no direct inheritance. The D2730G, E2404K and D544Y RYR1 substitutions were each found in single families and all 25 MHS subjects who were diagnosed as MHS by the IVCT, were mutation carriers. All 83 MH normal (MHN) individuals in the 12 families were not carriers for these novel mutations and the mutations were not found in 100 control samples. The functional effect of these four novel mutations is currently under investigation.

Association between the severity of angiographic coronary artery disease and paraoxonase-1 promoter gene polymorphismT(-107)C in Iranian population. *E. Javadi[1,2], A. Jalilian[2], M. Doosti[2], P. Amiri[1], B. Shariati[3]* 1) Shariati hospital, EMRC(Endocrine and Metabolism Research Center), Tehan, Tehran, Iran; 2) Department of Medical Biochemistry,school of medicine of Tehran university,Tehran ,Iran; 3) Department of community medicine,school of medicine,Tehran university of medical science,Tehran,Iran.

Increased plasma low-density lipoprotein-cholestrol(LDL-C)levels are associated with enhanced LDL oxidation that represents and additional risk for coronary artery disease(CAD).Paraoxonase-1(PON1)is an enzyme associated with the high-density lipoprotein(HDL)particle.It catalyses the hydrolysis of organophosphates and protects LDL from oxidative modification in vitro by hydrolyzing lipid peroxides,suggestive of a role for PON1 in the development of CAD.The present study tested the hypothesis that PON1 promoter plymorphismT(-107)C could be a risk factor for CAD in Iranian patients.Paraoxonase promoter genotypes were determined in 300 consecutive subjects(>40 years old)who underwent coronary angiography(150 subjects with 50% stenosis served as cases[CAD+]and 150 subjects with <20% stenosis served as controls[CAD-].Paraoxonae promoter genotypes were determined by PCR and BSTU1 restriction enzyme digestion .CAD+ subjects did not show any significant difference in the distribution of PON1 promoter genotypes as compared to CAD- subjects(p=.075)However the analysis of PON1 promoter genotypes distribution showed an higher percentage of (-107)TT among CAD+ compared with CAD-(P=.027).After controlling for other risk factors,the T(-107)C polymorphism had correlation with age(p=.027).After controlling for other risk factors,the T(-107)C polymorphism had correlation with age(p=.012),but did not show any correlation with other risk factors such as BMI,gender,smoking,diabetes,level of HDL-C,LDL-C,TG and TC.These data suggest that the TT genotype may represent a genetic risk factor for coronary artery disease in Iranian population.

Decreased expression of Hsp27 associated with cell toxicity in a cellular model of Machado-Joseph Disease. *M. Hsieh[1, 2], W.H. Chang[1], F.C. Wen[1]* 1) Dept Life Sci, Tunghai Univ, Taichung Taiwan, Taiwan; 2) Life Science Research Center, Tunghai Univ, Taichung, Taiwan.

Machado-Joseph disease (MJD) is an autosomal dominant spinocerebellar degeneration caused by the expansion of a polyglutamine tract within the gene product, ataxin-3. We have previously shown that increased oxidative stress and decreased expression of Hsp27 may be contributory factors for the pathogenesis of Machado-Joseph disease. In this study, we utilized neuroblastoma SK-N-SH cells stably transfected with full-length MJD with 78 polyglutamine repeats to further investigate the mechanism resulting in decreased expression of Hsp27 and how endogenous expression of Hsp27 influences neuronal cell survival. Results from semi-quantitative RT-PCR and 35S-methionine pulse-chase labeling revealed that decreased expression of Hsp27 in mutant MJD cells is due to defects in protein synthesis. These findings provide the first evidence that mutant ataxin-3 may be involved in deregulation of protein synthesis. In addition, in contrast to Hsp70, we demonstrated that Hsp27 degradation in SK-N-SH cells does not go through proteasome degradation pathway. Furthermore, t-butyl hydroperoxide was used to assess the tolerance of different passages of cells expressing mutant ataxin-3. Our results indicated that earlier passages of mutant cells were less tolerant to exogenous oxidative stress, which may be due to the decreased endogenous Hsp27. Consistently, we showed that over-expression of Hsp27 desensitizes SK-N-SH-MJD78 cells to poly-Q toxicity. These results support a model that full-length mutant ataxin-3 expression does significantly impair cells ability to respond to stresses, which may be partly due to decreased expression of Hsp27 together with increased cell toxicity in cells expressing mutant ataxin-3.

Further support for *KIAA0319* as a susceptibility gene for developmental dyslexia. *D. Harold[1], S. Paracchini[2], T. Scerri[2], M. Dennis[2,3], N. Cope[1], G. Hill[1], V. Moskvina[1], J. Walter[2], A.J. Richardson[2], M.J. Owen[1], J.F. Stein[2], E.D. Green[3], M.C. ODonovan[1], J. Williams[1], A.P. Monaco[2]* 1) Cardiff University, UK; 2) University of Oxford, UK; 3) National Human Genome Research Institute, Bethesda, MD.

The DYX2 locus on chromosome 6p22 is the most replicated region of linkage to developmental dyslexia (DD). Two candidate genes within this region have been implicated in the disorder: *KIAA0319* and *DCDC2*. Five variants within *DCDC2* have shown association with DD in a US and a German sample. We genotyped these variants in two independent UK samples from Cardiff and Oxford. Although nominally significant associations were detected between three *DCDC2* SNPs and reading traits in the Oxford sample, no association with DD was observed for any *DCDC2* variant in the Cardiff sample.

Having previously found evidence that variation in *KIAA0319* confers susceptibility to DD, we sought to refine this association by genotyping 36 additional SNPs in the gene. Ten SNPs, predominantly clustered around the first exon, showed the most significant association with DD in one or both UK samples, including rs3212236 in the 5flanking region ($P = 0.00003$) and rs761100 in intron 1 ($P = 0.0004$). The location of this association may indicate the presence of susceptibility variants influencing gene regulation.

The KIAA0319 and DCDC2 proteins share functional similarities: both appear to play a role in neuronal migration, a mechanism that has been implicated in the development of DD. We tested for epistatic interactions between variants in the two genes and observed a significant interaction between rs793862 in *DCDC2* and rs761100 in *KIAA0319* ($P = 0.007$).

Thus our data provide further support for *KIAA0319* as a DD susceptibility gene in the UK population. The evidence implicating *DCDC2* is inconsistent, but it may contribute to DD susceptibility through epistatic interactions with *KIAA0319*.

A Novel CFTR Mutation in a Korean Infant with Cystic Fibrosis. *J.H. Lee, G-H. Kim, J.M. Ko, H-W. Yoo*
Pediatrics, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea.

Cystic fibrosis (CF) is the most common lethal autosomal recessive disease in Caucasians, but rare in Asians. The mutations of cystic fibrosis transmembrane conductance regulator (CFTR) gene are responsible for CF. Up to date, 3 cases of CF have been reported and only one of them diagnosed based on the genotype of the CFTR gene. We encountered a 4-month-old Korean Infant with CF and his diagnosis was confirmed by CFTR gene mutation analysis. The patient was born to health parents of non-consanguineous marriage. The patient underwent surgical operation, due to meconium ileus at birth. He had chronic respiratory symptom, failure to thrive, fatty liver with hepatomegaly, and cholestasis. The mutations of the CFTR gene were identified in the patient and his parents. The patient was a compound heterozygote with a nonsense mutation of c.263T>G, resulting in an amino acid change of p.Leu88X in exon 3. It was previously described in a Korean patient with CF. The other is a novel mutation; c.2089-2090insA mutation (p.Arg697Lysfsx33) in exon 14. The mutation c.263T>G was inherited from his father, and the c.2089-2090insA mutation from his mother. His parents were apparently healthy carriers. We report a novel CFTR mutation in a Korean infant with CF.

Expression profile pathology of Alzheimer disease model mice is enhanced by low omega-3 polyunsaturated fatty acid diet: synapse is the intersection of the disease and diet. *T. Morihara[1], B. Teter[2], S. Ball[2], G.P. Lim[2], F. Calon[2], L. Zhao[2], F. Yang[2], M. Takeda[2], S.A. Frautschy[2], G.M. Cole[2]* 1) Psychiatry, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; 2) Neurology, University of California Los Angeles, North Hills, CA.

Background: Alzheimer Disease (AD) is a complex disease, which is affected by environmental factors. Diet including 3 polyunsaturated fatty acid is the one of the important environmental factors that were shown to impact on AD by epidemiological studies. We used AD model animals (APP transgenic mice), which allow to control environmental factors. Four gropes, APP Tg mice with standard chow, with low 3 chow and non Tg mice with standard chow, with low 3 chow were analyzed. We previously reported that the amyloid pathology which is the central pathology of AD was enhanced by low 3 diet in APP Tg mice (*J Neurosci* 2005 25:3032, *Neuron* 2004 43:633).

Results: To address the underlying interaction between these two complex factors, AD and diet, microarray analysis were performed. The number of genes affected by diet was 117 in APP Tg mice, which was 17 times more than in Tg-. The number of genes affected by APP Tg on the animals with standard diet was 305. In the animals with low 3 diet, 604 genes were affected by APP Tg. These observations suggested that low 3 diet and APP transgene enhance their expression profiles change each other. To reveal what kinds of genes were commonly changed by diet and APP transgene, GO annotation analysis were performed using EASE. 74 genes which were commonly changed by diet and APP transgene were compared to the genes generally affected by diet (117 genes) or APP transgene (604 genes). The enriched GO annotations in the commonly changed 74 genes were transmission of nerve impulse and synaptic transmission.

Conclusion: The two complex factors APP transgene (AD causative mutation) and low 3 diet enhance their expression profile each other. Synapse was identified as the intersection of the two factors.

A Fourth Phenotype for Autosomal Dominant Hypercholesterolemia. *A. Marques[1], M. Abifadel[1], J. Bonneau[1], K. Ougerram[2], Y. Zair[2], M. Devillers[1], D. Erlich[1], M. Krempf[2], C. Junien[1], A. Munnich[1], J-P. Rabès[1], C. Boileau[1], M. Varret[1]* 1) INSERM U781, Hôpital Necker-Enfants Malades, Paris, France; 2) INSERM UR539, Hôtel-Dieu, Nantes, France.

Autosomal Dominant Hypercholesterolemia (ADH), characterized by isolated elevation of LDL-cholesterol, is associated with high risk of premature cardiovascular disease. Three genes have already been implicated : LDLR (low density lipoprotein receptor), APOB (apolipoprotein B-100) and PCSK9 (proprotein convertase subtilysin kexin-like 9). We now report a large French ADH family in which involvement of these three genes was excluded and named the pathology HCHOLA4 in accordance with the official nomenclature. Our aim is to identify the new disease gene and to define the associated pathophysiology. A whole-genome scan, using 232 polymorphic microsatellite markers, located the HCHOLA4 gene at 16q22.1 in a 5.31 cM interval. Functional candidate genes in the critical interval were tested by sequencing but no causal mutation was detected. In vivo kinetics of apolipoprotein B-100-containing lipoproteins, conducted in 2 affected members, mainly showed a decrease in LDL catabolism like as observed in LDLR mutation carriers. Q-PCR analysis of LDLR expression in EBV-transformed lymphoblasts, cultured in standard and cholesterol-deprived mediums, showed that cells of two affected subjects do not reply to cholesterol deprivation by activating LDLR expression contrary to controls in whom the expression rate increases 2-fold. This lack of reply to cholesterol deprivation has also been described in LDLR mutation carriers. These results suggest that this novel form of ADH is due to an alteration, direct or not, in LDL receptor endocytosis or intracellular traffic. Furthermore, we performed two-dimensional electrophoresis for cytosolic and membrane proteins from lymphoblasts and fibroblasts and observed different profiles for affected subjects when compared to non-affected relatives. Mass spectrometry for twenty of the more significatively different proteins is in process. We expect to identify one or more proteins for wich the coding gene is localized in the 16q22.1 interval of interest.

Investigation of Candidate Genes for Attention-Deficit/Hyperactivity Disorder in the Chromosome 5p12-q11 Region. *N. Laurin[1], A. Ickowicz[2], T. Pathare[2], M. Malone[2], R. Tannock[2], R. Schachar[2], J.L. Kennedy[3], C.L. Barr[1,2]* 1) Cell & Molecular Division, Toronto Western Research Institute, Toronto, ON, Canada; 2) Brain and Behaviour Programme, The Hospital for Sick Children,; 3) Centre for Addiction and Mental Health, Department of Psychiatry, University of Toronto, Toronto,ON, Canada.

Attention-deficit/hyperactivity disorder (ADHD) is a highly heritable childhood-onset disorders characterized by age-inappropriate levels of inattention, hyperactivity and impulsivity. Three independent genome scans aimed at uncovering major risk genes for ADHD have been performed using affected sib pairs. Region 5p12-q11 was the only region presenting evidence of linkage in all screens. The aim of this research was to investigate candidate genes located in this region for their involvement in ADHD in our sample of families from Toronto. Four genes were selected according to their role in brain functions, their interaction with systems known to be important in ADHD, their expression pattern and/or results from animal studies. These are the genes for glial-derived neurotropic factor (GDNF), islet-1 (ISL-1), the hyperpolarization activated cyclic nucleotide-gated potassium channel-1 (HCN1) and a glial glutamate transporter (SLC1A3) previously implicated in ADHD. A total of 15 SNPs across these genes were genotyped and analyzed using the transmission disequilibrium test (TDT). The study sample consisted of 258 nuclear families ascertained through a proband with ADHD (311 affected children). Analysis of individual markers did not yield significant association for any of the markers. Moreover, no bias was revealed for any of the haplotypes observed for each gene. Finally, when analyzed in relation to dimensional symptom scores of inattention and hyperactivity/impulsivity, no strong association was demonstrated for individual markers or haplotypes. Our results do not support major involvement of ISL-1, GDNF or HCN1 in ADHD in our sample of families. In addition, we did not replicate previous finding implicating SLC1A3 in ADHD. Further genetic studies for susceptibility candidates in this region are necessary in order to identify a causal gene.

Genetic variation in myosin IXB is associated with ulcerative colitis. *K.A. Hunt[1], A.A. van Bodegraven[2], C.R. Curley[3], A.J. Monsuur[4], R.J. Playford[1], C.G. Mathew[5], C. Wijmenga[4], J.D. Rioux[3], D.A. van Heel[1], M.J. Daly[3]* 1) Institute of Cell and Molecular Science, QMUL, London, UK; 2) VU University Medical Centre, Amsterdam, The Netherlands; 3) The Broad Institute, Cambridge, MA; 4) University Medical Center Utrecht, The Netherlands; 5) King's College London School of Medicine, London, UK.

Background: Common variations in the 3 region of myosin IXB (MYO9B) have recently been associated with celiac disease (Monsuur, Nat Genet 2005). The mechanism by which these variations lead to increased susceptibility in celiac disease is unclear. MYO9B is known to be an atypical motor protein with a Rho-GTPase domain, is expressed in leucocytes and intestinal epithelial cells, and is involved in cytoskeletal modification and tight junction assembly.

Aims: We hypothesised that MYO9B variants might also promote susceptibility to other intestinal inflammatory diseases, including Crohns disease and ulcerative colitis.

Methods: 8 tag SNPs for the 3 MYO9B region were studied in three independently collected and genotyped inflammatory bowel disease case-control cohorts of Northern European descent, comprising in total 2717 cases (1197 Crohns disease, 1520 ulcerative colitis) and 4440 controls.

Results: Common variation in MYO9B was found to be significantly associated with inflammatory bowel disease in all cohorts examined (most associated SNP rs1545620, meta-analysis P = 1.9 x 10-6, OR=1.2), with the same alleles showing association as reported for celiac disease.

Conclusions: Interestingly, rs1545620 causes an amino acid change (Ala1011Ser) in the third calmodulin binding IQ domain of MYO9B. Unlike previous variants (in other genes) predisposing to inflammatory bowel disease, the association at MYO9B is considerably stronger with ulcerative colitis, although weaker association with Crohns disease is also observed. These data imply shared causal mechanisms underlying intestinal inflammatory diseases, and potentially other auto-immune diseases.

Clinical and molecular investigation of a patient with atypical Noonan syndrome. *A-M. Nystrom[1], G. Annerén[1], B. Stromberg[2], M-L. Bondeson[1]* 1) Department of Genetics and Pathology, Uppsala University, Rudbeck Laboratory, Uppsala, Sweden; 2) Women's and Children's Health, Uppsala University, Uppsala, Sweden.

The autosomal dominant disorder Noonan syndrome (NS) is characterised by heart defects, facial dysmorphism and short stature. Gain-of-function mutations of the gene *PTPN11* on 12q24 have been identified in around 50% of the NS patients and recently, missense mutations in *KRAS* on 12p12.1, have also been found to cause NS. Clinical overlap between NS and Neurofibromatosis type 1 (NF1) is well known. NF1 is characterised by cutaneous neurofibromas, cafe'-au-lait spots, Lisch nodules and freckling of axillary and inguinal regions and is caused by haploinsufficiency of the *NF1* gene, mapped to 17q11.2. Here, we report the clinical and molecular investigation of a patient with an atypical and severe form of NS. Besides the classical NS phenotype, the proband suffer from mild mental retardation, a type I Arnold Chiari malformation, scoliosis, malrotation of the intestines and of the genito-urinary tract, right-sided hydronephrosis and café-au-lait spots. The probands sister and father presented with café-au-lait spots (CALS) but with no other clinical manifestations of NS. Mutation analysis of the *PTPN11* gene revealed a recurrent *de novo* mutation, 853 T>C; F285L in the proband that was absent in the father and the sister. The result thus suggests that the CALS and NS in this family are caused by distinct genetic entities. Linkage analysis and direct sequencing of the *NF1* gene revealed a variant, 5425 C>T; R1809C in the proband. The variant which co-segregated with the CALS in the family was absent in 150 control chromosomes. Both *PTPN11* and *NF1* operate in the same pathway suggesting that the alterations identified in the *PTPN11* and *NF1* genes in our NS patient could explain her severe and atypical symptoms.

An Extra Structurally Abnormal Chromosome derived from a t(17;22)pat without phenotypic consequences. *C. Morales-Peydro[1,2], E. Margarit[1], I. Madrigal[1], A. Soler[1], A. Sánchez[1]* 1) Servei Bioquímica i Genètica , Hospital Clínic , Barcelona, Spain; 2) Fundació Clínic, Hospital Clínic, Barcelona.

We report on a healthy 35 year-old male referred for genetic counselling because of familial antecedents of mental retardation (paternal uncle). G-banded chromosome analysis on peripheral blood revealed the presence of an Extra Structurally Abnormal Chromosome (ESAC) in all analysed cells. Cytogenetic evaluation of his parents and brother was performed; the karyotype of his mother and his brother were normal while the karyotype of the father was 46,XY,t(17;22)(p13.3;q12). The der(22) chromosome of the father was cytogenetically identical to the ESAC identified in the patient. Unfortunately, the mentally retarded uncle had died at the age of 55 years due to cerebral vascular disease without chromosomal study. Additional analyses were performed to confirm the origin of the ESAC. Fluorescence in Situ Hibridation (FISH) showed the presence of 3 signals of TUPLE-1 probe (22q11), one of them hybridising on the marker. Multiplex Ligation-dependent Probe Amplification (MLPA) analysis, carried out using commercial subtelomeric MLPA kits (MCR-Holland), detected subtelomeric trisomy of 17p and 22p. These results confirmed the origin of the marker as a derivative chromosome 22, consequence of an abnormal segregation of the paternal t(17;22). The triplication of the 22q11 region is compatible with the Cat Eye syndrome, a chromosome anomaly that presents high variability in its clinical expression, including unaffected individuals. However, the most surprising finding was the lack of phenotypic repercussion of the 17p subtelomeric trisomy. In order to discard the existence of a normal cell line, due to the lost of the ESAC during post-zygotic development, a cytogenetic analysis of cultured epithelial cells exfoliated from the urinary tract has been performed. Exhaustive examination tests (abdominal ultrasound scan, cranial magnetic resonance and ophthalmologic and cardiac exploration) have been recommended to discard undetected anomalies. In the present case, providing genetic counselling is difficult due to the unpredictable clinical consequences of the ESAC in the next generation.

The Ghosal disease gene maps to chromosome 7q33. *B. Isidor[1], N. Dagoneau[1], C. Huber[1], D. Genevieve[1], B. Bader-Meunier[2], S. Blanche[3], C. Picard[3], M.C. De Vernejoul[4], A. Munnich[1], M. Le Merrer[1], V. Cormier-Daire[1]* 1) Dept of Genetics and INSERM U781, Necker Hospital, Paris, France; 2) Dept of Pediatrics, Kremlin Bicetre Hospital, France; 3) Dept of Pediatric Hematology, Necker Hospital, Paris, France; 4) Rhumatology, Lariboisiere Hospital, Paris, France.

Increased bone density disorders are a heterogenous group of conditions resulting from an imbalance between bone resorption and bone formation. The international classification of bone disorders has divided these conditions into two groups 1)those with no modification of bone shape and 2)those with metaphyseal and/or diaphyseal involvement. Among this last group, Ghosal disease also called hematodiaphyseal dysplasia(GHDD, OMIM 231095) is a rare autosomal recessive disorder first described in 1988 by Ghosal in five patients and further delineated in 1993 by Gumruck in two siblings. GHDD is characterized by diaphyseal dysplasia with some metaphyseal involvement in association with aregenerative corticosensitive anemia. The main differential diagnosis is Camurati-Engelmann disease (OMIM 131300), an autosomal dominant disorder due to TGF mutations, differing from GHDD by the absence of anemia and the presence of a marfanoid habitus, with muscular weakness and pain. We have had the opportunity to collect the DNA samples of two inbred families originating from Algeria and Tunisia and including five affected individuals ranging in age from 18 months to 34 years. All of them fulfilled the diagnostic criteria for GHDD. The presenting symptom in all the children (3/5) was pallor related to anemia while in one of the adults, anemia was detected by the systematic blood count. Using an homozygosity mapping strategy, we found linkage of the disease locus to chromosome 7q33(Zmax=4.21 at =0 at locus D7S2513) in a 3.4Mb interval defined by loci D7S2560 and AC 091742-GT. This region encompasses 34 genes and six of them were regarded as candidate genes based on their putative function i.e. ATP6V0A4, RAB19B, HIPK2, MLD1,BRAF, PTN. All these genes were excluded by direct sequencing. We hope that ongoing studies will help to solve the pathogenesis of corticosensitive anemia and diaphyseal dysplasia.

A second family with COG7 deficiency reveals a consistent phenotype including microcephaly, adducted thumbs, growth retardation, VSD and episodes of hyperthermia. *E. Morava[1], R. Zeevaert[2], E. Korsch[3], D.J. Lefeber[4], K. Huijben[4], S. Wopereis[4], G. Matthijs[2], R. Wevers[4]* 1) Department of Pediatrics, University Medical Center Nijmegen, Nijmegen, The Netherlands; 2) Department of Human Genetics, University of Leuven, Belgium; 3) Pediatric Hospital Amsterdamerstrasse, Köln, Germany; 4) Laboratory of Pediatrics and Neurology, University Medical Center Nijmegen, Nijmegen, The Netherlands.

Here we describe the clinical and biochemical characteristics of two additional patients diagnosed with Congenital Disorder of Glycosylation type II due to a defect in COG7; one of the 8 subunits of the Conserved Oligomeric Golgi complex. The patients presented with growth retardation, progressive, severe microcephaly, hypotonia, adducted thumbs, feeding problems by gastrointestinal pseudoobstruction, failure to thrive, cardiac anomalies, wrinkled skin and episodes of extreme hyperthermia. A combined disorder in the biosynthesis of N- and O- linked glycosylation with hyposialylation was detected. Western blot analysis showed a severe reduction in the COG5 and COG7 subunits of the Conserved Oligomeric Golgi complex. A homozygous, intronic splice site mutation (c.169+4A>C) of the COG7 gene was identified. The children presented with a similar phenotype as those previously described with the same mutation, except for the lack of skeletal anomalies and only a mild liver involvement in our patients. We suggest performing protein glycosylation studies and western blot for the different COG subunits followed by sequence analysis in children with progressive microcephaly, growth retardation, hypotonia, adducted thumbs and cardiac defects, especially in association with skin anomalies or episodes of hyperthermia.

Androgen insensitivity syndrome: several novel mutations in the androgen receptor gene associated with sex determination. *O.T. Mueller[1,2], H. Mason-Suares[3], R. Hitchcock[1], A. Root[2]* 1) Molecular Genetics Section, All Childrens Hospital, St. Petersburg, FL; 2) Pediatrics Department, University of South Florida, St. Petersburg, FL; 3) Eckerd College, St. Petersburg, FL.

Androgen Insensitivity Syndrome (AIS) is caused by loss-of-function mutations in the androgen receptor gene (AR). Mutations in AR result in variable clinically phenotypes from that of a clinically normal female with a blind vaginal pouch (complete AIS, CAIS), to an incompletely virilized male with ambiguous genitalia (partial AIS, PAIS), to sub-virilized males often with sub-fertility and gynecomastia (mild AIS, MAIS). In this report, we present mutation analysis of AR in seventy individuals with a 46,XY karyotype in whom the diagnosis of AIS was suspected. Pathogenic mutations in AR were identified in 19 patients, 10 of which have not been previously reported. Mutations detected included 12 amino acid substitution (missense) mutations, two chain termination (nonsense) mutations, three small deletions or insertions causinga reading frameshift and premature termination, one in-frame codon deletion, and one base change that created a novel cryptic donor splice site resulting in exon skipping. Two mutations including one nonsense and one missense were detected as mosaics with the normal sequence in the peripheral white blood cells analyzed. These cases of mosaicism have an ambiguous phenotype suggesting that somatic mutation may be a significant cause of phenotypic variability in partial AIS. Missense mutations were evaluated using online SIFT and PolyPhen assessment tools which use phylogenetic conservation of amino acid changes to predict structural and functional effects. In 8 of the 12 missense mutations, both tools supported a pathogenic effect. However, in the remaining four cases these tools were either not in agreement, or conflicted with the phenotype, and were not useful for interpretative purposes.

A powerful method for testing haplotype-disease association in genomewide studies. *B.E. Huang[1], C.I. Amos[2], D.Y. Lin[1]* 1) Department of Biostatistics, Univ North Carolina, Chapel Hill, NC; 2) Department of Epidemiology, MD Anderson Cancer Center, Houston, TX.

The analysis of genomewide association studies requires methods that are both computationally feasible and statistically powerful. Given the large-scale collection of single nucleotide polymorphisms (SNPs), one cannot afford to ignore the information contained in their interrelationships. In particular, utilizing haplotypes rather than individual SNPs and accounting for correlations in adjustment for multiple testing can lead to increased power. We have developed a statistically powerful and numerically efficient method for detecting haplotype-disease association in genomewide studies by sliding windows of SNPs over the genome. This method consists of an efficient algorithm to calculate a proper likelihood-ratio statistic for any given window of SNPs, along with an accurate and efficient Monte Carlo procedure to adjust for multiple testing. Simulation studies based on the HapMap data showed that the proposed method performs well in realistic situations. We applied the new method to a case-control study of 2,300 SNPs on chromosome 18 to test for association with rheumatoid arthritis. Several loci were identified as having possible effects on the disease, none of which would have been detected with existing methods.

Whole genome microarray analysis of gene expression in an imprinting center deletion mouse model of Prader-Willi syndrome. *N. Kibiryeva[1], D.C. Bittel[1], R.A. White[1], D.J. Driscoll[2], M.G. Butler[1]* 1) Children's Mercy Hospital and University of Missouri-Kansas City, Kansas City, MO; 2) University of Florida, Gainesville, FL.

Prader-Willi syndrome (PWS) is due to loss of paternally expressed genes in the 15q11-q13 region which directly or indirectly causes dysregulation that controls neurodevelopment and function. Little is known about the global effects on gene expression that might result. To further characterize genetic alterations we used whole genome microarrays to analyze gene expression in brain tissue from a PWS mouse model (on the C57BL/6J strain) resulting from a paternally derived imprinting center deletion (PWS IC deletion). Of more than 45,000 probes examined, 59% were detectable and 69 had a significant change in expression of at least 1.5 fold with a false discovery rate of 5% in brain of PWS IC deletion mice relative to normal littermates less than 24 hours old. The known paternally expressed genes from the PWS critical region had detection signals below the threshold in the PWS IC deletion mice but were clearly detectable in control littermates. Three previously unstudied transcripts, AK013560, BB314814 and BB182944, located in the mouse PWS critical region, chromosome 7B, were expressed only from the paternal allele and apparently under regulatory control of the imprinting center. The three genes with the highest expression relative to control littermates were pro-opiomelanocortin (*Pomc*, an obesity related gene) and two transcripts of unknown function. Upregulation of the *Pomc* gene has been reported previously in brain tissue from PWS deletion mice (TgPWS) and may contribute to their failure to survive. In addition, *Mc5r* gene (involved in eating behavior and obesity) was also upregulated in our PWS IC deletion mice relative to control littermates. Several of these genes were studied by quantitative RT-PCR which confirmed their expression status. In addition, a small cluster of genes on mouse chromosome 18B3 was reported to be upregulated by haploinsufficiency of the 7B region in TgPWS deletion mice. However, we did not find evidence of changed expression of these genes in our PWS IC mouse model.

Zoom-in CGH arrays for the characterization of variable breakpoint contiguous gene syndromes. *J.J. Johnston, R.L. Walker, S. Davis, P.S. Meltzer, L.G. Biesecker* NHGRI/NIH, Bethesda, MD.

Contiguous gene syndromes cause pathology via haploinsufficiency for adjacent genes. Some contiguous gene syndromes have stereotypical breakpoints, but others have variable breakpoints. In the latter, the extent of the deletions may be correlated with severity. The Greig cephalopolysyndactyly contiguous gene syndrome (GCPS-CGS) is a multiple malformation syndrome caused by haploinsufficiency of *GLI3* and adjacent genes. As well, non-CGS GCPS can be caused by deletions or duplications within *GLI3*. Although FISH can identify large deletion mutations in patients with GCPS or GCPS-CGS, it is not practical for identification of small intragenic deletions or insertions and it is difficult to accurately characterize the extent of the large deletions using this technique. We have designed a custom CGH array that allows identification of deletions and duplications at kilobase resolution in the vicinity of *GLI3*. The array averages one probe every 730 basepairs for a total of ~14,000 probes over 10 Mb. We have analyzed 16 individuals with known or suspected deletions/duplications. In 15/16 individuals (14 deletions and one duplication) the array confirmed the prior results. In the remaining patient, the normal array CGH result was correct and the prior assessment was a false positive qPCR. We conclude that high-density CGH array analysis is more sensitive than FISH analysis for deletion detection and provides clinically useful results on the extent of the deletion. We suggest that high-density array CGH analysis should replace FISH analysis for assessment of deletions and duplications in patients with contiguous gene syndromes caused by variable deletions.

Educational and Outreach Programs for Genomic Medicine in Mexico. *S. March, A. Lopez, J. Bedolla, C. Davila, V. Castellanos, A. Hidalgo-Miranda, I. Silva-Zolezzi, G. Jimenez-Sanchez* National Institute of Genomic Medicine, Mexico.

Mexico develops a national platform in genomic medicine. For this, the Mexican Congress created the National Institute of Genomic Medicine (INMEGEN) that develops scientific research in genomic medicine and has developed different educational programs for a wide range of groups, including graduate students and postdoctoral trainees, medical students, elementary and secondary school kids, as well as the general public. As a part of its graduate programs INMEGEN developed the first courses in genomics for Latin America: Introduction to genomic medicine, Genomic applications in medical pediatrics and Genomic applications in internal medicine. In these courses students develop research proposals using genomic tools to conduct research on a national health problem. In 2006 we initiated life broadcast to universities in 60% of the states of Mexico. We broadcast in real time academic lectures to audiences in Mexico, Central and South America, and Spain. In addition, we have developed and distributed over a dozen educational materials in Spanish related to genomic medicine. We produced a three comic series aimed to target population ages 11-14 yrs. The purpose of this effort is to offer basic education about the human genome, genomic applications to health care, as well as the ethical and legal implications of genomic medicine. These educational programs include a web-based component (www.inmegen.gob.mx) that offers information and services, including materials from our graduate courses, over 20 in-house publications, 100 video-lectures and several brochures. During 2005, our web portal received over 1.9 million visits and more than 1.3 million documents were downloaded from over 38 countries, mostly from Latin America. This electronic resource has turned into a major educational tool in genomic medicine for the Spanish-speaking world. In summary, Mexico develops a strong educational and outreach strategy as a key component of its national platform in genomic medicine. We predict that a knowledgeable society will be a major asset to successfully develop genomic medicine.

Using disease information in tagSNP selection for disease association mapping. *N. Liu* Dept of Biostatistics, Univ of Alabama at Birmingham, Birmingham, AL.

Selection of subsets of single nucleotide polymorphisms (SNPs) is important and challenging for genome wide association studies. Using such tagSNPs not only reduces cost in genotyping, but also increases efficiency and power in statistical analysis since redundant SNPs have been removed and the number of tests could be greatly reduced. In recent years tagSNP selection has drawn much attention and many methods have been proposed to effectively select these tagSNPs using various criteria. However, existing tagSNP selection methods do not take disease information into consideration and may result in inefficient selection of SNPs for the purpose of gene-disease association mapping. We propose a method for tagSNP selection that can take into account disease status in case-control studies. We first break down large marker sets into many disjoint pieces, and then use a tree-based method to search for tagSNPs. We evaluate the method using both simulated data and real data. The results show that the tagSNPs chosen using the proposed method have more power in certain situations.

Craniofacial And Growth Plate Abnormalities in a Mouse Model of Trichorhinophalangeal Syndrome Type I. *D. Napierala[1], K. Sam[1], R. Morello[1], Q. Zheng[1], T. Bertin[1], R. Shivdasani[3], B. Lee[1,2]* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Howard Hughes Medical Institute; 3) Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA.

To elucidate molecular mechanisms leading to Trichorhinophalangeal syndrome (TRPS) we studied Trps1 mutant mice with a deletion of the GATA-type DNA-binding domain of the Trps1 transcription factor (Trps1GT mice). Heterozygous Trps1GT mice phenocopy human TRPS type I. TRPS is a dominantly inherited craniofacial and skeletal dysplasia characterized by short stature, hip abnormalities, cone-shaped epiphyses, and premature closure of growth plates reflecting defects in endochondral ossification. 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. To quantify craniofacial changes in the Trps1GT mice we applied laser 3D surface scanning. This morphometric analysis revealed reduced size of mandible, and possibly also maxilla in the heterozygous Trps1GT mice. Consistent with a gene-dosage effect, the craniofacial abnormalities are more severe in homozygous Trps1GT mice. To elucidate the role of Trps1 in endochondral ossification we analyzed long bones of homozygous Trps1GT mice. Analysis of embryos across developmental stages revealed delayed onset of chondrocyte hypertrophy and slower progression of pre- and hypertrophic chondrocytes into terminal stage. Additionally, the Trps1 null mice demonstrated abnormal mineralization of the growth plate chondrocytes and perichondrium. Both mineralization and chondrocyte maturation are regulated by the Runx2 transcription factor, therefore we tested the potential interaction of Trps1 and Runx2 in vitro. Co-transfection experiments demonstrated that Trps1 represses Runx2-mediated transactivation of the reporter gene. Moreover, Trps1 forms complexes with binding sites of GATA transcription factors in the Runx2 promoter only in cells that do not express endogenous Runx2. Our data indicate that Trps1 regulates chondrocyte hypertrophy and mineralization and it could do so partially through repression of Runx2.

A genomewide search finds major susceptibility loci for nicotine dependence on chromosome 10 in African-Americans. *M.D. Li[1], T.J. Payne[2], J.Z. Ma[1], X.-Y. Lou[1], D. Zhang[1], R.T. Dupont[3], K.M. Crews[2], G. Somes[4], N.J. Williams[4], R.C. Elston[5]* 1) Psychiatric Medicine, University of Virginia, Charlottesville, VA; 2) ACT Center for Tobacco Treatment, Education & Research, University of Mississippi Medical Center, Jackson, MS; 3) Department of Criminology and Criminal Justice, University of Memphis, Memphis, TN; 4) Departments of Preventive Medicine and Dental Hygiene, University of Tennessee Health Science Center, Memphis, TN; 5) Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH.

Although several linkage studies on nicotine dependence (ND) have been conducted, all samples to date are primarily of European origin. In this study, we conducted a genomewide scan in 1261 individuals representing 402 nuclear families of African-American (AA) origin. We examined 401 microsatellite markers for ND, which was assessed by smoking quantity (SQ), the Heaviness of Smoking Index (HSI), and the Fagerström Test for ND (FTND). After performing linkage analyses using various methods implemented in the GeneHunter and S.A.G.E. programs, we found a region near marker D10S1432 on chromosome 10q22 between D10S1208 and D10S2470 that showed a significant linkage to Indexed SQ with a maximum LOD score of 4.17 at 92 cM, and suggestive linkage to HSI, SQ, and log-transformed SQ. Additionally, we identified three regions on chromosomes 9q31 at marker D9S1825 at 136 cM, 11p11 between markers D11S1993 and D11S1344, and 13q13 between markers D13S325 and D13S788 that met the criteria for suggestive linkage to at least one ND measure. Other locations on chromosomes 15p11, 17q25, and 18q12, exhibited some evidence of linkage (LOD >1.44). Some of these regions have been linked to smoking behavior at nominally significant levels in other studies, providing independent replication of the regions for ND in different cohorts. In summary, we found significant linkage on chromosome 10q22 and suggestive linkage on chromosomes 9, 11, and 13 for major genetic determinants of ND in an AA sample. Further analysis of these positive regions by fine mapping and/or association analysis is warranted.

Association of TNF -308 GA polymorphism and cancer risk. *K. Kohut[1], T. Kirchhoff[1], N. Ishill[2], A. Zelenetz[3], J. Lee[1], K. Lafaro[1], K. Offit[1, 3]* 1) Clinical Genetics Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY; 2) Department of Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, NY; 3) Lymphoma Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY.

BACKGROUND: In a recent study, the International Lymphoma Epidemiology Consortium analyzed 12 single nucleotide polymorphisms (SNPs) in 9 genes involved in immune and inflammatory processes, using 3586 cases of non-Hodgkin lymphoma and 4018 controls, from 8 centers in Europe, Canada, and U.S. The TNF -308 GA polymorphism was associated with increased risk of non-Hodgkin lymphoma, particularly diffuse large B-cell lymphoma. METHODS: To investigate whether geographically distinct cohorts resulted in biases due to population stratification, we compared population frequencies of the TNF -308 GA polymorphism in prior studies. We also analyzed 601 lymphoma, 780 breast cancer, 577 prostate cancer and 374 colon cancer cases along with 1039 unaffected controls in an Ashkenazi Jewish population, considered to be genetically homogeneous. TaqMan assay on ABI 7900 HT Sequence Detector and Sequence Detection Software 2.0 (Applied Biosystems) was used. Analysis included 2 or Fishers exact tests and unconditional logistic regression. RESULTS: Frequencies differed among geographically disparate cohorts. While there was no excess of A/A + G/A in diffuse large B-cell lymphomas in Ashkenazim (p=0.55), there was a trend for an excess of A/A genotypes (p=0.07). This finding supports association with this subtype of lymphoma, as observed by Rothman *et al*. A significant association of the TNF -308 GA allele with cancer risk was observed by performing simulations on data from prior studies. CONCLUSIONS: The G/A genotype was associated with prostate cancer risk (p=0.01), (OR 1.7 95%CI 1.04-2.8 (1 variant allele) and 3.6, 95% CI 0.4-34.6 (2 variant alleles), trend p=0.02). These findings are consistent with a prior observation of an increased risk in Korean prostate cancer cases heterozygous for G/A. We found no associations between G/A or A/A genotypes and risk of colon or breast cancer.*et al*.

Genetic and clinical epidemiology of pediatric idiopathic epilepsy in Newfoundland and Labrador. *K. Mahoney, S. Moore, D. Buckley, M. Alam, S. Penney, P. Parfrey* Memorial University of Newfoundland, St. John's, NL, Canada.

Epilepsy is a common neurological disorder with an incidence of 40 to 80 per 100 000. Approximately 40-60% of all cases of epilepsy are idiopathic. Most cases of idiopathic epilepsy follow complex inheritance patterns.
The objective of this study is to describe the incidence and genetic epidemiology of idiopathic epilepsy (IE) in Newfoundland.
All childhood cases of IE from 1997 to 2006 have been identified from the pediatric neurology clinic at the Janeway Hospital, the only childrens hospital in the province. Informed consent, family history, and medical information was obtained from parents of probands who are followed up in the clinic.
The incidence of IE for the population of children living on the Avalon region of the province from 1999 to 2003 was 80 per 100 000. This represents 56 newly diagnosed cases in a population of 13 779. This rate is approximately two to three fold greater than most populations of children in other developed regions.
121 individuals (116 families) are being followed up in the pediatric neurology clinic and are eligible to enter the study. 97 families were contacted and 86 agreed to participate. 72 of these families have provided family history data. To date, DNA samples have been obtained from 19 families.
At least 6 pedigrees are compatible with autosomal dominant inheritance with three or more affected individuals in three generations within three degrees of relation to the proband. 43 pedigrees have 3 or more affected individuals beyond three degrees of relation to the proband and thus are compatible with autosomal recessive or polygenic inheritance. In the remaining 23 pedigrees, the proband is the only known affected individual.
Further molecular studies are being undertaken in one family in which the pedigree is strongly supportive of autosomal dominant inheritance.
The incidence of epilepsy is high in Newfoundland and the genetic contribution to the etiology of epilepsy is also high.

Mass Spectrometric Identification of Proteins in Patients with Platelet Storage Pool Deficiencies. *D.M. Maynard[1], H.F.G. Heijnen[2], M.K. Horne[3], J.G. White[4], H.D. Edwards[1], L.C. Riney[1], A. Helip-Wooley[1], W.A. Gahl[1]* 1) SHBG, MGB, NHGRI, NIH, Bethesda, MD; 2) Dept. of Hematology and Cell Biology, Univ. Medical Center, Utrecht, The Netherlands; 3) Hematology Service of the Dept. of Laboratory Medicine, W.G. Magnuson Clinical Center, NIH, Bethesda, MD; 4) Univ. of Minnesota, Depts. of Laboratory Medicine and Pathology and Pediatrics, Minneapolis, MN.

Platelets contain three types of secretory organelles, i.e., alpha granules, delta granules and lysosomes. These organelles have specific molecular compositions, genetic diseases, ultrastructural morphology, kinetics of exocytosis, and secretory responses to different stimuli. A deficiency in granule-bound substances in platelets causes a group of congenital bleeding disorders known as storage pool deficiencies (SPDs). For some disorders, such as Hermansky-Pudlak syndrome (HPS), most of the genetic bases have been determined. For others, such as -SPD (Gray Platelet syndrome), only the clinical and histological states have been defined. In order to understand the molecular bases for such genetic disorders, we are using proteomics and mass spectrometry combined with biochemical verification to identify causative gene products and potential interacting partners. After platelet disruption, sub-cellular organelles were separated using sucrose gradient ultracentrifugation. Granule fractions were confirmed by electron microscopy and Western blotting using antibodies to granule-specific proteins. Protein fractions were separated by 1-D SDS-PAGE. Proteins bands were excised, digested in-gel with trypsin, and resulting peptides were analyzed by 1D-LC-MS/MS. Raw files were submitted to the NIH Mascot cluster using Mascot Daemon and were searched against the SwissProt-Trembl database. DBParser was used for data processing and report generation. Preliminary mass spectrometry data from control samples confirms the presence of alpha granule-specific proteins in the fraction identified by Western blotting and EM. Further experiments are underway to analyze other granule fractions and to investigate samples from patients with a variety of storage pool deficiencies.

Triplication of 4q32.1q32.1: A new syndrome with minimal clinial findings and Hirschprung disease? *M.J.M.Trip Nowaczyk[1], J.C. Wang[1], I. Teshima[2]* 1) Dept of Pathology and Molecular Medicine, McMaster Univ., Hamilton, Canada; 2) Dept of Pediatric Lab Medicine, Hospital for Sick Children Dept of Pediatrics, Univ. of Toronto, Toronto, Canada.

We present three brothers and their mother with triplication of 4q. They present with minimal facial anomalies resembling velocardiofacial syndrome or myotonic dystrophy. The oldest boy has hydrocephalus (HCP), right sided ptosis, the middle one has Hirschprung disease (HD) and the third has HD, HCP and bilateral talipes equinovarus. The three sons share with their mother the following facial features: long midface, myopathic face, wide nasal bridge, short nose, short palpebral fissures, and low set, small and squared off ears. All four have mild developmental delay. Cytogenetic analysis showed an abnormal chromosome 4 in the four patients. At 850-band resolution the segments between 4q31.3 and 4q32 were duplicated. FISH analysis of metaphase and interphase cells with labeled BAC probes showed three signals for clone RP11-192D11 (located within 4q32.1) on the rearranged chromosome. Only one signal for the other probes located within 4q31.3, 4q32.2, 4q32.3, and 4q33 was observed suggesting that the band 4q32.1 was triplicated. Thus, the karyotypes for the boys were 46,XY,dup(4)(q31.?3q3?2)mat.ish trp(4)(q32.1q32.1)(RP11-192D11+++). There are no previous reports of 4q triplication; there are more than 65 reports of duplications 4q, of which 23 can be classified as pure. Our patients closely resemble the three patients with dup 4q31.1q32.3 reported by Goodman et al. [1997] suggesting a phenotype associated with multiple doses of this band. Four previously reported patients with 4q duplications had HD: two with duplication resulting from t(4;14)(q31.3;p11.2) who had a very mild phenotype similar to our patients; one with complex rearrangement involving monosomy 1pter; and one with monosomy 9qter. The last two patients had multiple congenital anomalies and mental retardation, likely as a result of the associated monosomies. It is possible that a gene or genes at 4q32.1 may be involved in pathogenesis of HD. It also appears that multiple doses of band 4q32.1 are relatively well-tolerated.

Association of genetic variations in the *G-CSF* gene with lung function decline and cross-sectional levels in smoking induced COPD. *J. He[1], K. Shumansky[1], J.E. Connett[2], N.R. Anthonisen[3], P.D. Paré[1], A.J. Sandford[1]* 1) James Hogg iCAPTURE Ctr, Univ British Columbia, Vancouver, BC, Canada; 2) Division of Biostatistics, School of Public Health, University of Minnesota, Minneapolis, Minnesota, USA; 3) Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada.

Background: Granulocyte colony-stimulating factor (G-CSF) or CSF3 is an important survival and proliferation factor for neutrophils. Neutrophils release proteinases that break down connective tissue in the lung parenchyma and contribute to emphysema and decline of lung function. Recently, it was shown that a single nucleotide polymorphism (SNP) was associated with peripheral blood granulocyte count among workers exposed to benzene. Objective: To determine whether SNPs and haplotypes of *CSF3* are associated with change or level of lung function in smoking induced COPD. Methods: Three SNPs of *CSF3* were studied in 587 non-Hispanic whites who had the fastest (n = 281) or the slowest (n = 306) decline of lung function selected from among continuous smokers followed for 5 years in the NHLBI Lung Health Study (LHS). These SNPs were also studied in 1074 non-Hispanic whites with the lowest (n = 536) or the highest (n = 538) baseline lung function at the beginning of the LHS. Results: The *CSF3* -1719C/T was associated with baseline lung function in an additive model, odds ratio (OR) = 0.73, 95% confidence interval (CI) = 0.56 to 0.95, P = 0.018 after adjustment for confounding factors and was still significant after adjustment for multiple comparisons. There was also a significant association of *CSF3* haplotype with baseline FEV_1 levels (globe test p = 0.004 and 0.027 before and after adjustment for confounding factors, respectively). Conclusion: Genetic variations in *CSF3* were associated with lung function in COPD patients. Supported by the NHLBI Lung Health Study and the Canadian Institutes of Health Research.

A potential therapy for inherited neuropathy: Oral curcumin mitigates the clinical and neuropathologic phenotype of the *Trembler-J* mouse. *M. Khajavi, K. Shiga, W. Wiszniewski, J. Yan, G.J. Snipes, J.R. Lupski* Dept of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.

There have been tremendous advances in our understanding of the molecular genetic bases of inherited peripheral neuropathies during the last decade and a half. This has revolutionized diagnostics, enabling accurate and secure diagnosis, precise recurrent risk estimates, prenatal diagnosis and better management of patients. However, there has been a complete failure to translate these findings in molecular based therapeutics. 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. Here, we demonstrate that oral administration of curcumin, a dietary supplement, partially mitigates the severe neuropathy phenotype of the *Trembler-J* mouse model in a dose dependent manner. 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. Administering curcumin significantly decreases the percentage of apoptotic Schwann cells and results in increased number and size of myelinated axons in sciatic nerves leading to improved motor performance. Our findings indicate that oral curcumin supplementation is apparently sufficient to relieve the toxic effect of mutant aggregation induced apoptosis and improves the neuropathologic phenotype in mice suggesting a potential therapeutic role in selected forms of inherited peripheral neuropathy.

Mitochondrial haplogroups are associated with asthma and total serum IgE levels. *B.J. Klanderman[1,2], S. Mazza[1], J.C. Celedon[1,2,3], S.T. Weiss[1,2], B.A. Raby[1,2,3]* 1) Channing Laboratory, Department of Medicine, Brigham & Women's Hospital, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) Division of Pulmonary and Critical Care Medicine, Beth Israel Deaconess Medical Center, Boston, MA.

Maternal history of asthma and/or atopy is a major risk factor for the subsequent development of asthma and allergy in childhood. Although mitochondrial mutations have been implicated in several maternally inherited monogenic disorders, no studies of mitochondrial polymorphisms and asthma have been reported. We evaluated whether common mitochondrial haplogroups are associated with asthma and total serum IgE levels. 8 common mitochondrial single nucleotide polymorphisms (mtSNP) were genotyped in two cohorts of European ancestry: 512 adult women with incident asthma and 517 matching controls participating in the Nurses' Health Study (NHS) and 654 children ages 5-12 years with mild to moderate asthma participating in the Childhood Asthma Management Program (CAMP). Genotyping was performed using TaqMan probe hybridization assays. 93 random NHS samples were run in duplicate for all assays and demonstrated 100% concordance. In the CAMP Study, genotype data from probands mothers was also 100% concordant across all assays. Completion rates in both cohorts were > 95% for all markers. mtSNP 9055 was seen at higher frequency in NHS asthma cases (frequency 11.1%) than controls (8.0%, p = 0.02). Association analysis using haplo.score identified two haplogroups associated with asthma: one haplogroup at a frequency of 3.83% among cases compared to 1.27% among controls (p=0.0002) and another at a frequency of 9.97% among cases and 11.3% among controls (p=0.04). The CAMP Study is a case-only (family-based) cohort, thus precluding evaluation of mitochondrial SNP associations with asthma status. However, quantitative analysis of mitochondrial haplogroups identified two haplogroups of 11.0% and 1.87% frequency that were associated with log-transformed total serum IgE levels, an important intermediate phenotype in asthma and atopy (p=0.006 and 0.01, respectively). These data suggest that common mitochondrial haplogroups influence asthma diathesis.

NAT2 and NER genetic variants and susceptibility to sporadic prostate cancer in African Americans. *S.E. Hooker[1], C. Bonilla[1], C.A. Ahaghotu[2], R.A. Kittles[1]* 1) Human Cancer Genetics, Ohio State University, Columbus, OH; 2) Division of Urology, Howard University Hospital, Washington, DC.

Prostate cancer is a common malignancy that disproportionately affects African American men. The large ethnic differences in prostate cancer risk are unlikely to be completely explained by environmental factors such as diet and lifestyle. Instead environmental exposures may modulate prostate cancer risk in combination with genetic variation in genes responsible for chemical and dietary carcinogen metabolism and DNA damage repair. We report on single nucleotide polymorphisms in six genes involved in carcinogen metabolism and DNA repair in 254 African American prostate cancer cases and 301 healthy controls from Washington, DC. Genotyping was performed using MassARRAY for fourteen SNPs within the NAT2, ERCC1, XPF/ERCC4, XPG/ERCC5, and CSB/ERCC6 genes. Structured association analyses were performed using individual ancestry estimates to control for admixture stratification. Smoking status, BMI and age were included as covariates in the analyses. We found that individuals homozygous for an XPG/ERCC5 -72C/T promoter polymorphism had significant reduction in risk for prostate cancer (OR= 0.12; 95%CI=0.03-0.48). A haplotype trend regression test also revealed a protective effect for the haplotype bearing the T allele (P=0.003). In silico analyses suggest a functional implication for the promoter variant since it deletes a GCF transcriptional factor binding site responsible for the down-regulation of transcription. The protective effect of the promoter SNP on risk for Pca was independent of smoking. In contrast, none of the SNPs typed for NAT2, ERCC1, ERCC4 and ERCC6 showed significant association with risk. Additionally, tests for genotype interactions were not significant. We note that there may be other factors, such as dietary exposures which may modulate prostate cancer risk in combination with genetic variation within the NAT2 and NER genes. Our results, in combination with previous independent observations of LOH for ERCC5 in prostate tumors, provide further evidence of a role for XPG/ERCC5 in the etiology of prostate cancer.

Intravenous immune globulin treatment for Hereditary Inclusion Body Myopathy: A pilot study. *I. Manoli[1], S. Sparks[1], G. Rakocevic[2], G. Joe[3], J. Shrader[3], B. Sonies[3], C. Ciccone[1], D. Krasnewich[1], M. Huizing[1], M. Dalakas[2], W.A. Gahl[1]* 1) MGB, NHGRI, NIH, Bethesda, MD; 2) NINDS, NIH, Bethesda, MD; 3) RMD, NIH, Bethesda, MD.

Hereditary Inclusion Body Myopathy (HIBM) is an autosomal recessive, adult onset, non-inflammatory neuromuscular disorder with no effective treatment. The causative gene, GNE, codes for UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase, which catalyzes the first two reactions in the synthesis of sialic acid. Reduced sialylation of muscle glycoproteins, such as -dystroglycan and neural cell adhesion molecule (NCAM), is observed in HIBM. In this pilot study we treated 4 HIBM patients with intravenous immune globulin (IVIG), as a means of providing high quantities of sialic acid, considering that IgG contains 8mol of sialic acid/g. IVIG was infused as a loading dose of 1g/kg on two consecutive days followed by 3 doses of 400mg/kg at weekly intervals. The primary outcome was muscle strength assessed by Quantitative Muscle Testing; secondary parameters included a 6-minute walk test, grip and tongue strength, swallowing function, and the Human Activity Profile questionnaire. Muscle biopsies were obtained before and after IVIG. Mild improvements in the strength of certain muscle groups were recorded. Function of the right and left quadriceps improved by 13-154% and 8-48%, respectively, in 3 patients. Similarly, shoulder abduction improved by 24-79% on the right and 13-184% on the left in 3 patients. Esophageal motility and lingual strength improved in the 2 patients with abnormal barium swallows. Minimal to modest qualitative improvements in daily activities were experienced in 3 patients. No unexpected side effects occurred. Mild headaches and vomiting after loading with IVIG subsided within 48h. Muscle immunohistochemistry and western blot analyses for -dystroglycan, NCAM and PSA-NCAM did not demonstrate any appreciable changes. The absence of inflammation in HIBM muscle suggests that the noted mild benefits were not related to the anti-inflammatory or immunomodulatory effects of IVIG. The uses of IVIG and other sources of sialic acid are being explored as treatment options for HIBM.

Sequence analysis of small SMN1 mutations in SMA compound heterozygotes. *D.C. Mihal, S.J. Bridgeman, R.E. Pyatt, T.W. Prior* Pathology, The Ohio State University, Columbus, OH.

While 95% of clinically diagnosed Spinal Muscular Atrophy (SMA) patients have homozygous deletions of the SMN1 gene, the remainder are presumably compound heterozygotes with one deleted SMN1 allele and a small mutation in the other. The molecular identification of the later is complicated by the presence of the pseudogene SMN2, but can be critical in determining a complete genetic diagnosis. We therefore examined the detection of compound heterozygotes in individuals clinically diagnosed with SMA. Validation of SMN1 sequence detection was performed using ABI dye terminator sequence technology on a series of carriers with defined SMN2 copy numbers ranging from 0 to 5. Peak intensity ratios were created by examining the major and minor alleles and adjacent bases at two of the five single base changes that distinguish SMN1 from SMN2. The results indicated that SMN1 alleles at both positions were consistently detectable despite increasing SMN2 copy numbers. Using this approach, mutations in seven compound heterozygotes samples previously defined by the cloning and sequencing of SMN1 were confirmed by direct genomic sequencing. We then examined ten presumptive compound heterozygotes all meeting the following criteria: clinically diagnosed with SMA, SMN1 homozygous deletion negative, and possessing a single SMN1 gene copy by dosage analysis. These samples were then screened by sequence analysis for small mutations in SMN1 reported in the human gene mutation database. We identified mutations in three individuals consisting of a single base insertion in exon 2a, Y130C in exon 3, and an 11 base duplication in exon 6. Despite rigid criteria to define compound heterozygotes for sequence analysis, mutations were only identified in 30% of the individuals screened. The remaining 70% are ambiguous as they may possess unidentified mutations in regulatory or other relevant non-coding regions of SMN1, or may be carriers with a neuromuscular disease that symptomatically resembles SMA. Clearly, careful clinical discrimination is essential to assess the likelihood of these possibilities within this substantial portion of presumptive compound heterozygotes.

Enhanced linkage maps from family-based genetics studies. *C. He[1], X. Kong[1], S. Buyske[1], D.E. Weeks[2], T.C. Matise[1]* 1) Rutgers Univ, Piscataway, NJ; 2) University of Pittsburgh, PA.

Meiotic linkage maps are the foundation of linkage mapping for disease genes. Existing genome-wide linkage maps were built using only small collections of pedigrees, and so they have wide confidence intervals. The 95% CI for a 10cM map distance in Marshfield maps is from about 4 to 19cM. Incorrect marker order and map distances can lead to incorrect or imprecise results from linkage analyses, so there is a clear need for more accurate genetic maps for disease mapping studies. We have collected a very large sample of genotype data from disease-mapping studies genotyped by Mammalian Genotyping Service (MGS) in Marshfield. Our current sample includes genotypes for more than 18,000 individuals from over 4,500 pedigrees for markers on the Marshfield screening sets 8-11. These sets have average map resolutions of 8-10 cM. Our data collection has a total of 6.81 million genotypes and several ethnic groups are represented. We have cleaned numerous pedigree structure and genotyping errors, as well as verified the marker orders. We have used this dataset to a) test for population-specific distribution of recombination; and b) re-estimate the distances (including confidence intervals) on these screening set maps, both sex-averaged and sex-specific. The maps from Caucasian, Chinese, Hispanic samples are in quite good agreement with each other. We only found one map interval on chromosome 8p with significant difference between Caucasian and Chinese. Some comparisons between different Caucasian sub-populations have also been performed and no significant difference was found. The maps from the African-American sample have some significant differences with those from other populations. However this may be due to an apparently higher rate of genotyping errors present in this particular study. Our analyses result in screening set maps with improved accuracy, which can in turn be used to improve the accuracy of disease-mapping studies. The total map length of our enhanced maps is about 7% longer than Marshfield maps. Taking advantage of our large sample size, the 95% CI for a 10 cM map interval is only about 2cM long.

Detection of atypical duplications in 22q11.2 around the DiGeorge/Velocardiofacial region by array-based comparative genomic hybridization(Array CGH). *Z. Ou[1], X. Lu[1], H. Yonath[1], E. Roeder[2], V. Enciso[2], A.C. Chinault[1], A.L. Beaudet[1], S.W. Cheung[1], A. Patel[1]* 1) Dept. of Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Dept. of Pediatrics, UTHSCSA, San Antonio, TX.

The chromosome 22q11.2 region is susceptible to rearrangement, mediated by low copy repeats (LCRs). Three genomic disorders are caused by deletion or duplication in this region, DGS/VCF syndrome is the most common genomic disorder often associated with microdeletion on chromosome 22q11.2 region, resulting from non-allelic homologous recombination (NAHR) between LCR22 substrates, whereas cat-eye syndrome and duplication 22 syndrome are associated with tetrasomy, and segmental trisomy, respectively. We developed chromosomal microarray analysis (CMA) and designed the CMA to detect all rearrangements causing known genomic disorders including the DGS/VCFS region. The clones in the DGS/VCFS region have been selected to flank LCR22s. Seven patients with DGS/VCFS region duplications have been identified to date of which four were inherited from one of their parents. Three cases with an interstitial duplications map between the proximal LCR22-3b and the distal LCR22-5, span ~2Mb,and partially overlaps with DGS/VCFS typical 3Mb deletion region. In one case the duplication mapped between LCR22-3a and LCR22-3b and in another duplication between LCR22-3a and LCR22-4, both of which are also located within DGS/VCFS typical 3Mb deletion region. In two cases the duplication mapped between LCR22-4 and LCR22-5, which is outside the typical 3 Mb DGS/VCF associated deletion region. The breakpoints for none of these seven cases localize to LCR22-2 which are involved in the typical DGS/VCFS deletions. In 4 cases, the breakpoints map at LCR-3b which is not involved in the typical DGS/VCFS rearrangements. Our data further document the utility of clinical implementation of high resolution genome analysis using CMA. Such rearrangements are not observed by current clinical testing. Furthermore, we show that the genomic architecture within the DGS/VCFS genomic region appears to be recombination prone and subject to genomic instability causing multiple types of chromosome rearrangements in the DGS/VCFS region.

Evaluation of the clinical utility of a Family History Tool. *S.M. O'Neill[1], W.S. Rubinstein[1], L..S. Acheson[2], M.T. Ruffin[3], Family Healthware Evaluation Collaboration* 1) Evanston Northwestern Healthcare, Evanston, IL; 2) Case Western Reserve University, Cleveland,OH; 3) University of Michigan, Ann Arbor, MI.

The Family Healthware Tool (FHT) is a web-based program created by the Centers for Disease Control and Prevention(CDC)that collects family history of 6 common diseases, Coronary heart disease(CHD), Stroke(CVA), Diabetes(DM), and Colon(CC), Breast(BC) and Ovarian(OC)cancers. In addition to eliciting disease type and age of onset for first and second degree relatives, the program gathers information about an individuals behavioral risk factors and current screening practices. A risk assessment is performed for each of the diseases using complex algorithms[1] which generate tailored risk and preventive health messages for the user. The CDC selected 3 sites to evaluate the clinical utility of this new public health tool in over 6000 healthy participants between the ages of 35 and 65 ascertained through primary care practices. This randomized controlled trial examines the effect of the tool on risk perceptions, disease related attitudes and beliefs, and change in health behaviors. Case subjects complete a pretest and the FHT and changes are evaluated through a 6 month posttest. Controls will not complete the FHT until after their 6 mo. posttest. At the ENH site, 300 control subjects and 537 case subjects have enrolled. The average age of case subjects is 53 and most are Caucasian (90%) females (76%). Most cases (86%) chose to complete the tool online without assistance and the average completion time was 14.6 minutes. Overall, 453/537 (84.4%) have a strong(S) or moderate(M) family-history based risk for at least one of these diseases; CHD[S 36%, M 26%], CVA[S 16%, M 35%], DM[S 11%, M 28%],CC[S 1.5%, M 11%], BC[S 10%, M 15%], OC [S 4%, M 6%]. Use of the FHT algorithms suggests a substantial burden of family-history based risk in the adult primary care population, particularly for heart disease and stroke. Implementing the automated FHT as a standard part of clinical practice may identify subpopulations who would benefit most from targeted prevention strategies.

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Autism outcome extremes: biological but not social factors predict recovery. *M.K. Morgan, J.H. Miles, T.N. Takahashi* Med Gen Div, Univ of MO Hosp, Columbia, MO.

Autistic children exhibit a wide range of outcomes; a small portion recover to become independent adults, whereas others show minimal to no recovery. This study examines the extremes of the outcome curve: 7 in a recovered group (REG) vs 12 with poor outcomes (POG). Both groups were diagnosed with autistic disorder between the ages of 2 & 3 yrs. Selection criteria for POG included no functional language, high dependence, and 9 yr old. REG selection criteria were functionally verbal, attends regular classes, and independent functioning for age. Those with syndromes were excluded. Comparison of the groups revealed no differences in ethnicity, pre/perinatal or teratogenic problems. Family factors generally assumed to improve outcomes were all better in POG: high SES was over represented in POG (72.7 vs 50.0%) & low SES was less (9.1% vs 50.0%). More POG parents were married & probands were more apt to live with both parents (66.7% vs 42.9%). Though not significantly different, POG mothers reported less depression. The primary differences between the recovered & poor outcome groups were in biological measures. All dysmorphic probands were in POG (16.7%); no REG were dysmorphic. All seizure patients were in POG (8.3%). POG children were less apt to be normocephalic (33.3% vs 71.4%); 58.3% POG children were macrocephalic; 8.3% microcephalic. POG children had more regressive onsets (41.7% vs 28.6%). POG were mostly male (92%) and REG mostly female (57%). Pedigrees were analyzed to see if additive inherited tendencies impacted recovery. There were no differences in family loading for alcoholism, cognitive, language, or behavior disorders including ADHD or seizures. REG probands had a greater family history of autism (57.1 vs 16.7%) & more affected sibs (37.5% vs 14.3%). These results indicate there are fundamental biological & presumably genetic differences between autism that remediates and that which does not. Outcome appears to be an intrinsic component of the phenotype. Family & social factors did not associate with recovery. Analyses of educational and behavioral therapies are underway.

Autism probands with a mildly affected parent: a case-control study. *D.G. Ingram, T.N. Takahashi, J.H. Miles* Med Gen Div, Univ MO Hosp, Columbia, MO.

Identifying autistic traits in parents of autism probands may decrease sample heterogeneity, increase power for research analyses and allow us to study parent-of-origin effects. Twenty-seven autism probands were identified as having a parent with prominent autistic traits (study group, SG), compared with 252 probands not having an affected parent (comparison group, CG). Within the affected parents, 58% exhibited social deficits, 42% communication deficits, and 27% repetitive stereotyped behaviors. Groups did not differ in age, race, height, weight, head circumference, CARS score, dysmorphology, syndrome diagnosis, sex ratio, sib recurrence rate, abnormal EEG or MRI, or onset with regression. A greater proportion of SG families had high socioeconomic status (P=0.037). In terms of outcome measures, SG probands had higher IQ/DQs (P=0.009), a higher proportion of IQ/DQs > 70 (P=0.015), and a higher proportion of conversational language (P=0.016). Language and IQ/DQ scores were used to create an outcome classification of excellent, good, and poor. SG probands had more excellent outcomes (P=0.002) and less poor outcomes (P=0.006); outcome measure results did not change when probands < 8 years old were excluded from analysis. In addition to the parent with autistic traits, SG families had a higher prevalence of language disorders (P=0.045) and depression (P=0.050), but a lower prevalence of cognitive disorders (P=0.036). Comparing probands within the SG with an affected mother (M) vs. father (F) revealed trends towards parent-of-origin effects for excellent outcome (M=100% vs. F=41%, P=0.199) and conversational language (M=100% vs. F=54%, P=0.247). Overall, SG probands tended to have better outcomes, increased family history of neuropsychiatric disorders, and displayed evidence of parent-of-origin effects with better outcomes if the mother was affected. We speculate that parents autistic traits are a marker for specific genes that convey risk for autism, and that this genetic liability may work via epigenetic mechanisms.

X-Linked Creatine Transporter Deficiency Presenting as a Mitochondrial Disorder. *K. Limbo[1], C. Parker[1], J. Vockley[2], T. Wood[3], M. Friez[3], O.A. Abdul-Rahman[1]* 1) Department of Pediatrics, University of Mississippi Medical Center, Jackson, MS; 2) Department of Pediatrics, Childrens Hospital of Pittsburgh, University of Pittsburgh School of Medicine, Pittsburgh, PA; 3) Greenwood Genetics Center, Greenwood, SC.

Creatine transporter deficiency is an X-linked disorder characterized by developmental delay with severe language impairment, seizures, and hypotonia. Reduction in creatine uptake results in elevated urine creatine and CSF creatine deficiency. Mutations in the sodium-dependent creatine transporter (SLC6A8) are causative. We report a patient who was initially suspected of having a mitochondrial disorder, but was later found to have a creatine transporter defect. The patient is a 10 year-old Caucasian male born at term gestation with an uncomplicated prenatal course. By age 2, he had significant developmental delay and truncal hyptonia. An EEG showed epileptiform discharges, but no overt seizure activity was recognized. MRI of the brain was normal. Metabolic testing was performed and detected elevations of blood lactate, alanine, glycine, and C2- and C4-aclycarnitine. Urine organic acid analysis identified ethylmalonic acid and TCA cycle intermediates. Suspicion of a mitochondrial disorder prompted a muscle biopsy. Respiratory chain analysis, carnitine levels, CPT assay, coenzyme Q levels, and mitochondrial DNA testing of muscle were all normal. Blood mitochondrial DNA analysis was also normal. However, repeat serum chemistries demonstrated persistently low creatinine levels. Urine guandinoacetate was normal. However, urine creatine was significantly elevated. DNA analysis of SLC6A8 demonstrated a complex rearrangement which is still undergoing further characterization. Creatine uptake studies are currently pending. Review of the literature identified a second patient with a creatine transporter defect who initially presented with elevations of ethylmalonic acid, 3-methylglutaconic acid, and TCA cycle intermediates. Based on these two patients, we recommend testing for disorders of creatine metabolism in patients with a suspected mitochondrial disorder when no specific defect can be identified.

Association of -308A Allele of Tumor Necrosis Factor Gene (TNF?) with Asthma in Mexican Pediatric Patients.

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Asthma is one of the most important respiratory diseases in development countries. Genetic variations in the nucleotide sequence of genes with an important role in the antiinflamatory, inmunoregulatory response, have been associated to asthma in several populations. However, deep divergence in the distribution of some single base polymorphisms (SNPs) is noted en Caucasian, African-American, Asian and Hispanic-Latino ethnic groups. The aim of this study was to know whether IL4R?, TNF? and IL10 polymorphisms are associated with asthma in Mexican pediatric patients. We performed a case-control study in 82 Mexican patients with clinical diagnosis of asthma and 248 individuals without allergy and asthma history as controls. We genotyped 6 SNPs (IL4R?: Ser503Pro, Arg576Gln and TNF?: -308G/A, -238G/A, IL10: -1082G/A, -582C/A) using 5'exonuclease assay (TaqMan) for allelic discrimination. Polymorphisms in IL4R? and IL10 did not show association with asthma. However, the TNF? -308A polymorphisms was more frequent in asthma patients when they were compared with controls (P=0.04, OR=2.25; 95% CI= 0.97-4.83). Our preliminary results suggest that polymorphism in TNF? gene could play and important role in the susceptibility to development asthma. More studies are underway to confirm these results.

Loss of biallelic expression of 15q11-13 GABA-A receptor subunit genes in Rett syndrome and autism. *A. Hogart, R.P. Nagarajan, D.H. Yasui, K.A. Patzel, J.M. LaSalle* Medical Microbiology & Immunology and Rowe Program in Human Genetics, University of California Davis School of Medicine, Davis, CA.

Human chromosome 15q11-13 is a complex locus containing imprinted genes as well as a cluster of three GABA-A receptor subunit genes, *GABRB3*, *GABRA5*, and *GABRG3*. The GABR genes have relevance to Prader-Willi and Angelman patients with heterozygous 15q11-13 deletions and autism patients with maternal 15q11-13 duplications. GABRB3 protein expression is also reduced in Rett syndrome (RTT), caused by mutations in *MECP2* on Xq28. Although *Gabrb3* is biallelically expressed in mouse brain, the imprinting status of GABR genes in humans has not been conclusively determined. Therefore, we analyzed allelic expression of the 15q11-13 GABR genes in post-mortem human brain using SNPs within the coding region of each gene. Equal biallelic expression of at least one GABR gene was observed for 21 controls, demonstrating that these genes are not imprinted in human brain. Interestingly, 4/12 autism and 1/3 RTT samples expressed only one allele for one or more GABR gene, suggesting a variable loss of normal biallelic expression. To determine if allelic expression correlated with overall transcript levels, quantitative RT-PCR was performed. As a group, samples with loss of biallelic expression did not have significantly lower expression of *GABRB3* than those with biallelic expression, however, both autism and RTT samples had lower *GABRB3* transcript levels compared to controls. Since MeCP2 has been shown to positively regulate *GABRB3*, further studies were conducted to elucidate a role for MeCP2 in allelic expression. Chromatin immunoprecipitation and bisulfite sequencing demonstrated binding of MeCP2 to methylated intronic sequences in *GABRB3* and *GABRG3* without evidence for allele-specific methylation. Developmentally regulated homologous 15q11-13 pairing in neurons has been previously described to be dependent on MeCP2. Autism and RTT patients deficient for MeCP2 expression exhibit disrupted 15q11-13 pairing, suggesting that MeCP2 and *trans* interactions are necessary for normal gene expression of the 15q11-13 GABR cluster.

Proteomic profiling of hippocampal synaptoneurosomes in the mouse model of fragile X syndrome. *M. Nakamoto[1], D. Cheng[1], J. Peng[1], U. Narayanan[1], R. Alisch[1], H. Yi[2], S.T. Warren[1]* 1) Human Genetics; 2) Imaging Facility; Emory Univ, Atlanta, GA.

Fragile X syndrome (FXS) is a common form of inherited mental retardation and is caused by loss of FMRP. FMRP, an mRNA-binding protein, can suppress translation of target transcripts and the absence of FMRP results in excess translation of target mRNAs. Synaptic plasticity requires local protein synthesis of preexisting messages and in the absence of FMRP, electrophysiological measures of plasticity, such as mGluR5-induced long term depression (LTD) are exaggerated, consistent with the over translation of LTD-required protein(s). Indeed, mGluR5-induced LTD, which is sensitive to protein synthesis inhibitors in wildtype (wt) mice, is insensitive to the same inhibitors in *Fmr1* knockout (ko) mice. This suggests that in ko mice there are preexisting and constitutively expressed proteins that are normally translated locally in response to synaptic stimulation. We sought to identify such proteins in hippocampal synaptoneurosomes (SNs) from wt and ko mice utilizing a high throughput liquid chromatograph coupled with highly sensitive tandem mass spectrometer (LC-MS/MS). We compared mGluR5-stimulated wt SNs with unstimulated wt SNs to identify proteins normally induced by synaptic stimulation and compared unstimulated ko SNs with unstimulated wt SNs to identify proteins constitutively over expressed in the ko mice. We identified 7 proteins whose abundance was increased at least 2-fold in wt SNs upon stimulation and were at least 2-fold constitutively overexpressed in unstimulated ko SNs. The highest level of constitutive overexpression in ko SNs (8-fold) was eukaryotic elongation factor 1A protein (eEF1A), which showed a 2.4-fold increase in wt SNs upon mGluR5 stimulation. These results were verified by western blotting. Since eEF1A mRNA is a known FMRP ligand located in dendrites, our data suggest translational regulation of eEF1A by FMRP is lost in its absence. Moreover, overexpression of eEF1A may be responsible for the global increase of protein synthesis reported in ko mouse brain. These data provide further mechanistic insight into FMRP function and the consequence of its loss.

Primary Care Screening for Hereditary Cancers. *M. Kramer[1], J. Rispoli[1], T. Pollin[1], N. Khanna[2], S. DeLany[1]* 1) Genetic Counseling; University of Maryland, School of Medicine, Baltimore, MD; 2) Family Medicine; University of Maryland, Baltimore, MD.

Limitations in screening for hereditary cancer in primary care settings have been identified by several studies. In order to increase screening ability, physicians stated that they would like further education on screening patients for hereditary cancer. A study was conducted at the University of Maryland School of Medicine to determine what aspects of screening need improvement and what educational approaches would be preferred. 48 primary care physicians completed a questionnaire that asked about their practice and confidence in screening for hereditary cancers. They were also asked to assess cancer risk from four family history vignettes. Only 8% of physicians screened all patients regularly. A majority (88%) of physicians screened patients by asking about a presence or absence of a family history of cancer. Only 42% of respondents obtained a three-generation family history. Participants cited lack of time as the greatest barrier to hereditary cancer screening. No respondent correctly estimated risk as low, moderate or high, in more than 3 of 4 scenarios. Participants had the most difficulty assessing risk for low and moderate risk patients, resulting in overestimation of risk for inheriting a predisposition gene for cancer. Physicians (87%) stated that they would like more education on screening for hereditary cancer and that they would attend yearly lectures. Physicians indicated that they would particularly appreciate more information on cancer susceptibility tests and identifying high-risk patients. Only half of participants knew about local genetics services. These data indicate a need for further education of primary care physicians regarding both screening techniques and availability of resources and point toward some of the preferred means of providing this education.

Identification of PML-RARA rearrangement by RT-PCR and sequencing in an acute promyelocytic leukemia without t(15;17) on G-banding and FISH. *J. Han[1], K.E. Kim[1], K.H. Kim[1], J.S. Kim[2], J.I. Park[3]* 1) Department of Laboratory Medicine, Dong-A-University College of Medicine, Busan, Korea; 2) Department of Internal Medicine, Dong-A-University College of Medicine, Busan, Korea; 3) Department of Biochemistry, Dong-A-University College of Medicine, Busan, Korea.

t(15;17)(q22;q12) is seen in 70-90% of APL cases, leading to the formation of two reciprocal fusion genes, PML-RARA on the der(15) and RARA-PML on the der(17) chromosomes. A number of different variant translocations have been characterized in the remaining cases of APL. There are also APL patients with cytogenetically normal chromosomes 15 and 17. Combining molecular techniques such as RT-PCR, FISH, or sequencing has been a useful tool in identifying those abnormalities. FISH using a commercially available dual color, dual fusion probe could detect almost all PML-RARA fusions including variant and cryptic forms. We report a unique case of de novo APL with a cryptic PML-RARA rearrangement. This patient had a karyotype of 47,XX,+8 and no additional chromosomal abnormalities. Metaphase FISH of the patient did not show PML-RARA fusion signals. However, PML-RARA chimeric transcripts could be identified by RT-PCR and cDNA sequencing. A diagnosis of APL was made and she was treated with combination chemotherapy including idarubicin and all trans retinoic acid (ATRA). The chemotherapy was well tolerated and a complete hematologic remission was achieved four weeks later. Evidences of PML-RARA fusion gene could not be detected any longer and cytogenetic studies showed a normal karyotype. The newer FISH method is significantly superior to the previous FISH probes, accurately detecting cryptic, variants or complex translocations involving chromosomes 15 and 17. However, depending on the size of the insertion, the target could be so small that it does not hybridize with the probe or even hybridization itself might not generate a fluorescent signal large enough to be visualized by FISH. To resolve the presence or absence of a masked PML-RARA fusion, it may be necessary to further evaluate by using of other molecular testing.

Frequency of Thiopurine Methyltransferase alleles in Mexican population. *A. Inchaustegui, O.A. Perez-Gonzalez, L. Castellanos-Tapia, G. Jimenez-Sanchez* National Institute of Genomic Medicine, Mexico.

6-Mercaptopurine (6-MP), 6-thioguanine, and azathioprine are thiopurine drugs used in the treatment of patients with leukemia, autoimmune diseases and transplant recipients. Thiopurine methyltransferase (TPMT) catalyzes an S-methylation reaction in the metabolism of these agents. The activity of the TPMT enzyme is affected by the presence of polymorphisms in the *TPMT* gene. Polymorphisms in this gene have been documented in 2-10% of different populations around the world. The Mexican population has a unique genetic origin, more than 80% of the population is considered Mestizo, resulting from the admixture of any of 62 ethnic groups with Spaniards in the last 500 years. To establish the allelic and genotypic frequencies of functional polymorphisms (238G>C and 719A>G) in the Mexican population, we analyzed samples from 994 healthy Mestizo volunteers, 54.6% men and 45.4% women, from six states in separate areas of Mexico using allelic discrimination based on TaqMan technology (AB). Analysis of 238G>C (*TPMT*2*) showed 100% of the ancestral alleles. In contrast, analysis of 719A>G (*TPMT*3C*) showed an allele frequency of 5.2% for G with genotype frequencies of 9.6% for heterozygous (AG) and 0.4% for homozygous (GG) individuals. Results were confirmed by direct sequencing 11% of the samples. The presence of 719G allele is predictive of phenotype; individuals heterozygous 719A>G have intermediate enzyme activity, and subjects homozygous for this allele are TPMT deficient. Patients heterozygous in 719A>G are at intermediate risk of dose-limiting toxicity (Black AJ, et al. 2001. *Ann Intern Med* 129:716). These initial results will contribute to establish a screening program to detect those Mexican Mestizo patients with increased sensitivity to these drugs in order to adjust dose levels and decrease severe adverse effects. In addition, reduction in the amounts of 6-MP administered to these patients will result is a significant reduction of treatment cost. This initial analysis is part of the national platform of genomic medicine that Mexico develops for its population.

Midwives and genetics in Australia: current practice, anticipated needs. *S.A. Metcalfe[1,2], M. Bishop[1,2], Y. Bylstra[1], C. Gaff[3,4]* 1) Genetics Education and Health Research , MCRI, Melbourne, Australia; 2) Dept Paediatrics, The University of Melbourne, Australia; 3) Genetic Health Services Victoria, Australia; 4) Institute of Medical Genetics, Cardiff University, Cardiff, UK.

In Australia, the model of midwifery healthcare appears to lie somewhere between that of the USA and the UK. Midwives are an essential component of prenatal and postnatal care of women, yet their role in genetics in Australia has not been described, recognised or supported. Using both qualitative and quantitative approaches we investigated how genetics is incorporated into current and expected roles of midwives. Focus groups (9) were conducted with midwives (n=50) and 11 interviews with organisational managers and educators from the state of Victoria. Also, 2 focus groups were held with prenatal and neonatal genetic experts (n=10). Midwives discussed their experiences in dealing with genetic issues, while managers and experts were asked about their opinions on midwives practice, and what they expect their role to entail. Transcripts of discussions were analysed and major themes identified. Both managers and midwives recognised that midwives care primarily for normal or low risk pregnant women. Experts supported autonomy for discussing prenatal and neonatal screening tests, whilst managers saw midwives essentially as resource/information providers. Midwives reported their role in regards to genetics includes information provider, counsellor, and support person. Perceived boundaries with other health professionals was also discussed. Genetics seems to impact on the midwife role through the whole continuum of pregnancy, being defined mostly by organisational practice and population demographics, rather than personal experience or knowledge. Data from the qualitative part of the study was used to inform the development of a survey which has subsequently been validated. Items in the survey include areas of current practice, knowledge, skills, attitudes and requirements for education. The survey has been sent to 850 midwives, data is being collected and will be presented. Together the study will inform educational needs and policy regarding midwife practice of genetics.

Holoprosencephaly: Clinical and genetic study about 350 patients (1996-2006). *S. Odent[1], L. Pasquier[1], C. Dubourg[2], C. Bendavid[2], C. Henry[3], S. Jaillard[3], M.R. Durou[2], I. Gicquel[2], V. David[2]* 1) Genetique Medicale, Hopital SUD, Rennes Cedex2, France; 2) CNRS UMR6061, Groupe Genetique humaine, Universite Rennes1, 35 043 Rennes cedex, France; 3) Laboratoire de Cytogénétique, CHU de Rennes, Hopital Pontchaillou, France.

Holoprosencephaly (HPE) is the most common brain malformation resulting from incomplete cleavage of the prosencephalon (1 out of 16.000 live births; 1 out of 250 conceptuses). HPE is associated with a wide spectrum of craniofacial malformations ranging from lethal forms (alobar HPE with cyclopia) to less severe forms such as lobar HPE and normal face. The etiology is very heterogeneous involving environnemental factors, chromosomal abnormalities and at least 7 genes. Since 1996, our team has started to work on the wide clinical and genetic variability of holoprosencephaly. Patient samples (fetuses or children with normal karyotype) and clinical data (from HPE to microforms) were collected from medical teams in France and Europe. Systematic mutation analysis and genomic rearrangements of the five main genes (SHH, ZIC2, SIX3, TGIF, GLI2) were performed. About 19%sequence changes were identified among 350 DNA samples The familial cases confirmed an extreme clinical variability. Then, microrearrangements were detected by QMPSF, MLPA and CGH array (particularly in fetuses) improving the rate of molecular defects to a total of 30%;. Several patients samples within 2 microdeletions and/or duplications were identified supporting multiple-hit hypothesis involving others genetics and/or environnemental factors. We performed molecular prenatal diagnosis four times with fetal US scan and cerebral MRI screening. At last, we show involvement of cerebral malformations as Aprosencephaly/Atelencephaly linked to SIX3 mutations and cerebellar hypoplasia to SHH gene. Pan-hypopituitarism and cleft lip/palate were associated with GLI2. This work has improved molecular diagnosis rate in Holoprosencephaly and therefore the genetic counseling.

Expression profiling of retinoblastoma and meibomian cell carcinoma using cDNA microarray. *A. Kumar[1], S.K. Dorairaj[2], R. Prakash[2], C.P. Venkatesh[2], S. Chakraborty[1]* 1) MRDG, Indian Inst Science, Bangalore, India; 2) Minto Ophthalmic Hospital, Bangalore, India.

Development and progression of tumors is a multistep process dictated by expression of many genes. DNA microarray could facilitate identification of genes involved in tumor development by hybridizing cDNA samples from normal and tumor tissues on cDNA microarrays. Although it is well known that the loss-of-function of retinoblastoma protein causes retinoblastoma, it is not known as to how many other genes are involved in development and progression of retinoblastoma. Besides, the most common treatment available till date to treat retinoblastoma is enucleation of the affected eye(s). Therefore, finding targets for therapeutic intervention for retinoblastoma using microarray technique will be of paramount importance. Nothing is known about the genes involved in development and progression of meibomian cell carcinoma. The purpose of this study was to fill this gap using cDNA microarray technology. We have ascertained a total of 11 retinoblastoma (Rb) and five meibomian cell carcinoma (MCC) samples. We have also ascertained normal retina samples from eye globes which were discarded after removing cornea for corneal transplantation from individuals who donated their eyes. We have also collected normal eyelids from individuals who went through eyelid reconstruction surgery for a variety of reasons. We have isolated total RNAs from all of the retinoblastoma, meibomian cell carcinoma, normal retina and normal eyelid samples. We have analyzed three retinoblastoma samples using three 19K human microarray slides (University Health Network, Toronto). A comparison of the hybridization data showed up-regulation of 1,002 and down-regulation of 480 genes. Using semi-quantitative PCR, we have validated six genes so far. We have also carried out microarray analysis for two MCC tumors using human 8K microarray slides. Seventy genes were upregulated and 405 genes were downregulated. We have validated five genes so far in a panel of five MCC samples. The results will be presented and discussed. (Funding from ICMR, New Delhi is gratefully acknowledged).

A Novel mutation in the VDR gene in an Iranian patient with vitamin D-dependent Rickets type II. *V. Hadavi[1], N. Almadani[1], W. Wuyts[2], M.H. Kariminejad[1], H. Najmabadi[1]* 1) Kariminejad-Najmabadi Pathology & Genetics Center, Tehran, Iran; 2) Department of Medical Genetics, University and University Hospital of Antwerp, Antwerp, Belgium.

The vitamin D receptor (VDR), is the mediator of all genomic actions of vitamin D3 and it's analogs. Mutations in VDR results in target organ resistance to 1alpha,25-dihydroxy vitamin D [1,25(OH)2D3], the active form of vitamin D, and cause hereditary 1,25-dihydroxyvitamin D resistant rickets (HVDRR). This disease also called vitamin D-dependent rickets type II and is transmitted in an autosomal recessive mode. We report on a 5 years old girl affected with type II vitamin D-dependent Rickets, who appeared normal at birth but developed the clinical and biochemical features of calciferol deficiency with hypocalcaemia and rickets in the first year of life. She suffered from total alopecia and metaphyseal dysplasia in both hip joints and in pelvic. She was hospitalized at the age of four due to Diabetes Mellitus. Sequence analysis of all coding exons of VDR gene was performed and revealed a single homozygous point mutation in exon 7 causing a premature termination codon, Tyr295X. The Tyr295X mutation causes a truncation of the VDR protein, thereby deleting a large portion of the steroid hormone-binding domain (amino acids 295-424).

Triplication of the 1p36.3 Region in Two Siblings with Seizure Disorder. *S.-H.L. Kang[1], D. del Gaudio[1], P.A. Eng[1], M.L. Cooper[1], A. Scheffer[2], S. Vacha[2], C.A. Bacino[1], S.W. Cheung[1], T. Sahoo[1]* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Agilent Laboratories, Palo Alto, CA.

Duplications involving the terminal short arm of chromosome 1 are extremely rare. Deletions of 1p36 are one of the most common human microdeletion syndromes and have been extensively characterized. Genotype-phenotype correlations across the 10 Mb region frequently involved in the deletion syndrome suggests that there are multiple dosage-sensitive genes within this region. Constitutional duplications involving chromosome 1p described in the literature have been identified to be interstitial in nature involving variable segments, both in size and location, and consequently with variable phenotypic manifestations. To our knowledge, no cases of triplication of 1p36, and potentially familial in origin, have ever been reported. We describe two female siblings presenting with seizures, abnormal EEG and global developmental delay (at around 2 years of age) associated with regression in early childhood. Physical examination revealed no dysmorphic features. Array-CGH revealed a gain of greater than two copies of ~2.2 Mb including the telomeric region of the short arm of chromosome 1 (1p36.3). High-resolution G-banded chromosome and fluorescence in situ hybridization analyses were concordant with direct/tandem triplication of the terminal 1p36.3 segment. Additional studies including quantitative PCR for multiple amplicons across the putative segmental gain confirmed the presence of two additional copies of the 1p36 segment and provided a more precise estimate of the size and boundary of the rearrangement. The resulting gain includes increase in copy number of a number of genes that might be responsible for the phenotype. Interestingly, the potassium channel beta-subunit gene (*KCNAB2*) located approximately 6 Mb proximal to the 1p telomere, that has been implicated in the seizure disorder observed in some patients with deletions in 1p36, is not contained within the rearranged segment.

Detecting Epistatic Needles in Genome-Wide Haystacks. *J.H. Moore, B.C. White* Computational Genetics Laboratory, Department of Genetics, Dartmouth Medical School, Lebanon, NH.

The detection of epistasis is an important priority in the genetic analysis of complex human diseases. The most challenging epistatic effects to model are those that do not exhibit statistically significant marginal effects. Identifying these types of nonlinear interactions in the context of genome-wide association studies is considered a *needle in a haystack* problem. Given this complexity, it is unrealistic to expect that stochastic search algorithms will do any better than a simple random search. Our goal was to test this null hypothesis and then develop and evaluate a stochastic search algorithm that is capable of finding epistatic needles with the assistance of expert knowledge. We first developed a genetic programming (GP) approach to picking SNPs for epistasis evaluation using multifactor dimensionality reduction (MDR). This new GP-MDR approach was evaluated using simulated epistatic interactions of varying heritability (0.01, 0.025, 0.05, 0.1, 0.2, 0.3, 0.4) and minor allele frequency (0.2, 0.4) that were embedded in genome-wide datasets with varying numbers of SNPs (1000, 10000, 100000) and varying sample sizes (200, 400, 800, 1600, 3200, 6400). We found no evidence to suggest that GP-MDR performed better than random search on these simulated genome-wide datasets. Next, we modified GP-MDR to select SNP combinations for virtual recombination, mutation, and reproduction by the GP based on prior information about the quality of each SNP as assessed during a pre-processing analysis by the ReliefF filter algorithm. We found that the expert knowledge provided by ReliefF about which SNPs might be interacting significantly improved the ability of GP-MDR to identify epistatic SNPs in the absence of marginal effects over that of a simple random search. An important advantage of this approach is that any form of expert knowledge could be used to guide the stochastic search algorithm. For example, information about biochemical pathways, protein-protein interactions, Gene Ontology (GO), or even evidence from the literature could be used in addition to statistical measures such as ReliefF. (Funded by NIH R01s LM009012 and AI59694, PI-Moore).

Comprehensive analysis for large deletions in TSC1 and TSC2 in TSC patients: frequency, utility and reliability of MLPA, and genotype-phenotype correlations. *P. Kozlowski[1], P.S. Roberts[1], S. Dabora[1], D. Franz[2], J. Bissler[2], H. Northrup[3], K-S. Au[3], S. Jozwiak[4], D.J. Kwiatkowski[1]* 1) Dept Medicine, Brigham & Women's Hosp, Boston, MA; 2) U. Cincinnati, OH; 3) UT Houston, TX; 4) Children's Hospital, Warsaw, Poland.

Tuberous sclerosis is an autosomal dominant disorder caused by mutations in either of two genes, TSC1 and TSC2. Point mutations and small indels account for about 75% of mutations in unselected patients. Large deletion and insertion mutations are known to occur in TSC1 and TSC2, but their frequency is uncertain. We examined 269 TSC DNA samples (216 small-mutation-negative and 53 unscreened) for large deletion/duplication mutations using both commercial (MRC-Holland) and our own TSC1 and TSC2 MLPA probe sets. These combined probe sets permit interrogation of all TSC1/2 exons, as well as 15-50kb of flanking sequence. Large deletion mutations in TSC1 and TSC2 were identified in 50 (19%) of 269 TSC patients, of which 46 were in TSC2, and 4 were in TSC1. Only 12 deletions were intragenic in TSC2, and one in TSC1, so that 37 (74%) deletions extended beyond the 5, 3 or both ends of TSC1 or TSC2. Mutations were identified in 22% of small-mutation-negative and 6% of unscreened samples. Only 1 of the 50 (2%) samples showed evidence of duplication. Six of 49 (12%) deletions were mosaic, affecting ~30-60% of cells. All mosaic, single exon, and intragenic deletions were confirmed by LRPCR. In addition, all deletions were concordant between the two sets of probes for each gene. Genotype/phenotype analysis showed that 18 of 22 patients with TSC2 deletions extending 3 into the PKD1 gene had early onset polycystic kidney disease; further analysis continues. Break-points of intragenic deletions were randomly distributed along the TSC2 sequence. Our own 20-plex probe sets gave more robust performance than the 40-plex probe sets from MRC Holland. In conclusion, this comprehensive analysis indicates that large deletions in TSC1 and TSC2 account for about 0.5% and 6% of mutations seen in TSC patients, respectively, and that MLPA is a highly sensitive and accurate method for their detection.

Epigenetic differences in monozygotic Rett syndrome twin with different severity. *T. Kubota[1], Y. Chunshu[1], K. Endoh[1], M. Sohtome[1], M. Sasaki[2]* 1) Dept. Epigenetic Medicine, Univ. of Yamanashi, Yamanashi, Japan; 2) Dept. Child Neurology, National Center of Neurology and Psychiatry, Tokyo, Japan.

Rett syndrome (RTT) is an X-linked dominant disease caused by *MeCP2* mutations. MeCP2 is bound to the promoters of genes and controls their expression, suggesting that pathogenesis of RTT is mis-regulation of genes in the brain. Here we performed genetic and epigenetic analyses on monozygotic RTT twins with different severity. The twin sisters were 3.5 y.o. The older sister (OS) showed typical RTT features, such as epilepsy and hand wringing, by 2 y.o., whereas the younger sister (YS) was an atypical RTT, who had almost normal linguistic development by 2 y.o., but lost words thereafter. DNA polymorphisms at 6 microsatellite markers on various chromosomes were identical, indicating that twins were monozygotic. Both twin showed one base deletion in *MeCP2* [c.806delG (p.Gly269ArgfsX288)]. X-chromosome inactivation (XCI) patterns, obtained by methylation-specific PCR assay, were similar between twins in either lymphocytes (OS49:51, YS47:53), or hairs originated from the same embryonal origin (ectoderm) as CNS (OS40:60, YS51:49). Monozygotic twins with RTT did not show any apparent differences in genetic (mutation) and epigenetic (XCI pattern) factors. It has recently reported that DNA-methylation patterns become gradually different after birth between individuals. Thus, we are now investigating methylation at the MeCP2-target genomic sites, whether the sites are less methylated in severe OS than in milder YS, because less methylation can deteriorate the function of the preserved nearly half amount of normal MeCP2 in RTT.

De Novo Balancd Complex Chromosome Rearrangement (CCR) - 46, XY, t(8;10),t(8;12) associated with Autism Spectrum Disorder (ASD)- Cytogenetic, FISH and Microarray analysis. *D.S. Krishnamurthy[1], M. Susan[1], M. Naveed[1], S.K. Murthy[2], K. Sperling[3]* 1) Cytogenetics and Moleclar Cytogenetics, Canadian Specialist Hospital,DUBAI, UAE; 2) Cytogenetics Unit,Genetics Dept Al Wasl Hospital, DOHMS, DUBAI, UAE; 3) Institut fur Humangenetik University of Berlin, Berlin,Germany.

Autism Spectrum Disorder (ASD) is a rare behavioral phenotype defined by a qualitative impairment in reciprocal social interaction, impairment in communication and imaginative activity. About 1.7 % to 4.8 % of individuals with ASD have chromosome abnormalities, including unbalanced translocations, inversions, rings, and interstitial deletions and duplications. Balanced or unbalanced Complex chromosome rearrangements (BCCR/CCR) are rare structural abnormalities involving at least three chromosomes and three or more break-points. We report a 8-5-year-old boy with delayed speech and ASD associated with a BCCR involving chromosomes 8, 10 and 12. His Karyotype showed - 46,XY, der(8),t(8;12)(q22;q22), der(10), t(8;10)(q22;q25), del(12)(q22->qter). Teleomere FISH confirmed the the translocation brekpoints at terminal regions (8q;10q;12q).The family history was non-informative suggesting a possible de novo rearrangement. The karyotype in both the parents are normal. Fragile X (FMR1-gene)test was negative [30+1-1 repEats- normal range 6-49 repeats (2)]. FISH analysis for PWS (SNPRN LSI 15q11-q13) showed normal result. CGH analysis confirmed normal karyotype (rev ish XY). Microarray analysis confirmed no deletion or duplication of chromosomes 8,10 and 12.Balanced Complex de novo rearrangement may lead to disruption of genes at the breakpoints, position effect or cryptic imbalances in the genome. However, little is known about possible imbalances at the junction points. This case suggests that aberrations at 8q22, 10q25, and/or 12q22 may result in pervasive developmental disorder,associated with mild cognitive delay. The etiology and pathogeneis of ASD in balanced complex chromosome rearrangement, confirmed by array-CGH will be discussed.

Splicing analysis cassette vector system showed that either a polypyrimidine-tract or an exonic splicing enhancer is essential for correct splicing of dystrphin exon19. *Y. Habara[1], M. Doshita[2], Y. Yokono[2], M. Yagi[1], Y. Takeshima[1], M. Matsuo[1]* 1) Department of Pediatrics, Kobe University Graduate School of Medicine, Japan; 2) Kobe Pharmaceutical University, Japan.

Splicing signal is located not only at exon-intron boundary but also at deep into exons and introns in human genes. For this reason, it is sometime difficult and also confusing to find a responsible mutation of genetic disorder. We developed a vector assay system to examine mRNA splicing patterns in order to identify sequence elements essential for correct splicing. The vector expresses a reporter minigene under CMV promoter, containing two exons and adjacent intron which have a multiple cloning site (MCS) in the center. To analyze splicing patterns, a part of the target gene from patients, control or artificially mutated genes are ligated into the MCS of this vector. After making reporter fusion minigenes, plasmids are transfected into culture cells to express the minigenes. Then, cells are harvested and splicing patterns are analyzed by RT-PCR. We analyzed which element is essential for correct splicing of dystrophin exon19 by using this cassette vector system. Dystrophin exon19 was spliced as an exonic splicing enhancer (ESE) dependent manner; the exon19 was included to mature-mRNA when it had an ESE, and the exon19 was skipped when the ESE was eliminated from exon19 sequence. First, we confirmed that whether this ESE dependent manner is recapitulated in HeLa cells with this vector. Then we changed the polypyrimidine tract (Py-tract) sequences located upstream of exon19 to examine the relationship between ESE and Py-tract. It revealed that the ESE is not essential for correct exon usage when the activity of Py-tract is strong (10 nucleotides of U-stretch). On the other hand, when the exon has a full length ESE, 6 of 10 nucleotides alteration from pyrimidine to purine in Py-tract is not affected for its splicing pattern. It is suggested that either the good Py-tract or the fully active ESE is essential for correct splicing of dystrophin exon19. Also, it showed that this cassette vector system enable us to analyze splicing pattern by a single cloning and transfection steps.

The p53 codon 72 polymorphism is associated with primary open angle glaucoma in the Japanese population. *F. Mabuchi[1], S. Tang[2], K. Kashiwagi[1], Z. Yamagata[2], H. Iijima[1], S. Tsukahara[1]* 1) Dept Ophthalmology, Univ Yamanashi, Chuo, Yamanashi, Japan; 2) Dept Health Sciences, Univ Yamanashi, Chuo, Yamanashi, Japan.

Objective: Previous studies looking at the association of the p53 gene polymorphism and primary open angle glaucoma (POAG) revealed conflicting results. Additionally, there have been no studies in the Japanese population. We thus assessed whether genetic polymorphism of p53 was associated with POAG in the Japanese population.

Methods: Genomic DNA was examined in a cohort of 102 Japanese patients with POAG and 67 control subjects. The average age was 61.6 15.2 years (mean SD) for the POAG patients, and 66.1 9.4 years for the control subjects. The p53 genotype (a G to C substitution at codon 72 which changes an arginine to a proline residue) was determined using pyrosequencing analysis, and compared between POAG patients and control subjects.

Results: There was a significant difference in the genotype frequencies between the POAG and control groups (P = 0.0039 Chi-square test), and the frequency of the C allele was significantly higher in the POAG patients compared to the control subjects (36.8% vs. 24.6%, P = 0.023 Fisher exact test).

Conclusion: The p53 codon 72 polymorphism is associated with POAG and may be a maker for POAG in the Japanese population. Further studies in the other ethnic populations should be performed to elucidate the relationship between the p53 gene and POAG.

Identification of a genetic locus for Donnai-Barrow syndrome. *S. Kantarci[1], P.K. Donahoe[1], R.S. Hill[2], L. Al-Gazali[3], D. Lacombe[4], N. Chassaing[4,5], E. Bieth[5], G. Black[6], D. Donnai[6], C. Walsh[2], B.R. Pober[1,2]* 1) Massachusetts General Hospital,HMS, Boston, MA; 2) Children's Hospital,HMS, Boston MA; 3) FMHS, UAE University, Al Ain United Arab Emirates; 4) Hôpital Purpan, Toulouse France; 5) CHU Pellegrin, Bordeaux, France; 6) Marys Hospital, Manchester, UK.

Background: Donnai-Barrow syndrome (DBS)is a rare autosomal recessive [OMIM#222448] genetic disorder involving congenital diaphragmatic hernia (CDH), exomphalos, absent corpus callosum, hypertelorism, myopia, and sensorineural deafness. As part of our ongoing studies to uncover genes associated with CDH, we performed locus mapping on DBS. Methods: We recruited four DBS patients, with prior normal chromosome studies, from two distinct branches of a highly inbred family from the UAE. Array-based comparative genomic hybridization (aCGH) (Spectral Chip 2600) was performed on two patients. We applied the Affymetrix 10K SNP genotyping chip to identify areas of shared homozygosity among the affecteds; homozygous regions were confirmed using microsatellite analysis. Additionally, we obtained DNA from two previously reported multiplex DBS families from France on which we performed microsatellite analysis. We also recently recruited three DBS families, members of the original DBS kindreds, reported from the UK. Sequence analysis of candidate genes has been carried out in one affected member from each family (by SeqWright). Results:o In the UAE kindreds, aCGH results were normal. 10K SNP chip revealed several regions of shared homozygosity in the four patients, with a promising 10 cM homozygous block on chr.2. Microsatellite analysis narrowed the region to ~18 Mb on 2q23.3-q31.1. The highest single point LOD score was 3.2619. o Microsatellite markers on 2q23.3-q31.1 showed different haplotypes between affected and unaffected sibs in the French families. o Haplotype analyses of the UK families are underway. o Sequencing of 45 gene candidates in the chromosome 2 critical region has been completed for 10 genes. Conclusions: We identified a genetic locus in 2q23.3-q31.1 for DBS. Identification of the genetic basis of this monogenic CDH syndrome is likely to reveal a locus contributing to the more common polygenic form of CDH.

Gene Diversity at hypervariable polymorphic loci in African populations and its impact on estimating admixture components in admixed African populations of the American continent. *H. Lee, W. Niu, R. Chakraborty* Ctr Genome Information, Univ Cincinnati, Cincinnati, OH.

Gene diversity within Africa is the highest compared to other continents. As a consequence, there are criticisms of estimating admixture components in African populations using a single specific population from Africa as the source of their African gene pool. Using allele frequency data on 13 short tandem repeat (STR) loci used in DNA forensics, we examined the extent of gene differentiation at these loci between populations of the African continent, and the impact of the choice of ancestral African source population on admixture estimates in some admixed African populations of the continental USA. Allele frequency data were collected on eight sub-Saharan African populations from Kenya, Equatorial Guinea, Mozambique, Angola, and Rwanda. Admixed African populations included general US Blacks, Brazilian Blacks, and the three Caribbean populations (Trinidad, Jamaica, and Bahama). The gene diversity estimate within Africa (i.e., proportion of genetic variation due to between population differences) was 0.63%, which is almost 3-fold higher than that of the European populations published before. Dendrogram analyses also revealed this greater diversity within the African continent, although in terms of clusters, the admixed African populations clustered with the African populations. In terms of admixture estimates, however, the choice of specific African populations as the source of the ancestral gene pool did not change the admixture components appreciably. For example, for the US Blacks the proportion of African genes in their gene pool ranged between 78 to 89%, depending upon the choice of specific African populations. Of the three Caribbean populations, Trinidad showed comparatively smaller contributions (57 to 69%) of African gene pool. Our results indicate that even though between-population diversity within Africa is larger than that in European continent, this diversity is trivially smaller than the genetic difference between Africans and Europeans. Therefore, when estimating admixture proportons, choice of different indigenous African population make little difference.

Comparative Mouse Genomics Centers Consortium (CMGCC): Improved understanding of the biological significance of environmentally-responsive human polymorphisms. *K.A. McAllister, F.L. Tyson, E. Maull, G. Collman, CMGCC Steering Committee* Susceptibility and Population Health Branch, Division of Extramural Research and Training, NIEHS, NIH, Research Triangle Park, NC.

The National Institute of Environmental Health Sciences (NIEHS) Comparative Mouse Genomics Centers Consortium (CMGCC) was established with the goal of developing knockout and transgenic mouse models that harbor human single nucleotide polymorphisms (SNPs) and/or mutations in DNA repair and cell cycle control genes that could alter sensitivity to environmental agents. These models will be used to enhance our understanding of the biological significance of human DNA polymorphisms and their role in combination with environmental stressors resulting in disease outcomes including multiple cancers, aging-related diseases (including Werners Syndrome), diabetes, and obesity. This multidisciplinary Consortium is comprised of University Centers in Harvard Medical School, University of Texas, MD Anderson Cancer Center, University of Cincinnati Medical School, University of Texas Health Sciences Center at San Antonio, Buck Institute CMGC, and the University of Washington. The CMGCC has developed over 70 mouse models in various stages of validation. Validated mouse models and resources developed by the CMGCC are available to the scientific community and can be accessed at the CMGCC website, http://www.niehs.nih.gov/cmgcc. The CMGCC has also developed the Federated Mouse Database which includes in silico bioinformatics and comparative mouse phenotype assessment tools, as well as mouse model dissemination or repository data. Unique contributions of CMGCC include the utilization and comparison of different approaches to humanizing polymorphisms in mouse models, the development of high-throughput phenotyping technologies, and the use of short-term challenges of the mouse models to environmental agents. Of particular interest is a humanized mouse line with a p53 codon 72 polymorphism modulating the risk of developing several environmentally-influenced cancers and an XRCC1 mouse model with potential increased sensitivity to colon cancer when environmentally challenged.

Incorporating single locus tests into haplotype cladistic analysis in case control studies. *J. LIU*[1,2], *J. ZHANG*[1,2], *C.J. PAPASIAN*[1], *H. DENG*[1,2,3,4] 1) BASIC MEDICAL SCIENCE, UNIVERSITY OF MISSOURI, 2411 HOLMES STREET, KANSAS City, MO. 64108; 2) Department ORTHOPEDIC SURGERY,UNIVERSITY OF MISSOURI, 2411 HOLMES STREET, KANSAS City, MO. 64108; 3) LABORATORY OF MOLECULAR AND STATISTICAL GENETICS, COLLEGE OF LIFE SCIENCES, HUNAN NORMAL UNIVERSITY, CHANGSHA, HUNAN 410081, P. R. CHINA; 4) THE KEY LABORATORY OF BIOMEDICAL INFORMATION ENGINEERING OF MINISTRY OF EDUCATION AND INSTITUTE OF MOLECULAR GENETICS, SCHOOL OF LIFE SCIENCE AND TECHNOLOGY, XI'AN JIAOTONG UNIVERSITY, XI'AN 710049, P.R.CHINA.

In case control studies, genetic associations for complex diseases may be probed either with single-locus tests or with haplotype-based tests. Although different views have been expressed on the relative merits and preferences for the two test strategies, haplotype-based analyses are generally believed to be more powerful for detecting genes with modest etiological effects and may be useful for detecting rare causal variants. However, the main drawback of haplotype-based association tests is the large number of distinct haplotypes, which increases the degrees of freedom (df) for corresponding test statistics, thus reducing the statistical power. To enhance the efficiency and power of haplotype analyses, we propose a haplotype clustering analysis that is based on the haplotype cladistic analyses developed by Durrant et al. for decreasing the df. In our method, we use P values obtained in individual SNP locus association tests as a measure of weight. The weight will be used in computation of similarity measures, which are used in construction of distance metric between pairs of haplotypes. To assess our proposed new method, we perform computer simulation studies to compare the performance of 1) conventional haplotype-based approach, 2) cladistic analysis method (CLADHC) by Durrant et al., and 3) our weighted cladistic analysis method, under different scenarios. The results demonstrated increased statistical power and Positive Predictive Value (PPV) by using our weighted cladistic analysis method, when compared with the CLADHC method and the conventional haplotype-based approach. Therefore, our new approach has practical significance in the general field of human genetics.

Investigations of a candidate region for dyslexia on chromosome 7q32. *H. Matsson[1], H. Anthoni[1], M. Zucchelli[1], J. Schumacher[2], G. Schulte-Körne[3], I.R. Koenig[4], J. Nopola-Hemmi[5], H. Lyytinen[6], M.M. Nöthen[7], J. Kere[1,8], M. Peyrard-Janvid[1]* 1) Biosciences and nutrition, Karolinska Institutet, Huddinge, Sweden; 2) Institute of Human Genetics, University of Bonn, Germany; 3) Dept of Child and Adolescent Psychiatry and Psychotherapy, University of Marburg, Germany; 4) Institute of Medical Biometry and Statistics, University of Lübeck, Germany; 5) Dept of Pediatrics, Jorvi Hospital, Finland; 6) Dept of Psychology and Child Research Center, University of Jyväskylä, Finland; 7) Dept of Genomics, Life & Brain Center, University of Bonn, Germany; 8) Dept of Medical Genetics, Biomedicum, University of Helsinki, Finland.

Dyslexia is the most common childhood learning disorder and may have a significant impact on social development. The specific reading and spelling deficits are manifested in spite of normal intelligence, senses, education and social environment. Dyslexia shows genetic heterogeneity, and at least eight loci (DYX1-8) contributing to dyslexia phenotypes have been mapped and up till now, four candidate genes have been described. It is important to identify novel loci and investigate the functional significance of the genetic variation in dyslexia. Our previous genome scan suggested linkage of dyslexia to a region on chromosome 7q32 with a non-parametric linkage score of 2.8 in 11 Finnish families. The *FOXP2* gene, implicated in a severe speech and language disorder, is located within the linkage peak but no exonic mutations or SNPs were revealed in dyslexic family members in a previous screening. In order to identify the causal variant within the region, we performed a more detailed linkage analysis using 14 additional polymorphic microsatellite markers spanning the 7q32 region. The analysis of marker genotypes allowed an increased resolution of the linkage peak and the linked region was restricted to approximately 9 cM. We are extending the investigation by genotyping microsatellite markers on 251 German families with a total of 429 individuals with dyslexia. Analyses of the chromosome 7 region in this large independent sample set may refine further a locus for dyslexia and support further candidate gene analysis.

Iminoglycinuria: Molecular findings. *R. Kleta[1], T. Coskun[2], B. Tinloy[1], H.I. Aydin[2], M. Gunay-Aygun[1], H. Stanescu[1], I. Bernardini[1], W.A. Gahl[1]* 1) SHBG, MGB, NHGRI, NIH, Bethesda, MD, USA; 2) Department of Pediatrics, Division of Pediatric Nutrition & Metabolism, Hacettepe University Faculty of Medicine, Ankara, Turkey.

Disorders such as cystinuria (basic aminoaciduria) and Hartnup disorder (neutral aminoaciduria) have been instrumental in identifying the genes responsible for renal tubular transport of amino acids at the plasma membrane level. For iminoglycinuria, candidate genes like SLC6A20 and SLC36A1 have been proposed to be causative. This is because heterologous expression studies have shown that their gene products mediate uptake of proline and glycine, two of the amino acids improperly handled by the proximal tubules of individuals with iminoglycinuria. We identified two patients with iminoglycinuria by serum and urine amino acid analyses showing typically elevated fractional excretions of glycine, proline and hydroxyproline only. Sequencing of all coding exons (including the splice sites) of SLC6A20 and SLC36A1 showed no disease-causing mutations. Based on homology, expression, and function, another member of the SLC36 family, SLC36A2, was selected as a candidate gene for iminoglycinuria. Sequencing of all coding exons of SLC36A2 also revealed no disease-causing mutations. We conclude that amino acid transport genes other than the proposed candidates might be responsible for iminoglycinuria. We are pursuing patients and active collaborations to elucidate the basic genetic defect in iminoglycinuria.

Is disease severity in alkaptonuria modified by a common SNP in the organic anion transporter MRP4 / ABCC4?

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Alkaptonuria is a rare metabolic disorder of tyrosine catabolism in which the organic compound homogentisic acid (HGA) binds to connective tissue and causes darkened cartilage (ochronosis), joint destruction, and cardiac valve deterioration. In our investigation of more than 90 alkaptonuria patients, we previously identified 4 women with an ochronotic phenotype but normal HGA excretion. All had been treated with the tetracycline derivative minocycline. Tetracyclines are secreted into the urine by proximal tubular organic anion transporters. These transporters recognize a variety of drugs, xenobiotics, and organic acids, including p-aminohippurate (PAH). We had sequenced all coding exons of OAT1, OAT3, OAT4, and MRP4, in 2 of our 4 pseudo-ochronotic patients. Both exhibited a homozygous intronic acceptor splice site mutation, IVS2-5T>C, in MRP4. PAH clearance was abnormally low in the one patient we studied, demonstrating the deleterious effect of this mutation on organic anion transport. We generated a restriction site in MRP4 to screen for IVS2-5T>C. In 136 alleles from patients with alkaptonuria, we found the expected frequency of wildtype (70%) and minor (30%) alleles. We propose that this common MRP4 SNP, IVS2-5T>C, can potentially modulate the clinical severity of alkaptonuria, since HGA secretion depends upon an intact organic anion transporter. To pursue this, we measured plasma HGA levels in our alkaptonuria patients and correlated them with the presence of the MRP4 SNP. Our preliminary data support a potential influence of this SNP on plasma HGA levels. Our findings point toward a gene product that modifies the severity of alkaptonuria and could have a major impact on the clinical course of other organic acidurias, in which documented variability among affected siblings currently has no explanation.

Pitfalls in Homozygosity Mapping - Revisited. *G. Landoure[1], H. Stanescu[2], M.A. Knight[1], A.A. Taye[1], M. Arcos-Burgos[2], D. Pineda[2], W.A. Gahl[2], K.H. Fischbeck[1], R. Kleta[2]* 1) Neurogenetics Branch, NINDS, NIH, Bethesda, MD, USA; 2) MGB, NHGRI, NIH.

Myasthenia gravis is usually a sporadic condition, but approximately 4% of patients have a positive family history. We studied an Italian-American kindred in which the parents are first cousins; 5 of 10 siblings had autoimmune myasthenia. Affected family members had positive anti-acetylcholine receptor antibodies and other manifestations of autoimmune disease. The age of onset ranged from 50 to 79 years. The horizontal inheritance pattern and consanguinity suggested an autosomal recessive model, so we assessed linkage in the context of shared homozygosity. A genome-wide scan was performed using polymorphic markers spaced at 2 cM intervals. We probed 7 members of the family, i.e., 4 affected children, 2 unaffected children and one child with a less well defined phenotype. Using the Lander-Kruglyak multipoint linkage algorithm that includes homozygosity mapping (implemented in the Homoz) and considering affected individuals only, we found significant linkage with a LOD score of 3.28 for a region of shared homozygosity on chromosome 13. Taking unaffected individuals into account reduced the LOD score for this locus to 1.57. Haplotype reconstruction (using the SIMWALK software package) showed homozygosity in both affected and unaffected family members. A parametric multipoint analysis of the same data, without taking into account homozygosity (GENEHUNTER software), revealed a negative LOD score for this region. Instead, a single locus with a LOD score of 2.18 was detected on chromosome 2. Haplotype reconstruction of affected and unaffected individuals was consistent with this localization and was compatible with compound heterozygosity in affected individuals. In summary, even though homozygosity by descent appears more likely for recessive disorders in inbred families, the possibility of having two different disease-carrying alleles has to be considered. Our family with several affected children from a consanguineous marriage illustrates this principle.

Gitelman syndrome and sclerochoroidal calcifications: The critical importance of renal regulation in calcium homeostasis. *K. O'Brien[1], E. Tsilou[2], M. Lynch[1], C. Stuart[1], A. Jeong[1], R. Kleta[1], W.A. Gahl[1]* 1) SHBG, MGB, NHGRI, NIH, Bethesda, MD, USA; 2) Ophthalmic Genetics and Visual Function Branch, NEI, NIH.

Calcium homeostasis is critical for many intracellular functions. Hypocalcemia as well as hypercalcemia can present with acute life-threatening complications. Ionized and total calcium levels are tightly regulated within the vascular compartment. Gitelman syndrome is caused by mutations in the electroneutral sodium-chloride transporter gene SLC12A3 in the distal convoluted tubule of the kidney. In general, patients present with the unique combination of hypocalciuria and normocalcemia. Gitelman syndrome patients are often diagnosed accidently in late childhood or their twenties with hypokalemia, hypomagnesemia and metabolic alkalosis. More severe and distinct forms of renal tubular salt losing with neonatal or infantile presentations comprise Bartter syndromes. Here we present clinical, molecular and ophthalmological findings in a 60-year-old patient with Gitelman syndrome and her unaffected 62-year-old sister. The patient was compound heterozygous for two missense mutations in SLC12A3 and showed typical hypocalciuria. She had impressive peripheral sclerochoroidal calcifications without significant visual impairment. The blood compartments calcium homeostasis is maintained by well-defined mechanisms that involve vitamin D, parathyroid hormone, calcitonin, and the kidney. Our findings point to the possibility that chronic, long-term calcium overload, as occurs in Gitelman syndrome due to diminished renal excretion, can lead to ectopic precipitation of calcium in areas such as the retina. Enhancing urinary calcium excretion in patients with Gitelman syndrome might warrant consideration.

Ordered Stratification to Reduce Obesity Heterogeneity in Linkage Analyses of Type 2 Diabetes Quantitative Traits. *A.K. Manning[1], J. Dupuis[1], C. Lui[4], C. Fox[3], L.A. Cupples[1], J. Meigs[2]* 1) Biostatistics Department, Boston University, Boston, MA; 2) General Medicine Division and Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA; 3) The NHLBIs Framingham Heart Study and the Division of Endocrinology, Diabetes, and Hypertension, Brigham and Womens Hospital, Harvard Medical School, Boston, MA; 4) Department of Neurology, Boston University School of Medicine, Boston, MA.

Background: Heterogeneity in adiposity complicates detection of genomic loci predisposing to type 2 diabetes. Obesity may unmask diabetes susceptibility genes, or may produce non-genetic phenocopies. Methods: We conducted an ordered subsets linkage analysis (OSA) to diabetes-related traits (including fasting insulin and 28-year time averaged fasting plasma glucose (tFPG)) in 330 families of the Framingham Offspring Study. Subsets were constructed by adding one family at a time in increasing (lean family to obese) or decreasing (obese to lean) order, with the OSA LOD reported as the maximum LOD score observed in any of the subsets. Permutation p-values derived from 1000 random family orderings tested the hypothesis that ordering by obesity gave stronger linkage than random ordering. Results: On chromosome 1, with families ordered by increasing family mean waist circumference (WC), linkage to tFPG at 256 cM increased from 2.5 to 3.6 (p=0.02; 260 families with mean 100.1 cm). On chromosome 19, with families ordered by decreasing WC, linkage at 67 cM increased from 2.4 to 4.2 (p=0.03; 131 families with mean 96.0 cm). On chromosome 3, with families ordered by decreasing WC variance, linkage to fasting insulin at 79 cM increased from 2.9 to 4.8 (p=0.008; 186 families with SD 8.9 cm). Conclusion: At some genomic loci, obesity obscures linkage to diabetes-related traits. On chromosomes 3 and 19, where more obese families showed the strongest linkage to diabetes, diabetes-susceptibility genes may be unmasked by excess adiposity. These and chromosome 1 appear likely to harbor obesity-interacting diabetes susceptibility genes. Obesity effects should be considered in diabetes whole-genome association studies.

Telomere Shortening in T Lymphocyte Metaphases and Interphases and Individual Chromosomes 21 and 1, from Older Individuals with Down Syndrome and Dementia. *E.C. Jenkins[1], M.T. Velinov[1], L. Ye[1], H. Gu[1], D. Pang[1,2], D.A. Devenny[1], W.B. Zigman[1], N. Schupf[1,2], W.P. Silverman[1,3]* 1) NYS Institute for Basic Research in Devel. Disabil., Staten Island; 2) Taub Institute for Research on Alzheimer Disease Aging Brain, Columbia Univ, NY; 3) Kennedy-Krieger Institute, Johns Hopkins Univ, Baltimore, MD.

We have previously shown that telomeres, chromosome ends consisting of highly conserved TTAGGG repeats, are shortened in individuals with Down syndrome (metaphases and interphases from short-term whole blood cultures) when they have dementia. Using quantitative telomere protein nucleic acid FISH analysis, we have now extended our observations to a second sample. Subjects were ascertained through the NYS Developmental Disability Service System, and classified as demented if they developed progressive memory loss, disorientation, and functional decline over a period of at least one year. An additional study of a female with Down syndrome and clinical presentation similar to mild cognitive impairment (see ASHG 2005, #761) also showed reduced telomere size, thus confirming our initial study and further strengthening our hypothesis that telomere shortening is a candidate biomarker of dementia status. Also, we have compared light intensities within telomeres of chromosomes 21 (selected because of its triplication) and 1 (selected because of its size difference with 21) within 20 cells from three matched pairs of individuals (based on age and sex). Results indicated that chromosome 21 ($ps<0.008$, 0.0001, and 0.0001) and 1 ($ps<0.002$, 0.0002, and 0.006) telomere light intensities were significantly reduced in individuals with dementia. We also have examined p and q arms separately and found evidence that there may be some type of sentinel effect for specific chromosome arms in some individuals. (Note: Sentinel was used in MBC Online 2004;15(8):3709-18, to explore the possibility that just one or two sentinel telomeres may be responsible for replicative senescence induction.) (This work was supported in part by NYS OMRDD, Alzh. Assoc. grants IIRG-99-1598, IIRG-96-077; by NIH grants PO1 HD35897, RO1 HD37425, RO1 AG014673, and RO1 AG14771.).

A case of renal hypouricemia with nephrotic syndrome and acute renal failure. *K. Ichida[1], Y. Takeda[2], A. Abe[2], K. Hanaoka[1], S. Nakanishi[2], M. Umezu[2], M. Fukagawa[2], T. Hosoya[1]* 1) Div. of Kidney and Hypertension, Jikei Univ Sch Medicine, Tokyo, Japan; 2) Div. of Nephrology and Dialysis Center, Kobe University School of Medicine, Kobe, Japan.

Renal hypouricemia is an inherited and heterogeneous disorder characterized by increased uric acid clearance. Uric acid is reabsorbed via URAT1 on the apical membrane in the proximal tubules, and mutations in SLC22A12 encoding URAT1 cause renal hypouricemia. About ten percentages of the affected patients have exercise-induced acute renal failure as complication. Here, we present a renal hypouricemic case with nephrotic syndrome and acute renal failure. A 64-years-old Japanese female, having non-steroidal anti-inflammatory drug (NSAID) for lumbago, admitted with symptoms of edema and oliguria. Clinical examination showed autoantibody negative nephrotic syndrome and acute renal failure without hyperuricemia. After steroid pulse therapy for nephrotic syndrome and hemodialysis for acute renal failure, the renal function recovered to a normal range in a few weeks. Renal biopsy on the fiftieth hospital day demonstrated a minor glomerular abnormality with slight tubular inflammation. After the recovery of renal function, hypouricemia due to increase of uric acid clearance indicated that the patient was affected with renal hypouricemia. We identified homozygous G774A mutation in SLC22A12 of the patient. As the mechanism of acute renal failure accompanied by renal hypouricemia, renal reperfusion injury due to vasoconstriction has been proposed resulting from an exercise-induced increase in oxygen free radicals and a lack of urate, free radical scavengers. In this case, nephrotic syndrome and the usage of NSAID instead of exercise were suggested to decrease the renal flow leading to acute renal failure. This is the first report of renal hypouricemia with acute renal failure related with nephrotic syndrome and NSAID.

Lack of genotypic effect of polymorphism in C-Reactive protein gene on serum CRP level in a Japanese rheumatoid arthritis cohort. *K. Ikari, S. Momohara, E. Inoue, M. Hara, T. Tomatsu, H. Yamanaka, Y. Kawaguchi, N. Kamatani* Institute of Rheumatology, Tokyo Women's Medical University, Tokyo, Japan.

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease of unknown cause, with a prevalence rate of approximately 1%. C-reactive protein (CRP) is present during inflammation and is one of the main measures for disease activity of RA. CRP has an advantage over erythrocyte sedimentation rate (ESR), a traditional marker for inflammation because it is independent of physical properties such as age, sex, pregnancy, the size/shape of red blood cells or plasma composition. However, polymorphisms in CRP genes are reported to be associated with serum CRP level in recent studies. Supposing this association is true, using CRP to monitor treatment without any information on the genotype may mislead physicians on the best course of care. Therefore, we aimed to determine whether this CRP polymorphism is associated with serum CRP level in Japanese RA patients.

The study was a part of a Japanese RA cohort project that included over 4000 Japanese RA patients (IORRA: Institute of Rheumatology RA cohort). DNA samples were available from 1284 patients, of whom, 1128 were randomly selected for this study. Genotyping of the CRP polymorphism (rs1205) was performed using the TaqMan fluorogenic 5' nuclease assay. Disease Activity Score 28 (DAS28) was used to assess disease activity of RA since it was independent of CRP. A cross-sectional analysis using the DAS28 and serum CRP data in April 2003 in the cohort study was performed on 1021 patients (patients with missing data for DAS28 or CRP were excluded). Differences in serum CRP level and DAS28 among CRP genotypes were analyzed by regression analysis. There were no significant differences among CRP genotypes for serum CRP level or DAS28. The mean CRP level was 1.17, 1.22, and 1.21 for homozygote of a major allele, heterozygote, and homozygote of a minor allele, respectively while the mean DAS28 score was 3.49, 3.66, and 3.54, respectively. We conclude that CRP gene is not associated with serum level of CRP in Japanese RA patients; CRP can be used to measure disease activity of RA without testing the genotype.

Tagging single-nucleotide polymorphisms in excision repair cross-complementing group 1 (*ERCC1*) and risk of primary lung cancer in a Chinese population. *H. Ma[1], L. Xu[2], J. Yuan[3], M. Shao[4], Z. Hu[1], F. Wang[3], Y. Wang[4], W. Yuan[2], J. Qian[4], Y. Wang[2], G. Jing[1], X. Huo[1], F. Chen[1], Y.Y. Shugart[5], L. Jin[2, 4], Q. Wei[6], T. Wu[3], H. Shen[1], W. Huang[2], D. Lu[4]* 1) Department of Epidemiology and Biostatistics, Cancer Research Center of Nanjing Medical University, Nanjing, China; 2) Department of Genetics, Chinese National Human Genome Center at Shanghai, Shanghai, China; 3) Institute of Occupational Medicine, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China; 4) State Key Laboratory of Genetic Engineering, School of Life Sciences, Fudan University, Shanghai, China; 5) Epidemiology Department, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA; 6) Department of Epidemiology, The University of Texas M. D. Anderson Cancer Center, Houston, TX, USA.

Excision repair cross-complementing group 1 (ERCC1) is one of the core enzymes in the NER pathway, and polymorphisms in *ERCC1* may lead to altered DRC and therefore confer inherited predisposition to cancer risk. To test the hypothesis that common variants in *ERCC1* may be associated with lung cancer risk, we performed genotyping analyses for seven selected SNPs in *ERCC1* using TaqMan assay in a case-control study of 1010 patients with incident lung cancer and 1011 cancer-free controls in a Chinese population. We found that the variant genotypes of the rs3212948 C allele were associated with significantly decreased risk of lung cancer. Similarly a significant protective effect was also evident for the variant genotypes of rs1007616 C/T. Stratified analysis revealed that the protective effects of these two SNPs were both more evident among young subjects and subjects without family history of cancer. Consistently, when assessing each unique haplotype compared to the most common haplotype TAGCACG with the EM algorithm in *Haplo.stats*, the risk of lung cancer was significantly decreased among people who carry the haplotype TCCCATT with the variant rs3212948C and rs1007616T alleles. These findings indicate that *ERCC1* polymorphisms may contribute to the etiology of lung cancer.

East Asian-specific natural selection at the leukocyte immunoglobulin-like receptor A3 (*LILRA3*) locus. *K. Hirayasu[1,2], J. Ohashi[1], H. Tanaka[2], K. Kashiwase[2], M. Takanashi[2], M. Satake[2], K. Tokunaga[1], T. Yabe[2]* 1) Human Genetics, University of Tokyo, Tokyo, Japan; 2) Tokyo Metropolitan Red Cross Blood Center, Tokyo, Japan.

[Purpose] Leukocyte immunoglobulin-like receptors (LILR) are a family of activating and inhibitory receptors expressed on cells of the myeloid and lymphoid lineages. The *LILR* family is comprised of 11 functional genes encoded on chromosome 19. On the basis of the structural feature, LILR is divided into activating (LILRA1, -A2, -A4, -A5, -A6), inhibitory (LILRB1, -B2, -B3, -B4, -B5), and soluble (LILRA3) forms. Only the *LILRA3* gene exhibits a presence or absence variation due to a 6.7-kb deletion, which removes a leader peptide and all of the Ig-like domains of the gene. We previously reported that the allele frequency of the *LILRA3* deletion is extremely high (71%) in Japanese. In this study, we examined the geographic distribution of the *LILRA3* deletion in East Asians, and assessed the evolutionary significance of the *LILRA3* deletion, using the HapMap database.

[Methods] The *LILRA3* genotypes were determined by the PCR-SSP typing method in East Asians (Chinese-Korean, Man, Mongolian, and Buryat), and HapMap population samples (JPT, CHB, CEU, and YRI). F_{ST} values were calculated for all the polymorphic SNPs on chromosome 19 in the HapMap database.

[Results] High allele frequencies of the *LILRA3* deletion in East Asians (Chinese-Korean 84%, Man 79%, Mongolian 56%, and Buryat 76%) were observed, in contrast to the frequency reported for African (6%), Caucasian (26%), and South Asian (10%). The comparison of F_{ST} value for the *LILRA3* deletion with those for SNPs on chromosome 19 revealed that East Asians (JPT and CHB) and the others (CEU and YRI) were significantly differentiated for the *LILRA3* deletion polymorphism.

[Conclusion] Our results suggest that natural selection has acted to increase the allele frequency of the *LILRA3* deletion in East Asians, and lead us to speculate that susceptibility to some kind of disease such as infectious disease prevalent in East Asians might be a selective pressure at this locus.

TBX5 Gene Expression is Regulated Through Two Nkx2.5 Binding Elements. *G. Liguo, Q. Guangrong, X. Na, S. Kailai* Medical Genetics, China Medical University, Shenyang, Liaoning, China.

TBX5 gene is expressed in the developing heart, limb bud, lung and trachea at an early stage. TBX5 expression is precisely controlled in the developing heart and limbs in human and mice. Haploinsufficiency or overexpression of TBX5 gene causes heart abnormity. TBX5 interacts with other two cardiac transcription factors-NKX2.5 and GATA4, playing a central role during early cardiac development. But the regulation mechanism and pathway are unknown. Studies suggested that inter-regulation mechanisms maybe exist among the 3 transcription factors. As a marker of cardiac precursor cell differentiation, Nkx2.5 belongs to Homeobox gene family and plays a key role during cardiac development of fruit, mouse and human. Nkx2.5 regulates many downstream genes expression during heart formation. There is interaction between NKX2.5 and TBX5 transcription factors, but it is still not clear whether there exist upstream or downstream regulation between the two transcription factors. Our primary studies suggest that besides of interaction, NKX2.5 gene maybe locate upstream of TBX5 gene and regulate TBX5 gene expression during the regulative network involved in cardiac development. Using P-Match software, we predicted two possible Nkx2.5 binding elements at -139~-142 and -312~ -315 in 600bp sequence up to TBX5 gene transcription start site. The nucleotide sequences of special oligonucleotides and mutation probes were designed; Nuclear extracts were prepared from myocardium of mouse. Two Nkx2.5 binding elements were identified by electrophoretic mobility shift assay(EMSA). TBX5 promoter sequence of human and Nkx2.5 cDNA of mouse were cloned, respectively. Then we constructed luciferase report vector pGL3-TBX5 and eukaryotic expression vector pcDNA3.0-Nkx2.5. By transfecting different plasmids into COS7 cell line, the roles of Nkx2.5 proein to TBX5 gene was observed. The cell transfection results clearly demonstrate that 650bp up to transcription start site of TBX5 gene are able to drive luciferase reporter expression. The luciferase activity of COS7 cells increase significantly when co-transfection pcDNA-Nkx2.5 constructs.

Genome-wide microarray CGH analysis in patients with Aicardi syndrome. *T. Mizuguchi[1, 2], M. Kato[3], N. Matsumoto[1, 2]* 1) Department of Human Genetics, Yokohama City University , Yokohama , Japan; 2) Solution-Oriented Research for Science and Technology (SORST), JST, Kawaguchi, Japan; 3) Department of Pediatrics, Yamagata University School of Medicine, Yamagata, Japan.

Aicardi syndrome (AiS) is characterized by a clinical triad of callosal agenesis, infantile spasms and chorioretinal lacunae. AiS are generally found only in females. Importantly chromosomal abnormalities involving Xp22.3 were reported in some patients with AiS-like phenotype. These suggested that AiS may be an X-linked dominant disorder with hemizygous male lethality. Genetic analysis using familial cases is impossible due to severe outcomes of affected females, thus underlying causes of AiS remain unknown. We investigated eight AiS individuals by microarray comparative genomic hybridization analysis (array-CGH) containing 4234 FISHed BAC clones with a 0.75 Mb resolution across the entire genome. The aim of this study is to detect submicroscopic chromosomal abnormalities harboring a candidate locus/gene(s). We found a total of 28 chromosomal imbalances (gains or losses) among the patients by microarray CGH. Each patient possessed four to 12 copy number changes. Twenty of the changes were supposed to be copy number variants according to the public database of genomic variants, but eight were novel, including one on X chromosome. These changes are currently under investigation.

A link between hyperammonemia, potassium and water homeostasis and interastrocyte signaling. *U. Lichter-Konecki[1, 2], J.M. Mangin[1], V. Gallo[1]* 1) Center for Neuroscience Research, CRI,; 2) Division of Genetics & Metabolism, Children's National Medical Center, Washington, DC.

Acute hyperammonemia (HA) causes cerebral edema and severe brain damage in children with urea cycle disorders (UCDs) and in patients with liver failure. Chronic HA is associated with developmental delay and mental retardation in children with UCDs and with neuropsychiatric disturbances in patients with chronic liver dysfunction. The pathophysiology of the encephalopathy associated with these disorders has not yet been elucidated. Often treatment cannot prevent severe brain injury and neurological sequela. One of the cellular effects described in hyperammonemic encephalopathy (HAE) is astrocyte swelling. The swelling is considered to be causally related to the brain edema of acute HA.We studied the changes in metabolic and signal transduction pathways in acutely isolated astrocytes and brain tissue of mice with urea cycle disorders using microarray analysis. By crossing GFAP-EGFP transgenic mice with *Otc*^{spf} mice, we created a mouse with a urea cycle disorder, which also expresses EGFP in astrocytes, allowing for their purification by FACS. Expression analysis of FACS-purified astrocytes and cortex indicated changes in the expression of genes which mediate inter-astrocyte signaling and potassium and water homeostasis. These include the Connexin-43, the potassium channel subunit Kir4.1 and 5.1, and the water channel Aquaporin 4 genes. These findings indicate that proteins which facilitate potassium and water homeostasis and inter-astrocyte signaling might be potential targets for developing new therapies for HAE.

This work was supported by NIH-Mental Retardation and Developmental Disabilities Research Center Grant P30HD40677, by an Avery Research Scholar Award and a Research Advisory Council Award of the Childrens Research Institute.

Dynamic developmental changes of DNA methylation in the *Snrf-Snrpn* locus. *T. Kishino[1], K. Miyazaki[1], C. Mapendano[2], N. Niikawa[2], T. Ohta[3]* 1) Ctr. for Frontier Life Scie., Nagasaki Univ., Nagasaki; 2) Dept. of Hum. Genet., Grad. Sch. of Biomed. Scie., Nagasaki Univ., Nagasaki; 3) Inst. of Personalized Health Scie., Health Scie. Univ. of Hokkaido, Hokkaido.

The mouse *Snurf-Snrpn* locus has several multiple alternative transcripts, called *IC transcripts*, which initiate in exons (Un) that are distributed in a 500-kb region upstream of *Snurf-Snrpn* and share *Snurf-Snrpn* exons except exon 1 at the downstream region. Both of *Snurf-Snrpn* and *IC transcripts* are expressed highly in the brain exclusively from the paternal allele. We recently reported that *IC transcripts* were expressed only in the brain and ovary, especially in neurons and oocytes, whereas *Snurf-Snrpn* is known to be ubiquitously expressed in all tissues. The promoter region of *Snurf-Snrpn* has allele-specific differentially methylated region 1 (DMR1) established in gametogenesis and stably maintained during cell differentiation. Thus, the differential methylation at the DMR1 is supposed to be a parental mark for the imprinting domain. However, the mechanism of spatial and temporal expression of *Snurf-Snrpn* and *IC transcripts* is unknown. To investigate the epigenetic determinant of their spatiotemporal expressions, we analyzed the developmental changes of DNA methylation in U1 exon, and two DNase hypersensitivity sites (DHS) in DMR1; DHS1 in *Snrpn* 5 promoter and DHS2 in *Snrpn* intron 1. The bisulfite sequencing assay revealed that DHS1 was gametically methylated in oocyte, as has been reported before, however DHS2 was unmethylated during gametogenesis and became biallelically methylated during development in somatic tissues except in the brain. In U1 exon, both alleles were methylated in gamete and somatic tissues except in the brain. Interestingly, in the brain, the paternal DHS2 allele escaped methylation and paternal U1 allele was partially unmethylated especially in neurons. Recently DHS2 was reported to act as an enhancer of the *SNRPN* promoter and two upstream promoters U1A and U1B in human. Our results suggest that methylation status in U1 exon and DHS2 in DMR1 might be associated with spatiotemporal transcriptions in the mouse *Snurf-Snrpn* locus.

High density BAC clone mapping and visualizing genomic organization of satellite DNA by BAC fiber-FISH. *Y.C. Li[1], Y.M. Cheng[2], P.C. Hsu[1,2], T.S. Li[2], C.C. Lin[2]* 1) Dept Biomedical Sci, Chung Shan Medical Univ, Taichung, Taiwan; 2) Dept.Medical Research, China Medical University and Hospital.

The Indian muntjac (*Muntiacus muntjac vaginalis*) with 6 chromosomes in female and 7 in male is an ideal species for study chromosome rearrangement, comparative genomic, karyotype and genome evolution. A bacterial artificial chromosome (BAC) library of this species will be an invaluable resource not only for comparative sequence analysis, but also for the understanding of mechanisms involving centric fusion, telomere-mediated translocation, centromere inactivation, and genomic organization and alterations. We therefore have constructed a BAC library of male Indian muntjac. In total 164,736 individual BAC clones have been obtained. The average size of the inserts was estimated at 80 kilobases by analyzing almost 620 randomly chosen clones using *Not*I digestion followed by Pulsed Field Gel Electrophoresis (PFGE). Assuming that the Indian muntjac genome contains $2.6X10^9$ bp, the total library constructed corresponds to 5X genome coverage. The chromosomal location of over 700 BAC clones was each mapped to the Indian muntjac metaphase chromosomes by fluorescence in situ hybridization (FISH), thus providing a high density FISH BAC clone map of the species never been achieved. We have also investigated the genomic organization of several cervid centromeric satellite DNAs isolated from our laboratory using BAC fiber-FISH technology and some results of the study are presented in this communication. The genomic organization of these centromeric satellite DNAs should shed light on their structural aspect. Furthermore, the centromeric BAC clones mapped will also provide an excellent resource for the isolation of new centromeric satellite DNA sequences and their role in centromere function that can be evaluated by an artificial-chromosome assay. This could lead to the construction of function artificial mammalian chromosomes with gene therapy potential. This study was supported by grants from the National Health Research Institute (NHRI-EX94-9207SI) and from the National Science Council (NSC94-2320-B040-042).

Maternal uniparental disomy of the telomeric end of chromosome 16 is responsible for a malonic aciduria. *S. Malvagia[1], L. Papi[2], A. Morrone[1], MA. Donati[1], F. Ciani[1], E. Pasquini[1], H.R. Scholte[3], M. Genuardi[2], E. Zammarchi[1]*
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Malonic aciduria is a rare autosomal recessive disorder caused by a deficient activity of Malonyl-CoA decarboxylase. The human MLYCD gene encoding this enzyme has been mapped on chromosome 16q24.3. It is organized in five exons and 14 mutations have been reported so far. We report on a patient with arm and leg tremors since birth, who then developed tachypnea, pallor, and feeding refusal. On admission he was tachypnoic, hypotonic and soporous with a heart rate of 140/min. Metabolic investigations showed hypoglycaemia, mild hyperammonemia, grossly decreased total and free carnitine. Urine organic acid analysis revealed high a excretion of malonic acid consistent with malonyl-CoA decarboxylase deficiency, confirmed by reduced activity in cultured fibroblasts. High doses of carnitine and a diet low in lipids led to a reduction in malonic acid excretion. The child's clinical conditions markedly improved: growth was normal, and the cardiomyopathy was almost normalized. When he was 4 months old, he suddenly and unexpectedly died. No autopsy was performed. Molecular analysis of the MLYCD gene performed on the probands genomic DNA identified a homozygous c.655-659delACTG de novo small deletion. The same mutation was present in his mother, but not in his father. Paternity was confirmed by microsatellite analysis. We then investigated 14 markers of chromosome 16 in order to test the hypothesis of maternal uniparental disomy (UPD). Segmental maternal UPD was detected. Maternal isodisomy of 16q24 led to homozygosity for the mutant allele causing the patients disease. Since UPD can dramatically reduce the risk of recurrence, we would like to stress the importance of analysing parental DNA in the presence of homozygosity for genetic counseling.

Possible role of the *TNFRSF4* gene in the pathogenesis of womens essential hypertension. *Y. Mashimo[1], Y. Suzuki[1], K. Hatori[1], Y. Tabara[2], T. Miki[3], K. Tokunaga[4], A. Hata[1]* 1) Department of Public Health, Chiba University Graduate School of Medicine, Chiba, Japan; 2) Department of Medical Genomics, Ehime University School of Medicine, Ehime, Japan; 3) Department of Geriatric Medicine, Ehime University School of Medicine, Ehime, Japan; 4) Department of Human Genetics, Graduate School of Medicine, University of Tokyo, Tokyo, Japan.

Essential hypertension is a complex disorder that results from the interaction of a number of susceptibility genes and environmental factors. We have conducted screening of 121 candidate genes to identify responsible genes for hypertension. A part of candidate genes were chosen due to renal expression changes by long term salt loading detected by microarray experiment in mice. After several rounds of screening by case-control study using Japanese samples, SNPs of the *TNFRSF4 (tumor necrosis factor receptor, superfamily, member 4)* gene showed a significant association in female. We performed a detailed and duplicated association study and functional analysis of the gene. SNPs identification and linkage disequilibrium (LD) analysis of the entire gene have revealed that this gene is in one LD block and four tag SNPs in the 5 flanking region sufficiently consist major haplotypes. A four-SNP haplotype association study with 261 cases and 270 controls showed a significant result. In the hypertensives, the frequency of the C-C-A-A haplotype was significantly low (p0.05) and that of the C-T-G-A was slightly high (p=0.26) when compared with the controls. To confirm the association, another set of Japanese female samples (388 hypertensives and 584 normotensives) was analyzed. A significantly higher frequency of C-C-A-A and significantly lower C-T-G-A were observed in the cases than in the controls. On the other hand, the difference was not observed in male samples (299 hypertensives and 303 normotensives). Lower promoter activity of C-T-G-A haplotype determined by luciferase assay suggested the functional significance of this haplotype. Results of replicated association and functional analysis indicate an involvement of the *TNFRSF4* gene in the pathogenesis of female essential hypertension.

Identification of two sex-specific quantitative trait loci in chromosome 11q for hip bone mineral density in Chinese. *Q.Y. Huang[1], M.Y.M. Ng[1], C.L. Cheung[1], V. Chan[1], P.C. Sham[2], A.W.C. Kung[1]* 1) Department of medicine, The University of Hong Kong, Hong Kong; 2) Genome Research Center,The University of Hong Kong, Hong Kong.

Background: Chromosome 11q has not only been found to contain mutations responsible for the several Mendelian disorders of the skeleton, but it has also been linked to bone mineral density (BMD) variation in several genome-wide linkage studies. Furthermore, quantitative trait loci (QTL) affecting BMD in inbred mice and baboons have been mapped to a region syntenic to human chromosome 11q. The aim of the present study is to determine whether there is a QTL for BMD variation on chromosome 11q in the Chinese population. Methods: Nineteen microsatellite markers were genotyped for a 75 c region on 11q13-25 in 306 Chinese families with 1459 subjects. BMD (g/cm2) was measured by DXA. Linkage analyses were performed using the variance component linkage analysis method implemented in Merlin software. Results: For women, a maximum LOD score of 1.62 was achieved at 90.8 cM on 11q21 near the marker D11S4175 for femoral neck BMD; LOD scores greater than 1.0 were observed on 11q13 for trochanter BMD. For men, a maximum LOD score of 1.57 was achieved at 135.8 cM on 11q24 near the marker D11S4126 for total hip BMD. Conclusion: we have not only replicated the previous linkage finding on chromosome 11q but also identified two sex-specific QTL that contribute to BMD variation in Chinese women and men.

***KRAS, BRAF, MAP2K1/2* and *MAPK1/3* mutation analysis in cardio-facio-cutaneous (CFC) syndrome: genotype and phenotype correlation.** *Y. Narumi[1], Y. Aoki[1], T. Niihori[1], G. Neri[2], H. Cavé[3], A. Verloes[3], C. Nava[3], M.I. Kavamura[4], N. Okamoto[5], K. Kurosawa[6], H. Ohashi[7], R.C.M Hennekam[8], L. Wilson[8], G. Gillessen-Kaesbach[9], D. Wieczorek[9], P. Lapunzina[10], S. Kure[1], Y. Matsubara[1]* 1) Dept Med Genet, Tohoku Univ Sch Med, Sendai, Japan; 2) Istituto di Genetica Medica, Rome, Italy; 3) Hôpital Robert Debré, Paris, France; 4) Federal Univ of San Paulo, San Paulo, Brazil; 5) Osaka Med Ctr & Res Inst for Maternal & Child Health, Osaka; 6) Kanagawa Childrens Med Ctr, Yokohama; 7) Saitama Childrens Med Ctr, Saitama, Japan; 8) Inst of Child Health, London, UK; 9) Univ Essen, Essen, Germany; 10) Hosp Univ La Paz, Madrid, Spain.

Cardio-facio-cutaneous (CFC) syndrome is characterized by a distinctive facial appearance, cardiac defects, ectodermal abnormalities and mental retardation. Clinically, it overlaps with both Noonan syndrome and Costello syndrome, which are caused by mutations in two molecules of the RAS pathway (*PTPN11* and *HRAS* respectively). Recently, we discovered germline mutations in *KRAS* and *BRAF* in 19 of 43 patients with CFC syndrome, suggesting that dysregulation of the RAS/RAF/MEK/ERK pathway is a molecular basis for CFC syndrome. We here analyzed *KRAS* and *BRAF* in new 13 CFC patients and identified five *BRAF* mutations in eight patients. To characterize the further pathogenesis of CFC syndrome, we analyzed downstream molecules of *BRAF*, *MAP2K* (MEK) 1/2 and *MAPK3/1* (ERK1/2), in 29 patients without *KRAS* or *BRAF* mutations. We identified two mutations of *MAP2K1* in four patients and four new *MAP2K2* mutations in four patients. In total, mutations were identified in 35 of 56 (63 %) CFC patients (3 in *KRAS*, 24 in *BRAF* and 8 in *MAP2K1/2*). There was no significant difference in 81 clinical manifestations and calculated CFC indices among 3 patients with *KRAS*, 16 patients with *BRAF* and 6 patients with *MAP2K1/2* mutations. Relative macrocephaly, sparse curly hair, mental retardation and delayed speech were present in 90 % of mutation-positive patients. No mutations were identified in *MAPK3/1*. Further molecular analysis of RAS pathway will be necessary to identify new genes responsible for mutation-negative CFC patients.

Rod monochromacy: A genetic heterogeneity in the Tunisian population. *F. OUECHTATI[1], A. MERDASSI[2], K. DEROUICHE[2], L. LARGUECH[2], L. EL MATRI[2], S. ABDELHAK[1]* 1) Unit of Research Molecular Investigation of Genetic Orphan Diseases, Pasteur Institute of Tunis, Tunis, Tunisia; 2) Unit of Research Oculogenetics, Institute of Ophthalmology Hedi Rais, Tunis, Tunisia.

The complete achromatopsia named also rod monochromacy is a rare, congenital hereditary stationary disorder of retina featuring complete inability to discriminate between colours, diminished visual acuity in daylight, photophobia, horizontal pendular nystagmus which appear from the fifth month after the birth. . Its prevalence has been estimated at about 1 in 30,000 to 50,000. Achromatopsia is genetically heterogeneous with variable expressivity. The complete form is recessively inherited. The three achromatopsia genes are *CNGA3*, *CNGB3* which respectively encode for and subunits of the cGMP gated cation channel in cone cells and *GNAT2* which encodes the subunit of cone transducin. The transducin and the cGMP gated cation channel are important for the amplification and the transmission of visual signal from cones to the cortical regions. We report here a genetic analysis of a large consanguineous Tunisian family with nine individuals presenting with complete achromatopsia. Genetic linkage to *CNGA3* gene was investigated. For this purpose microsatellite markers overlapping this gene D2S2311 and D2S2175 were selected. Genotyping and linkage analysis excluded linkage to *CNGA3* gene. A previous study has shown that a sporadic Tunisian case with rod monochromacy shared the P372S mutation in *CNGA3* gene with a German patient. This result underlines the rich genetic background related to Tunisian population history which is characterized by a genetic heterogeneity as it has been reported in the Italian population for the same dyschromatopsia.

Validation and implementation of a panel of SNPs for large-scale zygosity testing and population genetics. *U. Hannelius[1], C.M. Lindgren[1,2], C. Lagerberg[3], V-V. Mäkelä[2], A. Lindstedt[2], L. Gherman[3], G. Tybring[3], J. Kere[1,2]* 1) Department Biosciences and Nutrition, Karolinska Institute, Huddinge, Sweden; 2) Clinical Research Centre, Karolinska University Hospital, Stockholm, Sweden; 3) Department of Medical Epidemiology and Biostatistics, Karolinska Institute, Stockholm, Sweden.

Background: Genetic fingerprinting is widely used in forensic sciences, paternity testing and in zygosity testing. Unambiguous identification of samples based on their DNA profiles strengthens the specificity and validity of genetic research.

Aim: To validate a panel of 50 SNP:s that can be used for large-scale zygosity testing as well for population genetic research on a range of different templates.

Results: We selected an initial panel of 50 highly polymorphic (MAF >20% in HapMap CEU population, phase I data) SNP:s including the AMELXY assay with a criterion of no inter-marker LD. 15 CEPH trios and 36 unrelated Coriell samples using DNA and whole genome amplified (Repli-G) DNA as template were genotyped in duplicates. Three SNP:s failed the initial validation. When considering all genotyped samples as well as HapMap data the global genotype concordance was 99.7% (SD 0.7%). Further, we genotyped 100 twin pairs and identified two pairs with wrongly assigned zygosity. The global success rate considering all genotyped samples was 98.4% (SD 1.4%). Preliminary findings show that DNA of lower quality yields lower success rates while the genotype concordance remains unaffected. The random match probability for full siblings being IBS=2 at all loci was 1.58e-10.

Conclusions: Compared to STR typing, the high multiplexing level and rapid analysis makes SNP typing a significantly more efficient and cost-effective way of large-scale DNA-profiling. We have started work on determining the genetic structure in the Swedish population using the validated SNP panel and a set of 2136 samples representative of the Swedish population.

Molecular Genetics of Primary-Angle Closure Glaucoma in a Chinese familyMolecular Genetics of Primary-Angle Closure Glaucoma in a Chinese family. *hui. li, xiao.hua dai, jin.yu liu, mu.gen liu, qing. wang* Center for Human Genome Resear, Huazhong University of Science and Technology, Wuhan, Hubei, China.

Purpose: Juvenile and late-onset primary open-angle glaucoma (POAG) has been associated with myocilin (MYOC) gene and CYP1B1 gene (2p21), with a predominantly autosomal dominant or recessive mode of inheritance respectively. Although there are some similarities in the phenotype of POAG and in particular primary angle-closure glaucoma (PACG), little is known about the role of MYOC and CYP1B1 in the causation of PACG. In this research, we therefore investigated the role of MYOC and CYP1B1 gene in PACG by screening the genes in a Chinese family with autosomal dominant. Methods: Complete ophthalmic examination and genomic DNA were obtained from a Chinese pedigree with 5 family members, in which 3 were confirmed PACG patients. Five polymorphic microsatellite markers, d10S547, d10S1653, d1S218, d2S367 and d2S2259, closely linked to the three know glaucoma genes optn, myoc and cyp1b1 were selected from the ABI PRISM Linkage Mapping Set MD10 panel for linkage analysis. The Chinese pedigree was screened for sequence alterations in the MYOC and CYP1B1 genes by polymerase chain reaction and automated sequencing. Two hundred unrelated individuals without glaucoma were studied as control subjects. Results: In our pedigree we have present three patients whose genomes harbor both MYOC and CYP1B1 sequence variants. These included the Arg46Stop mutation in MYOC gene and CG (L432V) polymorphism in CYP1B1 gene, which have been reported in individuals with POAG. However, both sequence alterations identified have not been identified in same control Chinese individuals. Conclusions: These observations suggest a possible role of MYOC and CYP1B1 in PACG, which probably do not cause glaucoma by inactivating either of normal MYOC and CYP1B1 function , but rather via digenic interaction and/or yet unidentified locus linked to the disease.

Identification of a novel locus for mitochondrial DNA depletion. *J. Mollet[1], J.P. Jais[2], E. Sarzi[1], D. Chretien[1], A. Munnich[1], A. Rotig[1]* 1) INSERM U781, Hosp Necker, Paris, France; 2) Service de Biostatistique et dInformatique Médicale. Hôpital Necker-Enfants Malades.

Mitochondrial DNA depletion syndrome (MDS) is a clinically and genetically heterogeneous condition characterized by reduction in mtDNA copy number responsible for multiple oxidative phosphorylation (OXPHOS) enzyme deficiency. In our series of more than 50 patients with severe mtDNA depletion, the disease-causing mutation has been identified in only 36% of the patients. We identified a large consanguineous family from North-Africa with multiple respiratory chain deficiency associated with mtDNA depletion. The four patients presented neonatal hypotonia, growth retardation and/or liver enlargement. Metabolic investigations revealed hyperlactatemia and highly elevated levels of urinary thymidine and uracil but these features were not consistently found in all patients. Biochemical analyses of skeletal muscle revealed OXPHOS deficiency in ¾ patients. A genome wide scan was performed using the GeneChip Human Mapping 10K 2.0 Array (Affymetrix) but did not revealed any region of homozygosity common to the four affected children. Nevertheless, a more careful examination of clinical, biological and enzymological data suggested the occurrence of two diseases in this family. Based on this hypothesis, we were able to identify a first region of homozygosity on chromosome 1 encompassing the DPYD gene encoding the dihydropyrimidine dehydrogenase. Mutations of DPYD were found in the three patients with high level of urinary thymidine and uracil. A second region common to the three patients with OXPHOS deficiency was found on chromosome 21 with a maximum multipoint lod-score of 3.597. Candidate genes are now under investigation. This work will hopefully lead to identification of a novel nuclear gene responsible for mtDNA depletion.

Mutational analysis of SCN2A gene in Italian families with Benign Familial Neonatal-Infantile Seizures (BFNIS).

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Benign autosomal dominant epilepsy syndromes of the first year of life include two main syndromes: benign familial neonatal seizures in which seizures start around day 3 and it is usually caused by defects in potassium channel genes KCNQ2 and KCNQ3, and benign familial infantile seizures which begins around 6 months of age but no genes have been so far identified. Recently, molecular studies have separated an intermediate variant of BFNIS, in which seizure onset varies from 2 days to 3.5 months and it is caused by mutations in the voltage-gated sodium channel alpha2 subunit (SCN2A) gene on chromosome 2. In this study, we wished to screen 2 families with BFNIS from southern Italy for mutations in SCN2A gene. Both families originated from southern Italy. The first family contained 3 affected individuals over four generations, in whom seizures always started around two months of age. In the second family with 4 affected individuals over four generations seizures started around day 17. Response to antiepileptic drugs and the outcome were good in both families. After informed consent, DNA was analyzed for mutations in the coding regions of SCN2A (28 exons) by PCR and sequencing. One proband from each family was screened. We have not found any mutation in the examined exons of the SCN2A gene. Mutations in SCN2A gene were reported in families with BFNIS of Italian, Canadian, Australian and American origin. Nonetheless, also in the original report some BFNIS families did not carry any mutations in SCN2A. Our data provide further evidence for genetic heterogeneity associated with BFNIS. It might be possible, however, that these families have SCN2A mutations in a nontranslated sequence. Supported by FIRB-MIUR anno 2001 RBNEO1XP4.

A variant in CDKN2a is associated with physical function in older people. *A. Murray[1], T.M. Frayling[1], A.J. Hurst[1], L.W. Harries[1], H. Song[2], K.T. Khaw[2], R. Luben[2], P.G. Surtees[2], S. Bandinelli[3], A.M. Corsi[4], L. Ferrucci[5], J.M. Guralnik[5], R.B. Wallace[6], A.T. Hattersley[1], P.D. Pharoah[2], D. Melzer[1]* 1) Peninsula Medical School, University of Exeter, Exeter, Devon, United Kingdom; 2) University of Cambridge, UK; 3) Italian National Research Council on Aging, Florence, Italy; 4) University of Florence, Italy; 5) National Institute on Aging, Maryland, USA; 6) University of Iowa, USA.

Aging is thought to be affected by a small number of biochemical pathways and recent evidence suggests that cell cycle arrest contributes, when senescence and cell loss exceeds cell regeneration. However, whether cellular senescence is a causal factor of age-related damage has been controversial, especially in humans. Cyclin-dependent kinases (CDKs) are involved in regulating the cell cycle and are in turn regulated by the CDKN family of inhibitors. Thus the CDKN genes are candidates for human aging.

Physical functioning is known to be highly heritable in old age and may be a better marker of the aging phenoptype than longevity. We used an initial study of 926 women aged 65 to 80yrs from the Norfolk site of the EPIC study and tested 25 tag SNPs in 5 CDKN genes. Physical functioning was measured by SF36 questionnaire. The rare allele of the rs2811712 SNP in CDKN2a ($p16^{ink4a}$) was associated with reduced limitation on the SF36 scale; odds ratio 1.38 (95%CI 1.06-1.79, p=0.015). The same allele of rs2811712 was associated with reduced physical limitation, measured by reported and performance tests, in 4 additional studies: two independent sets of respondents from the EPIC study (1916 individuals), 730 subjects from the Italian InChianti aging study and 419 subjects from the Iowa site of the Established Populations for Epidemiologic Studies in the Elderly (EPESE). The overall odds ratio was 1.31(95%CI 1.15-1.50), and was highly significant (p =7.4x10-5).

These data provide evidence for CDKN2a having a role in human age-related functioning, which may be mediated by influencing cell senescence.

Screening for the modifier gene(s) involved in phenotypic variation and malignant degeneration of neurofibromatosis type 1. *H.J. Kim, Y.R. Park, H.J. Jung, S.J. Park, S.Y. Jeong* Dept Medical Genetics, School of Medicine, Ajou University , Suwon, Korea.

Neurofibromatosis type 1 (NF1) is one of the most common inherited autosomal dominant disorders, with an estimated incidence of 1 per 3,500 births. NF1 is caused by mutations in the *NF1* gene which consists of 57 exons and encodes a GTPase activating protein (GAP), neurofibromin. NF1 is notable for its extreme phenotypic variability both within and between families and for a gradual malignant degeneration of normal and/or benign tumor cells within an individual. In addition to multiple allelism of the *NF1* locus, the influence of modifying gene(s) and the impact of stochastic events have been suggested as causes of this variability. However, there has been no clear evidence to explain the phenotypic variations among patients with an identical mutation. Korean NF1 patients also show a wide range of phenotypic variations. We screened 27 unrelated Korean NF1 patients for mutations and identified 25 distinct NF1 mutations. Mutation study revealed a wide spectrum of *NF1* mutations in Korean patients. A genotype-phenotype correlation analysis suggests that there is no clear relationship between specific *NF1* mutations and clinical features of the disease. For whole gene expression comparison among normal fibroblast, benign and malignant tumor cells, we used ACP (annealing controlled primer) based PCR method, GeneFishing DEG (differentially expressed gene) Screening, which substantially improves the specificity and sensitivity of PCR and eliminates the false positives and poor reproducibility of previous DEG discovery methods such as cDNA microarray. In this study, we performed DEG screening using RNAs from NF1 patient with 120 sets of ACP. We found total 64 DEGs that include 37 DEGs showing up-regulation and 27 DEGs showing down-regulation in malignant cells. We have cloned and sequenced 20 DEGs and 44 DEGs remain to be identified. A further functional analysis for the candidate DEGs may lead to identification of the key modifier gene(s). This study will contribute to a better understanding the phenotypic variability and the mechanism of the malignant degeneration of NF1.

Down-regulation of the Dopamine Receptor D2 (Drd2) in mice lacking Ataxin 1. *A. MATILLA-DUEÑAS[1], A. HUNT[1], M. HUBANK[1], J. HOLTON[2], T. REVESZ[2], A. PASTORE[3], R. GOOLD[1]* 1) Institute of Child Health, University College London, London, United Kingdom; 2) Institute of Neurology, University College London, London, United Kingdom; 3) National Institute for Medical Research, London, United Kingdom.

Ataxin 1 (Atxn1) is a protein of unknown function associated with spinocerebellar ataxia type 1 (SCA1) in humans, a neurodegenerative disease characterized by loss of balance and motor coordination (ataxia) due to progressive cerebellar dysfunction. The disease symptoms in SCA1 are caused by an expanded polyglutamine within Atxn1, which causes neurotoxicity in humans, mice, and flies by unclear gain-of-function mechanisms. Lack of Atxn1 in mice causes cerebellar motor deficits in the absence of neurodegeneration or apparent neuropathological abnormalities. To investigate the molecular mechanisms underlying cerebellar dysfunction in Atxn1-null mice, we generated their cerebellar gene expression profiles using oligonucleotide microarrays. We have identifed several genes implicated in the retinoid/thyroid hormone receptors signaling pathways dysregulated in Atxn1-null mice, including the dopamine receptor D2, the dopamine transporter, the retinol binding protein 1, and the thyroid hormone transporter transthyretin. Drd2 protein levels are severely decreased in the cerebellum and other brain regions in Atxn1-null mice. We provide evidence for transcriptional regulation by Atxn1 targeting the Drd2 promoter and we demonstrate that Atxn1 physically interacts and functions synergistically with transcription factor Sp1 to trans-activate Drd2 expression. The interaction and transcriptional effects are mediated by the AXH domain within Atxn1 and are abrogated by the expanded polyglutamine in mutant Atxn1. Our study provides evidence implicating ataxin 1 in the regulation of Sp1-dependent transcription and identifies molecular targets that could underlie the neurobehavioural deficits in Atxn1-null mice. Identifying the molecular pathways regulated by Atxn1 mediating cerebellar function can provide insights into the molecular mechanisms underlying cerebellar neurodegeneration in SCA1 and establish effective therapeutic strategies.

Genetic and acquired factors for ossification of the posterior longitudinal ligament of the spines in Japan; a case-control study. *G. Kobashi[1], K. Ohta[1], A. Hata[2], M. Washio[3], K. Okamoto[4], Japan OPLL Epidemiological Study Group*
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To elucidate risk factors of genetic variants and acquired factors such as lifestyles for ossification of the posterior longitudinal ligament of the spines (OPLL) in Japan, a case-control study was carried out. A self-administered questionnaire was obtained from 63 patients with OPLL in collaborate hospitals and age, sex and domicile-matched controls, 126 from hospital patients without OPLL and 126 from health check up in a town. Genotypes of vitamin-D receptor gene (*VDR*) F*ok*I genotype, lipoprotein lipase gene (*LPL*) H*ind*III genotype, and nucleotide pyrophosphatase gene (*NPPS*) IVS15-14TC were identified by use of PCR-RFLP methods. Frequency of *VDR* F*ok*I F allele was higher in OPLL (0.66) than in hospital controls (0.54) and population controls (0.56)(p0.05), while no significant differences were found in distributions of *LPL* H*ind*III genotypes and *NPPS* IVS15-14TC genotypes between cases and controls. An univariate analysis of the data from the questionnaire survey revealed high body mass after middle age, history of diabetes, history of hypertension, lack of body pliableness or stocky body shape, and some characters in Type A tendency were significantly associated with OPLL. A tree-based analysis to clarify confoundings among them revealed *VDR* F*ok*I F allele was associated with history of diabetes, suggesting that *VDR* F*ok*I F allele was the third factor of the association between OPLL and diabetes. *VDR* F allele was suggested to be associated with OPLL as well as diabetes. Further case-control studies in OPLL using candidate genes of hypertension are needed to elucidate the pathogenesis in OPLL and establish an effective intervention program for OPLL.

DNA array profiling of genes involved in differentiation of stem cell to spinal cord precursor cell in mouse. *H. Jeong, J.I. Ahn, H.J. Jeong* Molecular Pharmacology, NITR/KFDA, Seoul, Korea.

Stem cell is one of most promising therapeutic tools for neurodegenerative diseases such as Parkinsons and Alzheimer and so on. Stem cell therapy still has lots of obstacles to overcome to become a reliable therapeutic option. The biggest hurdle confronted is how to regulate correctly the pluripotency of stem cell to get intended cell type like motor neurons in this presented study. In this study, we isolated neural stem cells from E11.5 mouse spinal cord and differentiated them to motor neuron by using well known compounds like retinoic acid and BMP-2. The functionality of obtained cell was confirmed by a co-culture system of motor neuron and skeletal myotubes. To identify related genes in differentiation of stem cell to motor neuron, DNA microarray was employed to find genes whose expression level was significantly changed along the differentiation at day 2, 4 and 8. We isolated 1066 genes which had shown at least 2-fold expression at more than one day out of 23666 genes. These isolates included and some unexpected genes transcription factors (Nkx6.2, four and a half LIM domains, basic helix-loop-helix domain containing class B etc.) in addition to motoneuron-related genes such as ChPT1, PLD2, Nkx6-2 and NDE2. In this study, we identified groups of genes involved in differentiation of stem cell into motoneuron including genes with no known previous functions in stem cell differentiation, like Tbx20 and Lmo2 so on. Further investigation on these genes may shed light on the understanding of stem cell into spinal cord precursor cell that can facilitate studies of the mechanisms underlying induction and differentiation of stem cell to motoneuron.

Identification of prosencephalon-specific enhancer of SALL1: Comparative genomic approach using chick embryonic system. *K. Izumi[1], M. Aramaki[1], M. Uchikawa[2], H. Kondoh[2], Y. Naito[1], R. Kosaki[3], T. Takahashi[1], K. Kosaki[1]* 1) Department of Pediatrics, Keio University, Tokyo, Japan; 2) Graduate School of Frontier Biosciences, Osaka University, Suita, Japan; 3) Department of Clinical Genetics and Molecular Medicine, National Center for Child Health and Development, Tokyo, Japan.

Comparative genomics is a promising approach for disclosing regulatory elements that govern the unique spatio-temporal gene expression patterns of morphogenetic genes. Conserved non-coding genomic sequences have been considered as candidate regulatory elements. We performed a systematic survey for conserved non-coding elements (CNEs) belonging to the SALL1 gene, the causative gene of Townes-Brocks syndrome [TBS]. A comparison of the genomic sequences of humans and avians revealed 5 CNEs in the vicinity of the SALL1 gene. Genomic fragments corresponding to each CNE were inserted into reporter cassettes consisting of GFP cDNA and a minimal promoter. After CNE insertion, the construct was transfected using in vivo electroporation. If the CNE conferred enhancer activity at a specific location in the embryo, a GFP signal was detected in vivo. Among the 5 CNEs that were examined, one 623 bp-long CNE (CNE3) exhibited tissue-specific enhancer activity. At the neurula stage, the GFP was visualized in the prosencephalon. Histological analyses revealed that the GFP signal was present only on the ventral side of the prosencephalon. At the pharyngula stage, the GFP signal was confined within the anterior neural ridge, which represents the morphogenetic center regulating the patterning of the anterior neural plate. The GFP signal distribution in the forebrain recapitulated the SALL1 expression pattern previously demonstrated using in situ hybridization. This report identified, for the first time, an enhancer element of SALL1. Documentation of enhancer activity in the forebrain corresponds to the clinical observation that some patients with TBS have central nervous system dysfunctions. A comparative genomic approach, when combined with a chick embryo electroporation system, represents an effective method for identifying developmental regulatory elements.

Genome-wide association study of human narcolepsy using 500,000 SNPs. *T. Miyagawa[1], M. Kawashima[2], N. Nishida[1], J. Ohashi[1], R. Kimura[1], A. Fujimoto[1], M. Honda[3], Y. Honda[4], K. Tokunaga[1]* 1) Department of Human Genetics, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; 2) Department of Sleep Disorder Research, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; 3) Tokyo Institute of Psychiatry, Tokyo, Japan; 4) Sleep Disorder Clinic of Seiwa Hospital, Tokyo, Japan.

Human narcolepsy is a sleep disorder that is associated with multiple genetic and environmental factors. Narcolepsy affects 0.02-0.06% of the general population in the United State and Europe whereas 0.16-0.18% in Japan. A genetic factor strongly associated with the disorder has been found in the human leukocyte antigen (HLA) region: the HLA DRB1*1501-DQB1*0602 haplotype. However, it is suggested that narcolepsy susceptibility gene(s) other than HLA also exist because HLA DRB1*1501-DQB1*0602 haplotype carriers are about 12% in the Japanese general population, and the HLA alone cannot statistically explain all genetic factors. Therefore, to identify unknown narcolepsy susceptibility gene(s), we performed a genome-wide association study instead of a candidate gene approach. We genotyped about 500,000 SNPs in 110 narcoleptic patients and 191 healthy controls (Affymetrix GeneChip Human Mapping 500K Array Set) and performed case-control association analyses. The subjects investigated in this study were all Japanese living in the Tokyo area. We identified about 1,000 SNPs that were associated with human narcolepsy at significant p values of lower than 10^{-3}. Since it is possible to include a lot of false positive SNPs in this result, we are going to increase the sample sizes and perform a replication study. We also plan to compare the results in the present study with those in the genome-wide association study using about 23,000 microsatellite markers (Kawashima et al. Am. J. Hum. Genet. 2006).

Functional association of an intron variant of CXCR3 with the risk of asthma. *Y. Kim, J.W. Choi, M.Y. Hwang, H.S. Cheong, H.D. Shin, C.S. Park, B. Oh* Center for Genome Science, KNIH, Seoul, Korea.

Complex trait-diseases such as asthma have been known caused by accumulative effects of multiple genes that individually exert quantitative portions of the genetic influence. Genes known implicated in pathogenesis of the diseases could be possible candidates as the disease risk factors. Asthma is known as a respiratory disease characterized by airway hyper-responsiveness, increased numbers of lung mast cells, elevated mucous production, and pulmonary inflammation with prominent infiltration of lymphocytes and eosinophils. The chemokines and their cognate receptors play primary roles in chemotaxis mediating trafficking of leukocytes to the site of inflammation. By using a candidate gene approach, we have previously shown that single nucleotide polymorphism in the intron of CXCR3 (c. 12+234G>A) was statistically associated with the risk of asthma (OR=0.73 (0.58-0.93), P=0.009). To determine if this association may be attributable to alteration of the CXCR3 gene expression by the genetic variation in the intron region, we employed two-way approaches in this study: a reporter gene expression assay and quantitative analysis of the allele-specific expression level. Results from the reporter gene expression assay showed that Jurkat or HEK293 cells transfected with the reporter construct carrying the promoter and intron regions with minor allele of the variant expressed lower level of the reporter gene, relative to the cells with the constructs bearing either the promoter, intron, or both of the promoter and the intron with common allele of the variant. Quantification of transcription level revealed that the CXCR3 transcripts was lower in transformed lymphocytes from the minor allele-carrying individuals than ones from the common-allele carrier, which was consistent with the result from the reporter gene assay. These data, together with the previous data from the case-control study, suggested that the CXCR3 intron variant was functionally associated with the risk of asthma by affecting the expression level of the gene.

A confoundings of candidate single nucleotide polmorphisms for pregnancy-induced hypertention in Japan. *K. Ohta[1], G. Kobashi[1], H. Yamada[2], A. Hata[3], H. Minakami[2], S. Fujimoto[4], K. Kondo[5], Hokkaido PIH Epidemiological Study Group* 1) RadGenomics Research Group, Research Center for Charged Particle Therapy, National Institute of Radiological Sciences, Chiba, Japan; 2) Department of Obstetrics and Gynecology, Hokkaido University Graduate School of Medicine, Sapporo, Japan; 3) Department of Public Health, Chiba University Graduate School of Medicine, Chiba, Japan; 4) Tenshi Hospital, Sapporo, Japan; 5) The University of the Air, Chiba, Japan.

To clarify the context of the gene-gene interrelationship in the manifestation of PIH, we analyzed Met235Thr variant of the angiotensinogen gene (*AGT*) and Glu298Asp variant of the endothelial nitric oxide synthase gene (*NOS3*) in terms of 4G/5G variant of the plasminogen activator inhibitor gene (*PAI-1*). One hundred forty-five Japanese patients with PIH were matched with 357 Japanese normal pregnant controls according to age and parity. Genotypings of variants of *AGT*, *NOS3* and *PAI-1* were carried out using PCR-RFLP methods after extraction of genomic DNA from 1.0 ml of whole blood samples. In the subgroup of 4G/4G of *PAI-1*, the frequency of T235 of *AGT* was higher in PIH (0.78) than in controls (0.56) (p0.017), while in the subgroup of 4G/5G+5G/5G of *PAI-1*, no significant differences were found in the frequency of T235 of *AGT* between PIH (0.71) and controls (0.6). On the other hand, in the subgroup of 4G/4G of *PAI-1*, no significant differences were found in distributions of the frequency of GA+AA of *NOS3* between PIH (0.18) and controls (0.1), but in the subgroup of 4G/5G+5G/5G of *PAI-1*, the frequency of GA+AA of *NOS3* was higher in PIH (0.3) than in controls (0.15) (p0.017). Women carrying T235 of *AGT* had an elevated risk for PIH because the local AGT expression is elevated in developing spiral arteries in women carrying this allele. On the other hand, Asp298 of *NOS3* is associated with the reduction of NO in PIH. The present result suggests that coagulative tendency by 4G/4G of *PAI-1* may play a role in pathogenesis of PIH in the subgroup possessing TT of *AGT*.

Long-term expression and clinical improvement of Mucopolysaccharidosis I in dogs and cats from neonatal, intravenous, retroviral vector gene therapy. *M. Haskins[1], A. Traas[1], P. Wang[1], B. Wang[2], X. Ma[2], P. O'Donnell[1], R. Herati[2], B. Wang[2], K. Cullen[1], U. Proiuck[1], T. O'Malley[1], M. Sleeper[1], G. Aguirre[1], K. Ponder[2]* 1) Sch Vet Med, Univ Pennsylvania, Philadelphia, PA; 2) Sch Med, Washington University, St. Louis, MO.

Mucopolysaccharidosis I (MPS I) is a lysosomal storage disease due to deficient activity of alpha-L-iduronidase (IDUA). The MPS I cat has a 3 base-pair deletion and the MPS I dog a premature stop codon. We treated 9 MPS I cats and 7 MPS I dogs at 2-5 days of age intravenously with 0.5 to 10.1 X 10E9 transducing units/kg of a retroviral vector (RV) expressing canine IDUA from the human 1-antitrypsin promoter. **MPS I cats:** Seven treated cats had serum activity 30-fold normal (286416 U/ml) and 245-fold normal (23552763 U/ml) at 1 month (low and high RV dose, respectively). However, all cats lost expression by 3 months from a CTL response to the canine IDUA protein. Two additional MPS I kittens were treated with the immunosuppressive agent CTLA4-Ig for 2 weeks at the time of high dose RV therapy and have maintained 20- & 25-fold normal serum IDUA activity for more than a year. Liver biopsies at 4 months had IDUA activity 4- & 5.7-fold normal (90 & 123 U/mg protein). Glycosaminoglycans (GAG) were reduced to 3.9 & 6.0 ugm/mg protein (untreated 36.614.9, N=4; normal=1.20.9, N=4). Clinically, the two CTLA4-Ig/RV cats had improvements in facial dysmorphia, corneal clouding, stature, posture, and mobility. **MPS I dogs:** The treated dogs had a mean 23-fold normal serum IDUA activity (22-749 nmol/ml/hr). The 3 oldest dogs at more than 1 year of age have stable activity at 1.3-, 2.1-, and 41-fold normal. In liver biopsies, IDUA activity was 5-fold normal (133163 U/mg protein). GAG was reduced to 2.31.3 ugm/mg protein (untreated=264, N=3; normal=1.30.7, N=3). Clinically, the MPS I dogs had improvements in facial dysmorphia, corneal clouding, and mitral valve lesions. No treated dog had signs of cervical cord compression. **Conclusion:** These data show that neonatal, intravenous, RV gene therapy was successful in producing high, stable serum IDUA levels in MPS I cats and dogs, improving clinical disease.

Genetic teesting of nonketotic hyperglycinemia: Detection of genomic deletion within *GLDC* by multiplex ligation-dependent probe amplification. *S. Kure[1], J. Kanno[1], T. Hutchin[2], F. Kamada[1], A. Narisawa[1], Y. Aoki[1], Y. Matsubara[1]* 1) Dept Medical Genetics, Tohoku Univ Sch Medicine, Sendai, Japan; 2) Department of Clinical Chemistry, Birmingham Childrens Hospital, United Kingdom.

Nonketotic hyperglycinemia (NKH) is an inborn error of metabolism characterized by accumulation of glycine in body fluids and various neurological symptoms. NKH is caused by deficiency of the glycine cleavage multi-enzyme system with three specific components encoded by *GLDC*, *AMT*, and *GCSH*. The majority of patients are deficient of enzymatic activity of glycine decarboxylase, which is encoded by *GLDC*. Our recent study has suggested that there are a considerable number of *GLDC* mutations, which are not identified by the standard exon-sequencing method (Kure et al., Hum Mutat 2006;27:343-52). We have developed a screening system for *GLDC*, deletions by multiplex ligation-dependent probe amplification (MLPA). Two distinct cohorts of patients with typical NKH were screened by this MLPA method: the first cohort consisted of 45 families with no identified *AMT*, or *GCSH*. mutations and the second cohort was comprised of 20 patients from the UK. *GLDC*, deletions were identified in 16 of 90 alleles (18%) in the first cohort and 9 of 40 alleles (22.5%) in the second cohort. Fourteen different types of deletions of various lengths were identified, including one allele where all 25 exons were missing. We determined flanking sequences of interstitial deletions in five patients, and *Alu*-mediated recombination was identified in 3 of the 5 patients. These results suggest that *GLDC* deletions are a significant cause of NKH and that MLPA analysis is a valuable first-line screening for NKH genetic testing.

Investigation of the inducible nitric oxide synthase gene (NOS2A) polymorphisms in multiple sclerosis. *I. Manna[1], P. Valentino[2], A. La Russa[1], F. Condino[1], R. Nisticò[2], A. Clodomiro[2], M. Canino[2], R. Cittadella[1], A. Quattrone[1,2]* 1) Institute of Neurological Sciences (ISN) -CNR, Mangone, Cosenza, Italy; 2) Institute of Neurology, University Magna Græcia, Catanzaro, Italy.

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS), characterised by demyelinisation, gliosis and various degrees of axonal damage. Nitric oxide (NO) is a biological signaling and effector molecule and is especially important during inflammation. Inducible nitric oxide synthase (iNOS) is one of the three enzymes responsible for generating NO, and it is up-regulated in the CNS of animals with experimental allergic encephalomyelitis (EAE) and in patients with MS. The gene encoding iNOS, NOS2A, is located on chromosome 17q11.2-12, a region identified in genome wide screens as suggestive for linkage and association with MS. This study investigated the contribution of two functionally polymorphisms located in the promoter region of the NOS2A gene to susceptibility to MS. A group of patients with clinically definite MS (n = 115) were genotyped for a multiallelic pentanucleotide (CCTTT)n tandem repeat and a bi-allelic tetranucleotide AAAT/AAAAT repeat, and compared to group of healthy controls (n = 238). For the case-control study, patients and controls were genotyped for these polymorphisms using a PCR methods that involved the primer sense labelled with the fluorescent dye 6-fam. To determine the different alleles the PCR products were resolved on an ABI 377 automated DNA sequencer and analyzed using Genescan 3.1. For statistical analysis we collected (CCTTT)n alleles in two categories according to the number of repeats : Short alleles (S<10 repeats) and Long alleles (L>10 repeats). The differences among groups distribution were assessed using chi square test or exact test based on Monte Carlo method for sparse data. To assess differences in clinical features among subject with different genotype, one-way analysis of variance or Kruskal-Wallis test was used. No significant differences in allele or genotype frequencies for the NOS2A polymorphisms between MS patients and controls were observed ($p>0.05$), also when the stratified analysis for gender was performed. Lack of association was also observed when the data were stratified for specific clinical variables (age at examination, age at onset, disease duration and EDSS) ($p>0.05$). Statistical analysis demonstrates lack of association between the NOS2A promoter polymorphisms and MS and hence the gene does not appear to play a genetically significant role in MS pathology.

Aneuploidy risk evaluation according to the degree of analyte deviation in combined I. trimester screening. *M. Macek[1], M. Simandlova[1], S. Vilimova[1], R. Vlk[1], H. Cuckle[2], I. Spalova[1], A. Lashkevich[1], B. Cardova[1], M. Turnovec[1], J. Diblik[1], M. Havlovicova[1], M. Macek Jr.[1]* 1) Charles University Prague and University Hospital Motol, Prague, Czech Republic; 2) Leeds Screening Centre, University of Leeds, United Kingdom.

The aim of this study was to asess the efficacy of combined I. trimester screening by PAPP-A and free hCG, nuchal translucency measurements, LifeCycle/Elipse software, by the degree and number of marker deviations from 1.6-1.9 MoMs. 1415 I. trimester sera examinations were confirmed by cytogenetics. Aneuploidy risk categories (cat.) I.-IV. were defined according to marker number and degree of deviation, presuming the lowest aneuploidy risk in cat. I. and highest in cat. IV. Cat. I. (1.6-1.9 MoM) was detected in 46.2%, II. (0.5-0.6 and 1.9-2.0 MoM) in 11.7%, III. in 31.6% (only one marker >2.0 or <0.5), IV. in 10.7% (3-4 markers <0.5 and/or >2.0) of cases. In cat. I. only structural aberrations were detected in 2/688 (0.29%); in cat. II. only heterochromosomal aneuploidies (47,XXY; 46,XX/45,X) were found in 2/163 (1.23%); in cat. III. autosomal and heterochromosomal aneuploidies, including FRAXA syndrome, were detected in 6/468 (1.28%). Cat. IV. provided the highest 10/96 (10.4%) efficacy (+21,+13, triploidy, mosaics 45,X/46,XX and 47,XYY), compared to cat. I.-II. (p=0.00001). Combination of all markers in cat. III.-IV. assured 100% detection of all types of autosomal aneuploidies, whereas only number/degree of analyte deviations, 100% of heterochromosomal aneuploidies in categories II.-IV. Aneuploidy risk is also related to number/degree of marker deviations that increase detection rate of combined I. trimester screening: cat. I. without aneuploidy, cat. II. only with heterochromosomal aneuploidies, in cat. III. heterochromosomal and autosomal aneuploidies, with highest aneuploidy detection rate in cat. IV. Such screening strategy enables individualization of prenatal care and diagnosis. Genetic counseling is recommended in cat. II.-IV., prenatal diagnosis in III.-IV. according to results of clinical and ultrasound examinations and contingent screening in woman within cat. III.-IV. Supported by VZFNM MZO00064203.

SNP association study of esophageal squamous cell carcinoma in a high-risk population from China. *D. Ng[1], N. Hu[1], X.Y. Han[2], C. Giffen[3], Z.Z. Tang[2], A.M. Goldstein[1], M.P. Lee[4], P.R. Taylor[1]* 1) Genetic Epidemiology Branch, DCEG, NCI, NIH, DHHS, Bethesda, MD; 2) Shanxi Cancer Hospital, Taiyuan, Shanxi, PR China; 3) Information Management Services, Inc., Silver Spring, MD; 4) Laboratory of Population Genetics, CCR, NCI, NIH, DHHS, Bethesda, MD.

Esophageal squamous cell carcinoma (ESCC) incidence and mortality rates in north-central China are the highest in the world; rates in this region exceed the average Chinese rate by 10-fold and the US Caucasian rate by 100-fold. Geographic variation of ESCC rates in China suggests that environmental and/or lifestyle factors are major contributors to the etiology of esophageal cancer. In high-risk regions such as Shanxi Province, there is a strong tendency towards familial aggregation suggesting an etiologic role for genetic susceptibility and/or gene-environment interactions. To identify susceptibility genes that predispose to ESCC, we completed a pilot case-control whole genome SNP association study using the Affymetrix 10K SNP array in 50 ESCC patients and 50 matched controls from Shanxi Province. Using the generalized linear model (GLM) with adjustments for potential confounders and multiple comparisons, we identified 37 SNPs associated with disease assuming a recessive mode of inheritance, 48 SNPs assuming a dominant mode and 53 SNPs in a continuous mode. When the 37 SNPs identified from the GLM recessive mode were used in a principal component analysis, the first principal component correctly predicted 46 of 50 cases and 47 of 50 controls. To correct for 10,264 separate analyses, we used a Bonferroni-adjusted significance level of $P < 4.87187 \times 10\text{-}6$ to select combined SNPs identified from the GLMs of all three modes of transmission. Thirty-eight SNPs were identified that met the Bonferroni-adjusted significance level and were located in or near genes. To further validate these SNPs for genetic susceptibility/risk assessment, we performed a second study in which 300 new ESCC cases and their matched controls were genotyped using a multiplex oligonucleotide ligation assay to examine the 38 SNPs identified from the pilot case-control study.

Mutations in each of three Ribonuclease H2 subunits cause Aicardi Goutières Syndrome and mimic congenital viral brain infection. *A.P. Jackson[1], A. Leitch[1], B.E. Hayward[2], A. Garner[1], R. Parmar[2], E. Griffith[1], M. Ali[2], P. Lebon[3], D.T. Bonthron[2], C. Ponting[4], Y.J. Crow[2], The AGS consortium* 1) Medical Genetics, MRC Human Genetics Unit, Edinburgh, United Kingdom; 2) Leeds Institute of Molecular Medicine, University of Leeds, St Jamess University Hospital, Leeds, LS9 7TF, UK; 3) Service de Virologie, Hôpital Cochin, St Vincent de Paul, 82 Avenue Denfert Rochereau, 75674, Paris, France; 4) MRC Functional Genetics Unit, Department of Human Anatomy & Genetics, University of Oxford, South Parks Road, Oxford, OX1 3QX, UK.

Aicardi Goutières Syndrome (AGS) is an autosomal recessive neurological disorder whose clinical and immunological features parallel those of congenital viral infection. Using a combination of positional cloning, homology prediction and functional studies, we determine the human counterparts of the S.cerevisiae RNase H2 enzyme complex and identify pathogenic mutations in all three protein subunits, AGS2 (RNASEH2B, FLJ11712), AGS3 (RNASEH2C, AYP1) and AGS4 (RNASEH2A). Additionally, we show that these mutations may lead to reduced enzymatic function. Our findings thus demonstrate a role for this ribonuclease in human neurological disease, and suggest an unanticipated relationship between Ribonuclease H2 and immune response, which warrants further investigation.

A locus for familial skewed X chromosome inactivation maps to chromosome Xq25 in a family with a female manifesting Lowes syndrome. *M.A. Melis[1], M. Addis[1], C. Meloni[1], A. Cao[2], R. Congiu[1], S. Santaniello[1], M. Loi[3], F. Emma[4], O. Zuffardi[5, 6], R. Ciccone[5], G. Sole[2], M. Cau[1]* 1) Scienze Biomediche e Biotecnol, University of Cagliari, Cagliari, Italy; 2) Istituto di Neurogenetica e Neurofarmacologia, CNR, Selargius (CA) Italy; 3) Servizio di Neuropsichiatria. Azienda Ospedaliera G. Brotzu, Cagliari, Italy;; 4) Department of Nephrology and dialysis, Bambino Gesù, Children's Research Hospital, Rome, Italy; 5) Genetica Medica Università di Pavia, Italy; 6) IRCSS Policlinico San Matteo, Pavia, Italy.

In mammals X linked gene products can be dosage compensed between males and females by inactivation of one of the two X chromosomes in the developing female embryos. X Inactivation choice is usually random in embryo mammals , however several mechanisms can influence the choice determining skewed X inactivation. As consequence, females heterozygous for X linked recessive disease can manifest the full phenotype. Herein, we report a family with extremely skewed X inactivation that produced the full phenotype of Lowe's syndrome, a recessive X linked disease, in a female. The X chromosome inactivation studies detected an extremely skewed inactivation pattern with a ratio of 100:0 in the proband as well as in 5 out of seven unaffected female relatives in 4 generations. The OCRL1 de novo mutation, resides in the active paternally inherited X chromosome. The analysis of the minimal XIST promoter did not show DNA sequenze variation. Array-CGH revealed a normal profile of all chromosomes including the X. X chromosome haplotype analysis localized the familial skewed X inactivation locus to chromosome Xq25 with a maximum multipoint LOD score of 2.11 between markers DXS8059 and DXS8057. The description of this case adds Lowe's syndrome to the list of X-linked disorders which may manifest the full phenotype in females because of the skewed X-inactivation. Furthermore our study indicates that the skewed X inactivation in this family depends most likely on the inheritance of a dominant X-linked trait controlling X chromosome choice in X inactivation or cell proliferation.

Cytoplasmic aggregates and proteolytic cleavage of the 1A calcium channel in Spinocerebellar ataxia type 6. *T. Ishiguro, K. Ishikawa, T. Amino, T. Tsunemi, H. Mizusawa* Dept Neurology, Tokyo Medical & Dental Univ, Tokyo, Japan.

Spinocerebellar ataxia type 6(SCA6)is dominantly inherited ataxia clinically characterized by pure cerebeller symptoms, and pathologically characterized by nearly selective and progressive death of Purkinje cells. The causative mutation is the expansion of (CAG) repeat coding polyglutamine in the carboxyl terminal of the alpha1A voltage-dependent calcium channel. In this study, we show that a 75kD 1A carboxyl (C-) terminal fragment is cleaved from the full length calcium channel protein in western blots when 1A subunits containing either of 13 polyglutamine (Q13), 28 polyglutamine (Q28), or 165 polyglutamine (Q165) tracts were transiently expressed in PC12 cells. On immunohistochemistry using polyclonal antibody against the channel protein, C-terminal fragment was present both in the perinucler space and cytoplasm in cultured PC12 cells. On the other hand, the N-terminal part of the 1A was not always co-localized with C-terminal fragment. These cytoplasmic aggregates were morphologically similar to the 1C2 positive small granular aggregates seen in Purkinje cells of SCA6 patients brains. The present study may indicate that a certain proteolytic cleavage of the channel protein is underlying in the formation of calcium channel aggregates.

The TBX1 gene in the 22q11.2 deletion and duplication syndromes: a susceptibility factor for mental retardation.

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A mutational screen for TBX1 mutations was performed on 38 non-deleted patients that had clinical features compatible with the 22q11.2 deletion syndrome. Two rare variants were identified. The first is a de novo missense mutation in a patient with Tetralogy of Fallot, immunodeficiency and slight developmental delays. This mutation is localized in exon 9C and possibly affects a conserved amino acid within a transactivation domain. The second mutation is within the 5UTR region. This change affects two brothers with isolated congenital heart disease. Modelling studies show that this change affects the 5UT mRNA secondary structure. In addition, in vitro translation experiments showed this change to double the protein doses. Recently, patients referred for fragile-X were found to be carriers of duplications in the 22q11.2 region. Because the 5UT nucleotide change is functionally equivalent to a duplication of the TBX1 gene we screened 200 patients referred for fragile-X determination and detected 3 carriers of this change not detected in 400 healthy controls. However, affected carriers had all a normal carrier parent, which shows that the presence of the 5UT change is not sufficient to cause congenital heart disease or mental retardation. In conclusion, we report a new de novo mutation in the TBX1 gene responsible for cardiac defects as the main feature, and show that a rare variant affects TBX1 doses. Furthermore, we find that the change that increases the doses of TBX1 is a susceptibility factor to suffer features of the 22q11.2 duplication syndrome.

Analysis of BBS protein expression in an in vivo model organ system. *H. May-Simera[1], D. Jagger[2], A. Forge[2], P.L. Beales[1]* 1) Molecular Medicine Unit, UCL Inst Child Health, London, United Kingdom; 2) Centre for Auditory Research, UCL Ear Institute, London, United Kingdom.

Bardet-Biedl syndrome is a rare heterogeneous condition, characterized by obesity, polydactyly, renal abnormalities, retinal dystrophy and cognitive impairment. To date 11 genes have been identified (*BBS1-11*). Given that mutations in any of the BBS genes result in clinically indistinguishable phenotypes, it is likely that BBS proteins participate in common cellular processes. Several BBS genes have been associated with ciliary function and cognate proteins have been localized to the centrosome and basal body of ciliated cells. Previous analysis of BBS protein expression has predominantly centered on in vitro cell culture systems. Given the limited extrapolation of cell lines to live organisms we have focused attention on whole organ systems, in particular the mammalian cochlea. The developmental organization of sensory hair cells is under control of the kinocilium (a primary cilium) and its basal body and furthermore, the cochlear supporting cells are uniquely rich in microtubules. Our prior studies have identified that three Bbs mouse mutants (*Bbs1*, *4* and *6*) have hair cell defects likely associated with perturbation of planar cell polarity. However, the extent of these defects is unlikely to entirely explain the profound deafness exhibited by null mice. Therefore, we have further investigated the involvement of Bbs proteins in the auditory sensory epithelium. Within the organ of Corti, as expected, we observed a ubiquitous expression of Bbs6 within basal bodies. By contrast, in epithelial support cells there was strong but specific cytoskeletal expression of Bbs4 and Bbs2 in a distinct temporal pattern. These data suggest that some but not all Bbs proteins are tightly associated with microtubule function. We believe the inner ear presents an ideal organ structure for studying the developmental role of Bbs proteins. Owing to their microtubular enrichment, these support cells will be particularly useful to examine the involvement of low abundance cytoskeletal proteins.

The Norwegian Mother and Child Cohort Study (MoBa): a prospective cohort for genetic and epidemiological research. *R. Lyle[1], L.M. Irgens[1, 2], K. Haug[2], W. Nystad[1], R. Skaerven[1, 2], C. Stoltenberg[1], P. Magnus[1], The MoBa Study Group* 1) Division of Epidemiology, Norwegian Institute of Public Health, Oslo, Norway; 2) Medical Birth Registry of Norway, Locus of Registry Based Epidemiology, Department of Public Health and Primary Health Care, University of Bergen.

The Norwegian Mother and Child Cohort Study (MoBa) is a large-scale prospective pregnancy cohort for genetic epidemiological research. The objective is to collect data on exposures and health outcomes to critically test etiological hypotheses by estimating the association between exposures, genetic factors, and disease. The target population is all pregnant women in Norway, recruiting 100 000 pregnancies in 1999-2008. As of April 2006, ~70,000 pregnancies have been included. The participation rate is 42.7%, and the father participates in 82.9% of these cases. Four main types of variable are included in MoBa: 1. Questionnaire variables (~7000 questions in 6 questionnaries) 2. Data from biological samples (DNA, plasma, urine, RNA) 3. Derived variables from research groups 4. Exposure and outcome data acquired by record linkage with the birth, cancer, prescription, cause of death and vaccination registries. MoBa is unique in having recruited fathers, and routinely extracting DNA from mother, father and child. There are thus 10 000s of trios available for genetic research. MoBa allows researchers to have valid measures of both genes and the environment in the same study. An additional strength of MoBa is that trios allow great flexibility in study design and the types of research questions that can tackled. For example, the case-parent design, which opens the possibility of detecting effects of maternal genes, fetal genes and their interaction. Details of MoBa can be obtained online (www.fhi.no/morogbarn). The MoBa protocol is available (www.fhi.no/dav/14DFADA9F4.pdf) and includes information on consent, data collection and questionnaires. A set of guidelines for researchers applying for access to data and samples has been established and researchers are encouraged to use this unique resource. We will present the organisation of MoBa, including the biobank, details of ongoing research projects, and access procedures.

Beta adrenergic receptor (ADRB2) SNP and lung function in preschool children. *J. Hankinson[1], L. Lowe[2], S. John[1], A. Custovic[2], A. Woodcock[2], W. Ollier[1], A. Simpson[2]* 1) CIGMR, University of Manchester, United Kingdom; 2) North West Lung Centre.

Rationale: Specific airway resistance (sRaw), is a sensitive and reproducible measure of lung function in young children - higher sRaw indicating poorer lung function. Albuterol is an ADRB2 agonist and is the most common treatment used for wheezing illness. We investigated the relationship between a SNP in ADRB2 and pre and post albuterol sRaw at age 5 years in the setting of a population based birth cohort study. Methods: Lung function at age 5 years was assessed using plethysmographic measurement of sRaw at baseline and after bronchodilator [bd] (400 mcg albuterol). Results were also expressed as change in sRaw after bd as % of baseline sRaw (% change), and change in absolute value of sRaw after bd (absolute change). Wheeze in the last 12 months was assessed by parental questionnaire. Children were genotyped (n=636) for a SNP in the ADRB2 gene (rs1042713, Arg16Gly). Results were analysed using regression.Results: The SNP was in Hardy Weinberg equilibrium with a minor allele (Arg) frequency of 36.6%. Of the 636 children, 136 had wheezed in the previous 12 months. Wheezing children had poorer lung function at baseline (kPa/s, GM [95% CI] 1.26 [1.21-1.31] vs. 1.14 [1.13-1.16], $p<0.001$). Although both groups showed a significant improvement in lung function after bd ($p<0.001$) lung function was still poorer in wheezing children (kPa/s, GM [95% CI] 1.02 [0.99-1.05] vs. 0.97 [0.96-0.98], $p=0.001$).There was no difference in baseline or post bd lung function by genotype group ($p>0.2$). Amongst wheezing children however, minor allele homozygotes (Arg/Arg) showed a much smaller % change in sRaw post bd than the heterozygotes and common allele homozygotes (% change GM [95% CI] 1.99 [-11.55-15.52] vs. 18.38 [15.51-21.25], $p=0.003$) Similar results were seen when absolute change was considered ($p=0.014$). There was no difference in response to bd by genotype group amongst non-wheezing children ($p>0.4$) Conclusions: Amongst wheezing children, those homozygous for the minor allele showed a significantly smaller response to albuterol than wheezing children in the other genotype groups.

Accounting for genotyping errors in tagging SNP selection. *W. Liu[1], T. Yang[2], W. Zhao[1], G.A. Chase[1]* 1) Dept. of Health Evaluation Sciences, Penn State College of Medicine; 2) College of Information Sciences and Technology, Penn State University Park.

Many tagging SNP selection algorithms have been developed to optimally select the minimal informative subset of SNPs. One limitation of the existing algorithms is that they assume the reported genotypes are error free. However, genotyping errors are often unavoidable in practice. Many tagging SNP selection methods depend heavily on the estimated haplotype frequencies. Recent studies have demonstrated that even slight genotyping errors can lead to serious consequences with regard to haplotype reconstruction and frequency estimation. Here we present a tagging SNP selection method that allows for genotyping errors. Our method is a modification of the pair-wise r^2 tagging SNP selection algorithm proposed by Carlson et al. We replaced the standard EM algorithm in Carlsons method with an EM that accounts for genotyping errors in an attempt to obtain better estimates of the haplotype frequencies and r^2 measure. Through simulation studies, we compared the performance of our modified algorithm with that of the original algorithm. We found that the number of tags selected by both methods increased with increasing genotyping errors though our method led to smaller increase. We also studied the power of haplotype and single marker association tests using the selected tagging SNPs. We found that the power of haplotype association tests decreased dramatically with increasing genotyping errors. The power of single marker tests also decreased, but not at much as the decrease in haplotype tests. The power reduction in tests using tags selected from both methods was similar. Our method led to slightly higher power in haplotype tests, while Carlsons method led to higher power in single marker tests. When restricting the mean number of tags selected to be close to the baseline number, we still found reduction in power of the tests using the selected tags in the presence of genotyping errors. The power of all the tests resulted from our method was similar to that resulted from Carlsons method. These findings have implications for optimal selection of tagging SNPs in disease gene mapping.

Monozygotic twins of Smith-Magenis syndrome. *R. Kosaki[1], T. Okuyama[1], T. Tanaka[1], O. Migita[1], K. Kosaki[2]* 1) Clinical Genetics , National Childrens Medical Center, Tokyo, Japan; 2) Dept.of Pediatrics ,Keio Univ.School of Medicine,Tokyo.

Smith Magenis syndrome (SMS) is caused by an interstitial deletion of chromosome 17p11.2 involving the RAI1gene. Characteristic features include, broad squared face, midface hypoplasia, mild upslanting palpebral fissures, prognathism, upper lip eversion , short stature moderate mental retardation, sleep disturbance and behavior problems such as self injury . Among more than 150 SMS cases reported to date, all the cases have been sporadic except for a family in which the unaffected mothers were mosaic for 17p11.2 deletion. Sib pairs or twin pairs have not been reported. We here document first monozygotic twins of SMS. The male twins were born to a 26-year-old Japanese G2P1-3 woman with no previous medical problems. There was no consanguinity, or family history of mental retardation. The twins were delivered at 37 weeks of gestation. The placentation was diamniotic and monochorionic. At birth, the weight of twin A was 1812g, length 42.5cm, and head circumference 31.1cm. Apgar score was 5-7. The birth weight of twin B was 2564g, length 46cm, and head circumference 32cm. Postnatal courses of the twins were comparable. At the age of 3 years, the twins were referred to the genetic department because of developmental delay and self-injuring activities such as skin picking and nail yanking. Dysmorphic features, which were all concordant between the twins. At 5 years of age, the twin developed sleep disturbances. Karyotyping revealed deletion of (17)(p11.2p11.2). Identical twins have been reported in several multiple congenital anomaly syndromes such as Alagille syndrome and 22q11.2 deletion. Discordance in severity between the twin pairs in those reoprt pointed towards significant role of chance-like variation in the pathogenetic action of the mutated genes. In contrast, the SMS twins demonstrated a strikingly similar developmental and behavior patterns. The observation does not necessary indicate, but suggests a relatively large contribution of the genetic factors on the evolution of the SMS phenotype.

Genetic basis of autistic spectrum disorders: twin study of routinely-screened sample. *T. Nishiyama[1], S. Sumi[2], H. Taniai[3]* 1) Dept of Biological Information, Nagoya City Univ, Nagoya, Japan; 2) Nagoya Western Rehabilitation Center for Children with Disabilities, Nagoya, Japan; 3) Nagoya Child Welfare Center, Nagoya, Japan.

Background: A few twin study of autistic spectrum disorders suggested that these disorders were predominantly genetically determined, although only a small portion of them used statistically sound methods (Bailey et al.,1995).**Objective:** We examined the genetic structure of autistic spectrum disorders in an sample routinely screened in Nagoya city, Japan.**Methods:**A twin sample was ascertained through the annual routine screening for all cases with childhood autistic spectrum disorders, based on the criteria of pervasive developmental disorder (PDD) of *DSM-IV*. To examine the extent to which PDD fit specific models of genetic and environmental causation, these data were subjected to structural equation modeling by using the Mx software (Neale and Cardon, 1992). To correct for ascertainment bias we carried out a bivariate analysis of the final diagnosis, using categorical raw data option of Mx. The first statistical model tested incorporated the effects of three parameters: additive genetic (A), shared environmental (C), and non-shared environmental (E) factors. Successive models in which one or more of these variables were dropped from the full model were tested to examine the extent to which each model fit the observed data.**Results:** Fifteen pairs (7 monozygotic;MZ and 8 dizygotic;DZ) of twins were found for the study period of about two years. The concordance for PDD by pairs was 100% in the MZ and 25% in the DZ pairs. The structural equation modelling revealed that the AE model was best fitting(Akaikes Information Criteria; AIC, -1.110) among the models tested (AIC, 0.0 in the ACE model, 15.9 in the CE model, and 123.4 in the E model). The heritability estimate of the most favored model were 1.00 (95% confidence interval,0.99 to 1.00). **Conclusions:** It was confirmed that a broader phenotype such as PDD is likely under a high degree of additive genetic control, although our biased sampling may attribute to the present extreme findings.

185delAG in a Sri Lankan family with no Ashkenazi ancestry. *W. Meschino, H. Dorman, J. Furnival, J. Guo, J. Honeyford, D. Kennedy, M. Shama, J. Steer, K. Chun, D. Allingham-Hawkins* Genetics Program, North York General Hosp, Toronto, ON, Canada.

A 42 year old Sri Lankan woman presented to the familial breast/ovarian cancer clinic for counselling due to a history of invasive ductal carcinoma at age 37 followed by endometrial cancer at age 41. Family history revealed that her mother had developed breast cancer at age 37 and died of cancer at age 42. There were no other known cases of breast or ovarian cancer in the family. Screening of the BRCA1 and BRCA2 genes was performed using the protein truncation test plus direct sequencing of exons 2 and 5 of BRCA1. The common Ashkenazi Jewish (AJ) mutation, 185delAG, was found in exon 2 of BRCA1. A repeat analysis on a fresh specimen confirmed the finding. 185delAG is considered a "founder" mutation in the AJ population with a carrier frequency of ~1%. Carriers of 185delAG have a lifetime breast cancer risk of approximately 60%. Outside of the AJ population, however, this mutation is relatively rare. It has been observed in a group of non-Jewish Americans of Spanish descent from the San Luis Valley in Colorado as well as occasionally in other individual families with no known Jewish ancestry. This is the first report, to our knowledge, of 185delAG being identified in a Sri Lankan family. Haplotype analysis is pending to determine if the mutation is residing on the same haplotype as the common Ashkenazi mutation, another previously identified haplotype or one not previously described.

This family also presented an unusual counselling challenge in that when the younger, unaffected sister of our patient presented for counselling and predictive testing, she requested that the test results be given not to her but to her older brother, who attended the counselling session with her, as he is the paternal figure in the family. The counsellor was assured that the woman herself would return for full counselling but only after her brother had received her results and gently conveyed them to her. This is an example of the importance of flexibility and sensitivity to cultural traditions while delivering genetic services.

First reported case of CML in a patient treated with hydroxyurea for sickle cell disease (SCD). *A.S. Kulharya, A. Kutlar, K. Natarajan* Pathology, Medical College of Georgia, Augusta, GA.

Hydroxyurea (HU) is an effective treatment for controlling painful symptoms of SCD. Its effectiveness stems from its ability to be myelosuppressive, induce HbF, and modify red cell-endothelial cell interactions. No overt toxicity was observed in a study of 101 children with SCD treated with HU. There have been 4 reported cases of malignancy in SCD patients on HU therapy; 2 of these were found to be unrelated to the treatment. None of theses cases were of CML. We present the case of a 29 year old black male with SCD diagnosed with chronic phase CML. He has been treated with HU since 1997. His disease course has been remarkable for two thrombotic cerebrovascular accidents (CVA), both before the age of eight and an ICH in Nov 2004. His only other SCD related event has been gallstones requiring a cholecystectomy at age 18. He has been relatively free of vaso-occlusive episodes with no other symptoms. He has been on a chronic exchange transfusion regimen for his history of CVA. He developed transfusional iron overload initially treated with Desferoxamine and later with ICL670 (Exjade) in the context of a clinical trial. He has been occasionally non compliant with iron chelation and HU therapy. He first presented in Oct 2005 with an elevated WBC count which subsequently decreased. In Jan of 2006, his WBC count was in excess of 100,000 with an abnormal differential. Platelet count and Hb were essentially unchanged from baseline. He underwent a bone marrow aspirate and biopsy for flow cytometry, histopathology, and cytogenetic analysis. A peripheral blood analysis by FISH with a dual color dual fusion probe suggested the presence of an atypical abnormal clone with BCR/ABL fusion. However, routine cytogenetic analysis of bone marrow demonstrated a typical Ph chromosome in all the cells analyzed. The patient was started on imatinib mesylate at a dose of 400mg a day and has done well since then. The diagnosis of CML in this patient is unusual considering that HU is commonly used in the treatment of chronic myeloid disorders to stabilize the blood cell count.

***Slc25a19* is a mouse mitochondrial transporter necessary for viability, neural tube closure, erythropoiesis, and maintenance of mitochondrial thiamine pyrophosphate pools.** *M.J. Lindhurst[1], G. Fiermonte[2], S. Song[3], E. Struys[4], F. De Leonardis[2], A. Chen[1], A. Castegna[2], N. Verhoeven[4], C.K. Mathews[3], F. Palmieri[2], L.G. Biesecker[1]* 1) National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 2) Department of Pharmaco-Biology, Laboratory of Biochemistry and Molecular Biology, University of Bari, Bari, Italy; 3) Department of Biochemistry and Biophysics Oregon State University, Corvallis, OR; 4) Department of Clinical Chemistry, VU University Medical Center, Amsterdam, The Netherlands.

A missense mutation in *SLC25A19* causes Amish lethal microcephaly (MCPHA), which is characterized by severe microcephaly, brain malformations, -ketoglutaric aciduria and premature death. Previous data suggested that SLC25A19, also called DNC, was a mitochondrial deoxyribonucleotide transporter. We generated a knockout mouse model of *Slc25a19*. These animals had 100% prenatal lethality by E12. Affected embryos at E10.5 have a neural tube closure defect with ruffling of the neural fold ridges, a yolk sac erythropoietic failure and elevated -ketoglutarate in the amniotic fluid. We found that these animals have normal mitochondrial ribo- and deoxyribonucleoside triphosphate levels, suggesting that transport of these molecules is not the primary role of SLC25A19. We identified thiamine pyrophosphate (ThPP) transport as a candidate function of SLC25A19 through homology searching and confirmed it using transport assays of the recombinant reconstituted protein. The mitochondria of *Slc25a19*$^{-/-}$ and MCPHA cells have undetectable and markedly reduced ThPP content, respectively. The reduction of ThPP levels causes dysfunction of the -ketoglutarate dehydrogenase complex, which explains the high levels of this organic acid in MCPHA. In addition, pyruvate dehydrogenase activity is also decreased suggesting that transport of this cofactor into the mitochondria is important for central nervous system development and supports the hypothesis that the developing brain has a higher need for oxidative metabolism than other organs.

Pooled whole genome association genome scanning: validation using 100k Centurion and 500k Mendel Affymetrix microarray sets. *Q.R. Liu, T. Drgon, C. Johnson, D. Walther, G.R. Uhl* Molecular Neurobiology Branch, NIDA/NIH, Baltimore, MD.

Association genome scanning is of increasing interest for identifying chromosomal regions, genes and gene variants that contribute to vulnerability to complex disorders. Pooling strategies can efficiently perform allele typing, preserve confidentiality and reduce costs. We have helped to pioneer microarray-based pooled association genome scanning using 1.5K and 10K Affymetrix SNP arrays (Uhl et. al., 2001, AJHG; Liu et. al., 2005 PNAS). We now describe validation of pooled genotyping using early access versions of Affymetrix 100K and 500 K arrays and report the variation that we have obtained in comparing results of pooled with those of individually-determined genotypes. We obtained hybridization intensity data from perfect-match cells from these arrays and performed background subtraction, normalization, and arctangent transformations of A vs B allele hybridization intensity ratios. We compared values obtained from different pools of DNAs and compared them with expected allele ratios, the fraction of A and B alleles obtained from individually genotyping the individuals whose DNAs were used at 1:1; 1:5 and 1:15 ratios in these validating pools. Data from 150,000 informative SNPs was used yielded correlations between pooled and individually-determined genotypes of r2 = 0.95. This value rises to 0.99 if the 10% of the most poorly performing SNPs are removed. These values, and other validating results, appear to be at least as favorable as alternative approaches that use RAS values (Kirov et. al., 2006, BMC Genomics). They provide correlations that improve upon those reported using hybridization with long PCR amplimers and large-format wafer arrays (Hinds et. al., 2004, Hum Genomics). Adjacent presentations illustrate use of this approach in elucidating chromosomal regions and genes that contain alleles which appear to influence vulnerability to addictions. (Support: NIH-IRP, NIDA, DHHS).

Newborn screening for cystic fibrosis in the Czech Republic: results from a pilot study. *A. Holubova[1], F. Votava[2], V. Skalicka[3], V. Vavrova[3], D. Zemkova[3], P. Kracmar[2], M. Libik[1], J. Camajova[1], T. Piskackova[1], M. Macek[1]* 1) Charles University Prague, CF Center University Hospital Motol, Prague, Czech Republic; 2) Department of Pediatrics, University Hospital K. Vinohrady, Prague, Czech Republic; 3) Department of Pediatrics, University Hospital Motol, Prague, Czech Republic.

According to epidemiologic / genetic studies the incidence of CF in our country is 1:2700 newborns. Thus, given the current birthrate ~35 new cases of CF should annually be detected. However, registry data demonstrated that 1/3 of CF patients remains clinically undiagnosed and that the age at diagnosis has markedly increased (prior to 1998 median: 0.58; between 1999-2005: 1.2 years). Therefore, in II/2006 we started two tier (IRT/DNA) pilot CF neonatal screening project (NSCF) comprising Bohemian regions, representing ~2/3 of our population. Altogether 45,453 newborns were examined during the initial 9 month period. In 545 cases (1.2%), who had IRT concentration above the continuously adjusted cut off level, we examined the most common CFTR mutations. The diagnosis of CF was established in 5 newborns (2x F508del/F508del; 2x F508del/G551D; 1x F508del/R117H-7T - mild CF) and these children were subsequently referred to CF Centres. Furthermore, we detected 42 newborns with 1 CFTR allele (35x F508del, 2x CFTRdele2.3/21kb/, 2x N1303K, 1x G551D, 1x I507del and 1x I148T). Until now 27 follow up sweat tests (ST) were performed (26x 30mmol/L, 1x 30-40mmol/L). IRT recall was carried out in 14 children with IRT 200ng/mL and with a non-detectable CFTR allele - 11 children had negative IRT recall and 1 child was positive (ST negative). From these results the incidence of CF can preliminarily be adjusted to 1:9090 newborns. However, these results can be skewed due to lower number of newborns tested, effect of prenatal diagnosis and/or false negativity of NSCF. Supported by IGA MZCR 8236-3, VZFNM MZO00064203(6112).

Fine mapping of a linkage region on chromosome 17q13 reveals GABARAP and DLG4 are associated with vulnerability to nicotine dependence in European-Americans. *X.-Y. Lou[1], J.Z. Ma[1], J. Beuten[1], D. Sun[1], T.J. Payne[2], M.D. Li[1]* 1) Department of Psychiatric Medicine, University of Virginia, Charlottesville, VA; 2) ACT Center for Tobacco Treatment, Education & Research, University of Mississippi Medical Center, Jackson, MS.

A two-stage association study was conducted for a genomic region on chromosome 17q13-13.2 that was reported by us to harbor susceptibility gene(s) for nicotine dependence (ND). We first used ten SNPs in six genes within the region to determine which SNP/gene is associated with ND, which was assessed by smoking quantity (SQ), the Heaviness of Smoking Index (HSI) and the Fagerström Test for ND (FTND), in a total of 2037 subjects from 602 nuclear families of either African-American (AA) or European-American (EA) origin. Individual SNP analysis revealed that SNPs rs17710 and rs222843 in the GABARAP exhibited significant association with at least one age- and gender-adjusted ND measure in the EA sample and rs222843 still remained significant with adjusted FTND after correction for multiple testing (P = 0.009 under both dominant and recessive models). Although no SNP in the DLG4 was found to be significantly associated with ND, we found a G-G haplotype with a frequency of 14.2% formed by SNPs rs2242449 and rs507506 that showed significant inverse association with three ND measures (P = 0.003, 0.015 and 0.024, for SQ, HSI and FTND, respectively). We also found an A-A haplotype at a frequency of 8.8% formed by SNPs rs17710 and rs222843 in the GABARAP, that showed significant association with three ND measures (P = 0.006, 0.019 and 0.024, for SQ, HSI and FTND, respectively). To confirm these findings with a better coverage of the genes of interest, we conducted second stage association analysis by genotyping four more SNPs for GABARAP and nine for DLG4 on the same sample. Our second stage of association analysis results supported our finding of significant association of the two genes with ND. No significant association was detected in the AA sample. In summary, our two-stage association analysis results indicate that the GABARAP and DLG4 genes are involved in the etiology of ND in EA smokers.

Knock-in mouse model of Usher type IC. *J. Lentz[1], F. Pan[1], S. Ng[1], P. Deininger[2], B. Keats[1]* 1) Dept Genetics, Louisiana State Univ HSC, New Orleans, LA; 2) Dept Epidemiology, Tulane Univ Health Sciences Center, New Orleans, LA.

Usher syndrome type I is characterized by deafness at birth, vestibular dysfunction and progressive retinitis pigmentosa beginning in early adolescence. A cryptic splice site mutation (216GA) in exon 3 of the *USH1C* gene on chromosome 11p, which encodes a PDZ-domain protein, harmonin, was found in Acadian Usher type IC families in south Louisiana. *In vitro* analysis using constructs containing the mutant 216A and subsequent analysis of patient cells lines revealed a 35 base deletion. In order to analyze the impact of this frame-shift mutation, we created a knock-in mouse model containing the human 216GA mutation. A targeting construct was made containing 5 and 3 homology arms, each 4kb in length, and a 650 base pair fragment containing exons 3 and 4 from human *USH1C* cloned from an Acadian patient homozygous for the 216A mutation. 129SvEvTac embryonic stem cells were electroporated with the targeting construct, and after 10 days of neomycin (neo) selection, clones were screened by PCR and Southern blot analysis for homologous recombination. Two clones positive for targeted insertion were microinjected into C57BL/6 blastocystes and transplanted into pseudo-pregnant females. Chimeras were bred with cre-deletors for simultaneous deletion of the neo gene and germline transmission of the 216A allele. Four subsequent generations of offspring have now been genotyped. All homozygous *Ush1c*216A (216AA) mice are hyperactive, display circling and head tossing behavior, and do not display a Preyer reflex at 21-25 days old. They are similar in size and weight to their heterozygous and wild type litter mates, and both male and female homozygous *Ush1c*216A mice are fertile. Heterozygous mice show no behavioral phenotype. RT-PCR analysis of the cochlea and retina from 216AA mice shows the same 35 base pair deletion characteristic of Usher IC patients. Assessment of hearing and vision defects, as well as gene and protein expression are in progress.

Met80Ile polymorphism of the APOBEC1 gene, relating to plasma HDL cholesterol level, is a risk and modifies age-at-onset of male Alzheimer disease. *K. Kamino[1], R. Kimura[1], M. Yamamoto[1], A. Nuripa[1], T. Tanaka[1], T. Kudo[1], H. Akatsu[2], K. Kosaka[2], H. Yamagata[3], T. Miki[4], K. Urakami[5], Y. Wakutani[6], K. Wada[6], K. Nakashima[6], H. Kawakami[7], R.C.P. Go[8], R. Perry[8], T.D. Bird[9], G.D. Schellenberg[10], M. Takeda[1]* 1) Post-Genomics & Diseases, Osaka University Gradate School of Medicine, Suita, Osaka, Japan; 2) Choju Medical Institute, Fukushimura Hospital, Toyohashi, Aichi, Japan; 3) Preventive Medicine, Ehime University Graduate School of Medicine, Toon, Ehime, Japan; 4) Geriatric medicine, Ehime University Graduate School of Medicine, Toon, Ehime, Japan; 5) Biological Regulation, School of Health Science, Faculty of Medicine, Tottori University, Yonago, Tottori, Japan; 6) Neurology, Institute of Neurological Sciences, Faculty of Medicine, Tottori University, Yonago, Tottori, Japan; 7) Clinical Neuroscience and Therapeutics, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan; 8) Epidemiology, University of Alabama at Birmingham, Birmingham, AL; 9) Neurology, University of Washington, Seattle, WA; 10) Neurology, Pharmacology, University of Washington, Seattle, WA.

Apolipoprotein B mRNA-editing enzyme (APOBEC1) gene encodes catalytic enzyme, editing APOB-100 into APOB-48 mRNA by cytidine deamination. Since the APOBEC1 gene is located in a risk locus of Alzheimer disease (AD) at 12p, the Met80Ile polymorphism (rs2302515) in catalytic domain was subjected to case-control studies composed of 958 AD and 1361 controls in three ethnic backgrounds. Meta-analysis supported that the Ile/Ile genotype is a risk for male AD (O.R. = 1.51, 95%C.I. = 1.09-2.12), and also relates to earlier age-at-onset ($p<0.01$). On the other hand, the Ile/Ile genotype was gradually increased after 70 years old in population-based controls, and related with higher plasma HDL cholesterol level ($p<0.05$). Our results indicates that the Ile/Ile genotype of the APOBEC1 gene is a novel risk factor and modifies age-at-onset of AD, and plasma cholesterol profile related to the genotype suggests the etiological difference between AD and atherosclerosis.

Axonal swellings and impaired axonal transport in a mouse model of spastic paraplegia linked to spastin mutation. *A. Tarrade[1, 6], C. Fassier[1, 6], S. Courageot[1], D. Charvin[1], E. Mouisel[1], J. Vitte[1], A. Thorel[2], N. Fonknechten[3], N. Roblot[1], D. Seilhean[4], A. Diérich[5], J.J. Hauw[4], J. Melki[1]* 1) Molecular Neurogenetics Laboratory, INSERM U798, University of Evry, 91057 Evry, France; 2) Centre des materiaux, Evry; 3) Genoscope, Evry, France; 4) Laboratoire de Neuropathologie, Groupe Hospitalier Pitié-Salpétrière, Paris, France; 5) IGBMC, Illkirch, France; 6) Equal contribution.

Hereditary spastic paraplegia (HSP) is a neurological disorder characterized by axonal degeneration of corticospinal tracts. The most prevalent form of autosomal dominant HSP is linked to spastin (Sp) mutations. Homologous recombination in murine ES cells has been used to create a deletion of Sp exons 5 to 7 leading to a premature stop codon and the lack of the AAA domain. Examination of spinal cord revealed focal axonal swellings in descending and ascending tracts of mutant but not control mice. Electron microscopic analysis of the axonal swellings has shown an accumulation of organelles and cytoskeletal components suggesting an impairment of axonal transport in vivo. The frequency of axonal swellings was increasing with age in agreement with late and slowly progressive motor defect of mutant mice. Primary cultures of cortical motor neurons derived from embryos showed a localization of spastin in both the cell body and neurites. Mutant cortical neurons have normal viability and neurite outgrowth but develop focal swellings in the distal part of neurites. Our data strongly suggest for the first time that spastin, a protein involved in microtubule severing, is essential to maintain neurite integrity and axonal transport in vivo. Further investigations in our cellular model will help in clarifying the molecular pathway linking spastin, microtubule severing and axonal transport. In spite of the evidence for their involvement in distinct processes, mutations of paraplegin, PLP, KIF5A or spastin (this study) are leading to similar axonal degeneration and transport defect. The availability of a cellular model of spastic paraplegia should help in finding components able to stabilize or rescue neurite defects.

Truncation of the *Kcnq1ot1* non-coding RNA uncovers dual mechanisms of imprinting for *Cdkn1c*. *M.J. Higgins[1], G.V. Fitzpatrick[1], E.M. Pugacheva[2], Z. Abdullaev[2], V.V. Lobanenkov[2], J.Y. Shin[1]* 1) Department of Cancer Genetics, Roswell Park Cancer Inst, Buffalo, NY; 2) Molecular Pathology Section, Laboratory of Immunopathology, National Institute of Allergy and Infectious Diseases, NIH, Rockville MD 20852.

A cluster of imprinted genes in 11p15.5 are involved in the cancer-predisposition condition Beckwith-Wiedemann syndrome (BWS) and in some sporadic tumors. This genomic region consists of two independently regulated subdomains; the telomeric region, includes the maternally expressed *H19* gene and the paternally expressed *IGF2* gene and is regulated by a CTCF-dependent chromatin insulator, while the centromeric subdomain contains 8 imprinted genes regulated by the KvDMR1 imprinting control region (ICR). Loss of methylation at KvDMR1, a frequent epimutation in BWS, is associated with silencing of the *CDKN1C* tumor suppressor gene and is the probable cause of the disease. Previous studies suggest that KvDMR1 regulates imprinted expression by functioning as a chromatin insulator or silencer, or by acting as the promoter for the non-coding *Kcnq1ot1* RNA. Using cell culture-based reporter assays, we show that the enhancer-blocking element and the *Kcnq1ot1* promoter are completely separable. Furthermore, EMSA and chromatin immunoprecipitation demonstrate the presence of two binding sites for the CTCF. To test whether the non-coding RNA has a function in imprinting, we inserted a poly(A) cassette downstream of the *Kcnq1ot1* promoter to truncate the transcript. Following paternal transmission of this mutation, all genes under the control of KvDMR1 lose imprinting in the placenta. In contrast, while most of these genes exhibit widespread loss of imprinting in the embryo, *Cdkn1c* retains maternal-specific expression in some fetal tissues despite global loss of paternal methylation at its promoter. These results indicate that, while most imprinted genes in this domain are regulated exclusively by the noncoding *Kcnq1ot1* RNA, in some cell types *Cdkn1c* is silenced by an RNA-independent mechanism, perhaps by a CTCF modulated chromatin insulator. Thus KvDMR1 is the first ICR shown to repress the same gene by two distinct mechanisms.

Expression analysis of microRNAs during eye development in mouse. *M. Karali, V. Marigo, S. Banfi* Telethon Institute for Genetics and Medicine, Naples, Italy.

microRNAs (miRNAs) are a class of small (21-25 nucleotide), endogenous RNAs that negatively regulate gene expression by binding to target sites in the 3 UTR of messenger RNAs. Although they have been found to regulate developmental and physiological processes in several organs and tissues, their role in the eye transcriptome is completely unknown. We decided to perform detailed expression analysis for fourteen miRNAs that have been previously found to be expressed in the developing eye in non-mammalian vertebrates (zebrafish). We determined their spatiotemporal localization in the murine eye during development by RNA in situ hybridization (ISH) using LNA (locked-nucleic-acid)-modified DNA oligonucleotide probes. We analysed their expression at embryonic stages E10.5 (by whole-mount ISH) and E16.5 and on postnatal eyes at P0, P8 and adult. The analysed miRNAs are expressed in the eye with diverse and only partially overlapping patterns, which may reflect their role in the differentiation of retina precursors, establishment of cell-fate and, ultimately, development of the stratified adult retina and other ocular structures. To begin understanding the molecular mechanisms underlying the regulation of miRNA expression, we also analysed the genomic location of the miRNA primary transcripts. The analysis revealed that most eye-expressed miRNAs overlap with or are in the near vicinity of uncharacterized transcripts derived predominantly from eye cDNA libraries. Expression analysis by RNA ISH in adult murine eye showed that some of these transcripts share very similar cellular distribution with their corresponding miRNAs, suggesting that miRNAs may share common expression regulatory elements with their host genes. The precise determination of the expression patterns of these miRNAs will be instrumental for a better understanding of the mechanisms controlling gene expression in the eye both in physiological and in pathological conditions.

Bronchoscope-Guided, Targeted Lobar Aersolization of HDAd into the Lungs of Baboons: Exceedingly High Pulmonary Transduction Throughout the Entire Lung with Negligible Toxicity. *P. Ng[1], P. Hiatt[2], R. McConnell[2], D. Palmer[1], Y. Zuo[1], D. Dimmock[1], M. Finegold[3], A. Beaudet[1], N. Brunetti-Pierri[1]* 1) Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Dept Pulmonary Pediatrics, Baylor Col Medicine, Houston, TX; 3) Dept Pathology, Baylor Col Medicine, Houston, TX.

Uniform delivery of gene therapy vectors to all lung lobes will be important for CF gene therapy. This important objective has not been achieved in large animals. Therefore, we have developed an approach to specifically deliver vector into each of the lung lobes; an intracorporeal nebulizing catheter is inserted into a bronchoscope to permit visual targeted aerosolization of vector specifically into each lung lobe. Using this approach, 2 ml of 0.1% LPC containing $1x10^{12}$ vp of HDAd-K18LacZ was sequentially aerosolized into each lung lobe of a baboon. Halfway through the procedure a slight, transient and fluctuating decrease in O_2 saturation of no more than 2% was noted that did not warrant supplemental O_2. The procedure was otherwise uneventful and well tolerated with quick recovery. No changes in chest X-rays taken at 6 h, 24 h and at necropsy 72 h post-vector were observed compared to baseline. At necropsy, the lungs were stained with X-gal to reveal extensive transduction of the epithelium in the large and small airways in all lung lobes. Substantial transduction was exclusively restricted to the airway epithelial cells and submucosal glands, the target cells for CF gene therapy. Assessment of the proximal major bronchi from each lung lobe revealed that ~20% of the airway epithelial cells were transduced in the LUL, 90% were transduced in the LML, LLL, RUL, RML and RLL, and ~80% were transduced in the AC. Approximately 70% of the epithelial cells in the tracheal sections examined were transduced. These unprecedented results demonstrate that exceedingly high levels of transduction of the airway epithelial cells and submucosal glands throughout all lung lobes in a large animal can be achieved with negligible toxicity and should pave the way towards successful clinical CF gene therapy.

Novel technical approaches to prenatal diagnosis of genetics disorders. *D. Louesdon[1], C. Bernabe[1], E. Feyereisen[2], R. Frydman[2], A. Benachi[3], A. Yamgnane[3], Y. Dumez[3], A. Munnich[1], J. Steffann[1], J.P. Bonnefont[1]* 1) Department of Genetics, INSERM unit U781, Hopital Necker,Paris, France; 2) Obstetrics and Reproductive medicine unit, Hopital A. Béclère, Clamart, France; 3) Obstetrics unit, Hopital Necker, Paris, France.

The development of early, non invasive, simple, and low-cost procedures for PND is an important challenge for medical genetics. Amniocentesis and chorionic villous sampling, currently the methods of choice, are implemented late during the gestation (10-14 weeks), carry a fetus loss risk (0.5-1.0 %), and remain costly. Optional techniques based on the isolation of fetal DNA or fetal cells from maternal blood or transcervical sampling have not proven to be suitable for a routine implementation so far. Thanks to the presence of trophoblastic fetal cells in the endocervical canal of pregnant women at as soon as 5 weeks of gestation, we developed two approaches based on analysis of nucleated elements from transcervical cell samples, retrieved by endocervical lavage (16 women) or by cytobrush (19 women) at various terms of gestation. Presumptive fetal cells (syncitiotrophoblastic/ cytotrophoblastic cells) were isolated, based on their morphological characteristics, using an inverted microscope. Single cell DNAs were subjected to whole genome amplification, and subsequently haplotyped along with parental DNAs, using multiplex polymorphic markers. 60 presumptive fetal cells from 19 women (8-11 weeks of gestation) were retrieved by cytobrush. half of the DNAs failed to appropriately amplify. Only 2 of the remaining ones were shown to be fetal in origin, indicating that this approach is currently not suitable for a routine application. 99 presumptive fetal cells from 16 women (6-11 weeks of gestation) were retrieved by endocervical lavage. Prior to 8 weeks of gestation, 38 of the 66 cells were shown to be fetal (8/9 women). After 8 weeks of gestation, 24 of the 33 cells were shown to be fetal (4/7 women). This approach enabled us to carry out 3 successful early PND for various disorders (6-8 weeks of gestation). We are currently testing feasibility of fetal cells isolation by either approach at earlier terms of gestation.

Chromosome map reveals stage-specific gene signatures and identifies genetic hotspots during murine embryonic gonad development. *T.L. Lee[1], D. Alba[1], V. Baxendale[1], O.M. Rennert[1], W.Y. Chan[1, 2]* 1) Laboratory of Clinical Genomics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD; 2) Departments of Pediatrics and Biochemistry & Molecular Biology, Georgetown University School of Medicine, Washington D.C.

Despite the identification of key genes like Sry to be integral during embryonic gonad development, the chromosomal activity and stage classification in the genomic context is still poorly understood. To understand the genetic events of gonad development, we performed Serial Analysis of Gene Expression (SAGE) to profile the transcriptome in male embryonic gonads at E10.5, E11.5, E12.5, E13.5, E15.5 and E17.5 with an average of 152K coverage. Instead of resorting to static descriptive analysis, we developed algorithms to assign the SAGE tags to the corresponding chromosomal position based on the Unigene assignments and displayed in chromosome graphic format using the completed mouse genome information. Chromosomal hotspots with significant changes at any time point will be highlighted based on co-localization of gene expression. This is useful for determining the importance of specific chromosomal region at particular time point and the potential consequences due to chromosome duplication or deletion. Furthermore, the biological implication of significant chromosomal clusters will be extracted and further analyzed by linking to different mutation and functional annotation databases. Altogether, we have identified more than 70,000 genes in the six gonad libraries, far beyond the Affymetrix microarray coverage. Importantly, we observed a prominently increase in global genomic activity from E10.5 to E17.5, and identified important chromosomal regions related to the developmental processes and validated by established mouse models with developmental disorders. Taken together, the representation of male gonad SAGE data in transcription maps format is a new way of visualizing transcriptome data and allows rapid identification of chromosomal segments important in male embryonic gonad development. These candidate regions represent possible treatment targets and as markers in early diagnosis for male developmental disorders.

Expansion of ALFRED, the ALlele FREquency Database. *K.K. Kidd[1], H. Rajeevan[1], K.-H. Cheung[1,2], R. Gadagkar[2], S. Stein[1], U. Soundararajan[1], J.R. Kidd[1], A.J. Pakstis[1], P.L. Miller[2]* 1) Department of Genetics; 2) Center for Medical Informatics; Yale University School of Medicine, New Haven, CT 06520-8005 USA.

ALFRED (http://Alfred.med.yale.edu) is an actively curated database designed to make allele frequency data on anthropologically defined human population samples readily available to the scientific community. It currently (June 1, 2006) has data on 1,688 polymorphisms and 498 populations with a total of 50,296 frequency tables (one population typed for one site). The database is updated on a daily basis. Over the past year the types and quantity of data have increased considerably and the unique collection of allele frequencies in ALFRED has been integrated into the broader set of genetic databases by links *from* PharmGKB and dbSNP *to* ALFRED. Among various searching option enhancements is the ability to display gene/locus information in chromosomal order in addition to alphabetical order.

The number of allele frequency tables has almost doubled in the past year. Currently, the frequencies for hundreds of genetic markers typed by Marshfield on many populations are being loaded into ALFRED. Genotype data is now also stored in ALFRED. Genotype tables for only 47 polymorphic sites typed on 40 populations have been entered so far but more are being added as quickly as possible. ALFRED description pages can now be accessed from dbSNP by direct URL links from rs# definition/description pages. When ALFRED has frequency data for a polymorphism with an rs#, the frequency section of the dbSNP page indicates Additional frequency data available with an active link to the site page in ALFRED. From that page frequency data can be displayed in several different formats and/or downloaded as text or in XML format. Similar links also exist from PharmGKB to the relevant ALFRED locus page and site page. As new data are added to ALFRED, those links are updated. The newest display option in ALFRED provides pie charts displayed on a global map. The GIS format has been upgraded and allows selection of populations by geographic region as well as the display of frequencies. ALFRED is supported by US NSF BC0096588.

Significant associations between MTHFR gene polymorphism and obesity traits were found in a large family sample. *X.G. Liu[1,2], L.J. Zhao[3], D.H. Xiong[3], R.R. Recker[3], H.W. Deng[1,2,3]* 1) The Key Laboratory of Biomedical Information Engineering of Ministry of Education and Institute of Molecular Genetics, School of Life Science and Technology, Xi'an Jiaotong University, Xian, Shaanxi, 710049, China; 2) Department of Orthopedic Surgery and Basic Medical Sciences, School of Medicine, University of Missouri- Kansas City, Kansas City, Missouri, 64108-2792, USA; 3) Osteoporosis Research Center and Department of Biomedical Sciences, Creighton University, Omaha, Nebraska, 68131, USA.

Background: It is well established that Methylene tetrahydrofolate reductase (MTHFR) palys an important role in bone metabolism. Studies indicated that MTHFR indirectly correlated with obesity. This study was to test the hypothesis that MTHFR is associated with obesity traits.

Materials and Methods: 1,873 US Caucasians with European origin in 405 nuclear families were recruited. Fat mass, lean mass, and percentage fat mass (PFM) were measured with dual x-ray absorptiometry (DXA). Body mass index (BMI) was calculated from weight to height square. Five selected single nucleotide polymorphisms (SNPs) around the MTHFR gene were genotyped. family-based association test (FBAT) and QTDT (quantitative transmission disequilibrium test) were performed to test the association between the MTHFR gene and the four obesity-related phenotypes.

Results: In within-family-association analysis implemented in QTDT, significant association were found between rs17037390 (located at intron4 of MTHFR gene) and fat mass (P=0.0075), lean mass (P=0.0122), BMI (P=0.0132) and PFM (P=0.0136). These associations were all confirmed in the FBAT analysis by P-value of 0.0051, 0.0123, 0.0068, and 0.011362, respectively. After correcting for the overly conservative Bonferroni correction, the associations remained significant between rs17037390 and fat mass (P=0.0257), BMI (P=0.0345) in FBAT test, and between rs17037390 and fat mass (P=0.0375) in QTDT analysis.

Conclusion: The MTHFR gene is associated with obesity.

X-chromosome inactivation patterns in multiple sclerosis: a twin study. *B.M. Herrera[1, 2], K.M.E. Morrison[1, 2], S.V. Ramagopalan[1, 2], M.R. Lincoln[1, 2], S. Orton[1, 2], M.J. Chao[1, 2], M.Z. Cader[1, 2], G.C. Ebers[1, 2]* 1) Wellcome Trust Centre for Human Genetics, Oxfordshire, United Kingdom, OX3 7BN; 2) Department of Clinical Neurology, University of Oxford, United Kingdom, OX2 6HG.

Multiple Sclerosis (MS), like many other autoimmune diseases has a higher prevalence among females (female: male ratio of ~2.4:1). There is clear evidence of a maternal parent-of-origin effect which remains unexplained. X-linked transmission of susceptibility has been previously proposed; however linkage and association studies have provided some inconclusive support for a susceptibility locus for MS on the X-chromosome. In order to compensate for gene dosage, female embryos undergo a random process of X-chromosome inactivation (XCI). The inactivation is stable and transmitted to the progeny cells. Studies have shown skewed XCI to be a possible operating factor in autoimmune diseases. In this study we investigate the role of skewed XCI patterns in MS. We used a cohort of 188 concordant and discordant twin pairs from the CCPGSMS (95 MZ and 93 DZ) to investigate XCI patterns; we analyzed the androgen receptor (AR) locus using a fluorescent HpaII/polymerase chain reaction assay. The lack of success from high density linkage and association studies in the search for susceptibility genes to MS, suggests that it is time to move away from the Mendelian paradigm and take into account secondary inheritance mechanisms which have so far been overlooked in studies of complex disease. Similarly to other studies, our results indicate that skewed XCI mosaicism may play a role in the pathogenesis of multiple sclerosis.

Extreme phenotypic variations within a family with SALL1 mutations: Isolated preaxial polydactyly to Goldenhar syndrome-like phenotype. *K. Kosaki[1], R. Kosaki[2], H. Samejima[1], C. Torii[1], R. Fujimaru[3], H. Yamada[4], K. Iijima[2]* 1) Keio Univ, Dept Pediatr, Tokyo, Japan; 2) Natl Ctr Child Health & Development,Tokyo, Japan; 3) Osaka City Kita Hosp, Dept Pediatr, Osaka, Japan; 4) Osaka City General Hospital, Dept Pediatr, Osaka, Japan.

Townes-Brocks syndome (TBS) is a genetic disorder caused by mutations in SALL1 whereas Goldenhar syndrome (GS), which phenotypically resembles TBS, is considered to be a non-genetic disorder caused by a vascular accident in the embryonic stapedial artery. We report extreme phenotypic variations within a family with SALL1 mutations; the elder sister presented with a TBS-like phenotype including external ear anomalies, preaxial polydactyly, and anteriorly placed anus, whereas the younger sister presented with a GS-like syndrome phenotype including atretic ear canals, mandibular hypoplasia, and right preaxial polydactyly as well as epibulbar dermoid. The mother had triphalangeal thumbs but was otherwise structurally normal and the father was asymptomatic. Analysis of the SALL1 gene revealed that both daughters were heterozygous for non-sense mutation L419X within the first zinc finger domain. In addition, both daughters were heterozygous for a missense substitution P270L. The P270 residue is highly conserved among various vertebrate species and the P270L substitution was not present among 100 control individuals. The mother was heterozygous for L419X and the father was heterozygous for P270L. Hence, the daughters were compound heterozygotes for L419X and P270L. Obviously, heterozygosity for the P270L does not, in itself, have any detrimental effect because the father was devoid of any features attributable to the SALL1 mutation. However, the P270L may be functionally relevant in that the P270 residue is highly conserved and may have contributed to the severity of the phenotype in the daughters who had concurrent L419X mutation. To our knowledge, the younger daughter is the first patient with a SALL1 mutation to exhibit classic GS-like phenotype with epibulbar dermoid. The observation lends further support to the concept that GS is an etiologically heterogeneous disorder that may have a genetic basis in some cases.

Experience with the uptake of preimplanatation genetic diagnosis (PGD) for the purpose of family balancing. *S.L. McAdoo, R.R. Sharp, L. McCullough, S.A. Carson, P. Amato, J.E. Buster, J.A. Hamilton, F.Z. Bischoff, P.L. Cisneros, J.L. Simpson* Baylor College of Medicine, Houston, TX.

INTRODUCTION: Couples having one or more children of a given sex may desire a child of the opposite sex for purposes of family balancing. The publics opinions on gender preferences and selection have been surveyed often, but data have not been elicited from couples actually willing to pursue the laborious and costly methods (IVF with PGD) to do so. The objective of this study was to characterize couples willing to consider undergoing PGD for family balancing. METHODS: Press releases were disseminated through multiple media outlets. Those responding underwent a telephone screen to determine eligibility. Exclusion criteria included couples without prior children, couples medically ineligible for IVF, and couples who require assisted reproductive methods to achieve pregnancy. At that time, limited demographic information was collected and a short overview of the study and necessary technology provided. RESULTS: A total of 158 couples were screened. Of these, 24 (15%) did not meet eligibility, 64 (41%) failed to return our follow-up phone calls or could not be re-contacted, 19 (12%) declined (typically due to cost). There remained 51 couples (32%) with full expectations of proceeding to the interview stage. Subjects consisted of 58% Caucasian, 12% Hispanic, and 4% each of Middle Eastern, African-American, or Asian, and 18% of mixed ethnicity. Approximately two-thirds were from Texas. The group was religiously diverse: Protestant 38%, Catholic 29%, no religion 16%, Jewish 4%, other 13%. Mean ages were 36 years (male) and 33 years (female). Eighty-four percent had at least 2 prior children of one sex, and 16% had only one child. Twenty-eight (56%) desired a boy. It is of note that out of the initial group of 51, only 18 actually came for the scheduled visit. CONCLUSION: Family balancing and gender selection elicit wide-spread public interest, but few couples seem committed to proceeding once informed of procedure details, financial costs, and physical demands of PGD. Open access to PGD for family balancing is unlikely to generate huge demands in the U.S.

Novel mutations in four patients with pyruvate carboxylase deficiency. *S. MONNOT[1, 2], V. SERRE[1], B. CHADEFAUX-VEKEMANS[3], S. ROMANO[4], P. DE LONLAY[4], J. AUPETIT[3], V. PAQUIS[2], A. MUNNICH[1], J.P. BONNEFONT[1]* 1) Medical Genetics, INSERM unit U781, Hopital Necker, Paris,; 2) Medical Genetics, Hopital Archet 2, Nice,; 3) Metabolic Biochemistry, Hopital Necker, Paris,; 4) Pediatrics, Hopital Necker, Paris, France.

Isolated pyruvate carboxylase (PC) deficiency (Biotin-unresponsive) is a rare metabolic disorder inherited in an autosomal recessive way. Three phenotypes have been described, based on seriousness of the clinical presentation. Only 6 mutations of the PC gene have been reported so far. We screened the PC gene in four unrelated families with PC deficiency. Three Caucasian patients presented with the French form of the disease,of whom two died in the first week of life, and the third one at 7 months. Another patient, born to consanguineous parents from Saudi Arabia, currently 2 years old, suffered mental retardation and recurrent lactic acidosis episodes (American form). PC enzymatic activity was undetectable in all patient fibroblasts. Direct sequencing of the 23 exons of the PC gene on genomic DNA identified 6 novel variants, namely, heterozygous c.911AG (exon 7, p.Tyr304Cys) and IVS7-1GT for patient 1, homozygous c.467GA (exon 3, p.Arg156Gln) for patient 2, heterozygous c.1748GT (exon 12, p.Arg583Leu), and c.2876dupT (exon 17) for patient 3, and heterozygous c.3392_3400del9nt (exon 20) for patient 4. Scanning of one hundred control chromosomes failed to detect any of the missense mutations. cDNA sequencing showed that the splice mutation and the duplication resulted in RNA decay, while the remaining mutations had no apparent effect on the mRNA. At the protein level, *i)* missense mutations were located in highly conserved amino-acid domains of the protein, *ii)* we took advantage of the similarities of the PC with the biotin carboxylase subunit of *Aquifex aeolicus* PC and the *Propionibacterium shermanii* transcarboxylase to model the putative impacts of these mutations on protein conformation. Taking into account available molecular data, it appears that truncating mutations are predominantly found in the French phenotype, while missense mutations are more frequently associated with the American form of the disease.

Localization of chronic obstructive pulmonary disease susceptibility genes on chromosome 2q. *C.P. Hersh, R. Lazarus, B.J. Klanderman, B.A. Raby, A.A. Litonjua, D.L. DeMeo, J. J. Reilly, S.T. Weiss, E.K. Silverman* Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Rationale: In the Boston Early-Onset Chronic Obstructive Pulmonary Disease (COPD) Study families, significant evidence for linkage of COPD-related traits has been demonstrated on chromosome 2q. To date, only one potential COPD susceptibility gene in the region has been identified (SERPINE2).

Methods: In the first phase, we genotyped 1402 SNPs across the 20 Mb linked region in 309 cases with severe COPD from the National Emphysema Treatment Trial and 330 control smokers without airflow obstruction. In the second phase, we genotyped 914 SNPs in significant regions from phase 1 in the same cases and controls. To follow-up significant results from phase 2, 122 SNPs were genotyped in 949 individuals from 127 families in the Boston Early-Onset COPD Study.

Results: Based on single SNP and haplotype association analysis of phase 1, we focused on 50 regions for phase 2. After phase 2 genotyping, the evidence for association increased in 11 of these regions. In one of the 11 regions, spanning 300kb, 9 SNPs in two genes, XRCC5 (X-ray repair complementing defective repair in Chinese hamster cells 5; ATP-dependent DNA helicase II) and PECR (peroxisomal trans-2-enoyl-CoA reductase), showed replicated associations in the family-based study.

Conclusions: Dense fine mapping of chromosome 2q has identified two genes with replicated association for COPD-related traits. In order to validate and generalize these results, studies in additional populations are ongoing.

Support: NIH grants HL080242, HL71393, HL075478; ALA Career Investigator Award; Alpha-1 Foundation.

The PTPN22 gene and type 1 diabetes in children. *YJ. Lee[1, 2, 5], CY. Huang[1], SG. Shu[3], FS. Lo[4], CL. Lin[2], CK. Chen[2], ZC. Wang[2], HF. Liu[2], CC. Chu[2], M. Lin[2], FY. Huang[1,5]* 1) Dept Pediatrics; 2) Dept Medical Res, Mackay Memorial Hosp, Tamshui, Taipei; 3) Dept Pediatrics, Taichung Veterans General Hospital, Taichung; 4) Div Endocrinology, Dept Medicine, Chang Gung Children's Hospital, Taoyuan; 5) Dept Pediatrics, Taipei Medical University, Taipei; Taiwan.

dbSNP rs2476601, a functional SNP (C/T) in the PTPN22 gene, is associated with type 1 diabetes (**T1D**) and other autoimmune diseases in both Caucasians and Sardinians. However, this SNP is not present in Japanese but a SNP in the promoter region (rs2488457) of the gene was found to be associated with T1D in Japanese and Koreans. Thus, it is worthwhile to investigate the PTPN22 gene in other ethnicity. **Subjects** The patients were 374 unrelated children (178 boys, 196 girls) with T1D. Their age of diagnosis was 7.3 3.9 (0.3 - 16.2) years. The controls were 303 hospital personnel and individuals having a routine health examination or minor surgery. None had a history of autoimmune disease. All individuals were Chinese living in Taiwan. The individuals studied gave informed consent and the study was approved by the Institutional Review Board. **Methods** The genotype of dbSNP rs2476601 was C/C in all 30 of the patients and 29 of the controls using both RFLP and sequencing from both directions. Thus, this SNP did not exist in Chinese investigated. Then 4 SNPs including rs2488457 (promoter), rs1217419 (IVS2), rs2797415 (IVS15) and rs1217412 (Exon 21), scattering in the PTPN22 gene were genotyped using RFLP or TagMan (ABI) method. GAD-Ab and IA2-Ab were measured in most patients. Those with positive autoantibodies were considered to have type 1A diabetes **(T1AD)**. **Results** There were no significant difference in the frequencies of genotype, allele and phenotype in the 4 SNPs between T1D patients and controls. Similar findings were also noted between patients with T1AD and controls except for a marginally significant difference was detected in T phenotype of SNP rs1217412 between patients with T1AD and controls [187 (67.3%) vs 181 (59.7%), OR 1.39, 95% CI 0.99-1.95, p=0.06.] **Conclusions** The PTPN22 gene might not be associated with T1D in Chinese children. Genotyping rs1217412 and other SNPs in a larger dataset is necessary.

TLR4 and cardiovascular and metabolic phenotypes in myocardial infarction survivors. *F. Nyberg[1,2], T. Illig[3], M. Kolz[3], T. Bellander[1,4], F. Forastiere[5], K. Katsouyanni[6], J. Pekkanen[7], J. Sunyer[8], A. Peters[3], AIRGENE Study Group* 1) Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden; 2) Medical Sci/Epidemiology, AstraZeneca R&D Molndal, Molndal, Sweden; 3) Institute of Epidemiologie, GSF-Forschungszentrum, Neuherberg, Germany; 4) Department of Occupational and Environmental Health, Stockholm County Council, Stockholm, Sweden; 5) Local Health Authority RME, Department of Epidemiology ASL, Rome, Italy; 6) Department of Hygiene and Epidemiology, University of Athens, Athens, Greece; 7) Department of Environmental Health, National Public Health Institute (KTL), Kuopio, Finland; 8) Institut Municipal d'Investigació Médica. (IMIM), Barcelona, Spain.

Background: TLR4 has been suggested as a candidate gene for cardiovascular disease and metabolic phenotypes (coronary heart disease, acute coronary syndrome, myocardial infarction (MI), hyperlipidemia-induced atherosclerosis etc) and may modify the treatment effect of statins. Since TLR4 has many functions and affects many pathways it is of interest for understanding disease mechanisms to explore how TLR4 associates with different cardiovascular and metabolic phenotypes in MI survivors

Methods: We recruited 1003 MI survivors aged 37-81 years in 6 countries across Europe for follow-up of inflammatory markers. At baseline, history of coronary heart disease and other comorbidities was assessed, as well as blood lipids. Candidate genes were genotyped for selected SNPs.

Results: Several SNPs in TLR4 were associated with angina pectoris, arrhythmia, total cholesterol, HDL cholesterol as well as the ratio of HDL to total cholesterol at baseline.

Discussion: This study in a sample of MI patients provides evidence that TLR4 is of importance for cardiovascular and metabolic phenotypes in MI survivors. In particular, TLR4 seems to be intimately involved in aspects of lipid regulation, supporting its reported role in modifying the treatment effect of statins.

Beta Evaluation of the NanoChip 400 *CFTR* assay for Cystic Fibrosis carrier detection in a tertiary care hospital.
N.I. Lindeman, P. Flatley, D. Hall, P. Jarolim, J.A. Longtine Pathology, Brigham & Women's Hospital, Boston, MA.

Background: The NanoChip 400 (Nanogen, Inc., San Diego, CA) is a semi-automated array-based hybridization system that uses microelectronics to address individual biomolecules to different positions on a microchip array for multiplexed hybridization testing of amplified DNA. The ability of this system to perform testing for Cystic Fibrosis carrier assessment was evaluated on clinical samples from patients at the Brigham and Women's Hospital (Boston, MA) to aid in establishing performance characteristics for CE-IVD labeling. **Methods:** Whole blood samples from patients undergoing CF carrier screening were separated into two aliquots, one of which was sent to a referral laboratory (Genzyme Genetics, Framingham, MA) for diagnostic testing, while the other was stored in a refrigerator until analysis. 65 samples, 21 with and 44 without *CFTR* mutations, were tested in duplicate using the NanoChip 400, using beta reagents and protocols supplied by the manufacturer. In addition, 12 DNA samples from cell lines with *CFTR* mutations were also tested. Results were compared with those obtained by the reference laboratory and by in-house testing with an INVADER (Third Wave Technologies, Madison, WI) assay. **Results:** All samples (100%) gave the correct results, in both duplicate measurements, when compared with the reference laboratory and the INVADER results. The assay was technically straightforward, although the manual set-up procedures required a moderately high level of technologist concentration to prevent human error, and hardware failures involving the waste container, reagent sensor, and one lot of cartridges necessitated repeat analysis of 3 of 11 assay runs. Overall technologist time was comparable to the INVADER assay. **Conclusions:** The NanoChip 400 beta *CFTR* assay performed accurately and precisely, and no diagnostic errors were encountered in 65 clinical samples and 12 cell line samples. All 23 mutations on the ACOG/ACMG-recommended panel were genotyped properly. Minor modifications to hardware and software could improve the overall system performance, by minimizing instrument failure and the potential for human error during set-up.

Target DNA Production for Chip Resequencing. *D. T. Okou, M. E. Zwick* Human Genetics, Emory University School of Medicine, Atlanta, GA.

Resequencing genomic regions using high-density resequencing arrays (RAs) requires efficient methods of target DNA production. Current RAs can resequence up to 300kb per chip. We are exploring methods of target DNA production that are scalable to large genomic regions. One approach, Transformation-Associated Recombination (TAR) cloning technique allows direct isolation of up to 300 kb of specific chromosomal regions and genes from mammalian genomes. The technique is based on homologous recombination between a vector containing a gene-specific sequence and genomic DNA fragment during co-transformation into yeast spheroplasts. It results in the rescuing of chromosomal regions in yeast as linear or circular Yeast Artificial Chromosomes (YACs). We will present data contrasting TAR cloning with traditional Long PCR (LPCR) methods of target DNA production from both BACs and human genomic DNA. The performance of 300kb RAs using target DNA generated from these methods will be presented.

Genome scanning of chromosome 21 identifies DYRK1A gene as a risk factor for late-onset Alzheimer disease. *R. Kimura[1], K. Kamino[1], M. Yamamoto[1], T. Tanaka[1], T. Kudo[1], H. Akatsu[2], H. Yamagata[3], T. Miki[4], M. Takeda[1]* 1) Post-Genomics and Diseases, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; 2) Choju Medical Institute, Fukushimura Hospital, Toyohashi, Aichi, Japan; 3) Preventive Medicine, Ehime University Graduate School of Medicine, Toon, Ehime, Japan; 4) Geriatric Medicine, Ehime University Graduate School of Medicine, Toon, Ehime, Japan.

Late-onset Alzheimer disease (LOAD) is caused by genetic and environmental factors upon aging. Chromosome 21 is triplicated in Down syndrome, known as a condition of very early-onset AD, and genome scanning of familial LOAD also supported a risk locus at chromosome 21. To elucidate risk loci at this chromosome, we performed a genome scanning of chromosome 21, using SNPs spaced at 100kb-interval including at least one SNP in each coding region. By linkage disequilibrium (LD) mapping using 378 AD and 375 population-based controls, eight loci indicated significant LD with LOAD by allele frequency at $p<0.05$. The most significant association was found in DYRK1A (dual-specificity tyrosine regulated kinase 1A) gene ($p<0.003$), encoded within Down syndrome critical region (DSCR). The transcript of the DYRK1A gene in AD hippocampus was significantly higher than that of non-demented control. In SH-SY5Y cells, beta amyloid induced the expression of Dyrk1a protein, and overexpression of the DYRK1A gene increased phosphorylation of tau at Thr212. These results indicate that the DYRK1A gene is a genetic factor of LOAD encoded at chromosome 21, mediated by the interaction with beta amyloid and tau phosphorylation.

A clinical retrospective study of orofacial clefting frequency in different ethnic populations from the UCSF craniofacial clinic database shows Hispanics have a high frequency of additional anomalies. *E.W.Y. Hsieh[1], R-F. Yeh[2], S. Oberoi[3], K. Vargevik[3], A.M. Slavotinek[1]* 1) Department of Pediatrics, Division of Genetics, UCSF School of Medicine, San Francisco, CA; 2) Center for Bioinformatics and Molecular Biostatistics, UCSF, San Francisco, CA; 3) Center for Craniofacial Anomalies, UCSF, San Francisco, CA.

Opitz G/BBB syndrome is a multiple congenital anomaly syndrome characterized by developmental anomalies affecting the midline including hypertelorism, cleft lip and/or cleft palate (CLP), and hypospadias. This syndrome and orofacial clefting greatly affect the Hispanic population but their relative frequency in this population is unknown. Our goal is to determine if there are ethnic differences in the frequency of patients who have CLP, either as part of a syndrome, such as Opitz G/BBB, or as an isolated finding. We performed a retrospective analysis of 296 medical records of CLP patients referred to the UCSF craniofacial clinic. We recorded information about patient age, sex, type of cleft, presence of additional anomalies, syndrome diagnosis if present, and ethnicity, and analyzed our results using chi-squared tests. Our analysis revealed that the maternal ethnic distribution of patients with CLP with additional anomalies is different from the ethnic distribution of the patient population at UCSF craniofacial clinic. Hispanics represented 35.96% of the UCSF craniofacial clinic patients with CLP and multiple congenital anomalies while they constituted only 34.71% of the UCSF craniofacial clinic total patient population (p-value of 0.0476). There were a total of 90 CLP cases with additional anomalies in the 296 medical records analyzed, 35 of which were of Hispanic ethnicity (38.9%). Only 36/90 (40%) of CLP patients belonging to all ethnicities who had additional anomalies received a diagnosis, most frequently Van der Woude syndrome (10/36 = 27.78%) and Opitz syndrome (4/36 = 11.11%). In conclusion, amongst the UCSF craniofacial clinic patients with CLP, Hispanic CLP patients had a higher than expected frequency of additional anomalies, warranting careful examination and evaluation for signs of a syndrome diagnosis.

Population genetic data set of hundreds of thousands of SNPs free of ascertainment bias. *A. Keinan[1], J.C. Mullikin[2], N. Patterson[3], D. Reich[1,3]* 1) Department of Genetics, Harvard Medical School, Boston, MA; 2) Genome Technology Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 3) Program in Medical and Population Genetics, The Broad Institute, Cambridge, MA.

One of the hoped-for products of the International Haplotype Map Project (HapMap) was a large data set that could be used for making inferences about human demographic history. Unfortunately, the first 1.1 million SNPs (Phase I) were identified using different strategies by each of the participating centers and in ways that changed over time, which made it very difficult to make inferences about demographic history from these data. From Phase II of HapMap, however, we were able to identify a subset of SNPs that were free of ascertainment bias and useable for demographic analysis. We took advantage of the fact that for Phase II, essentially every SNP that was available in public databases at the time of marker picking was chosen for genotyping.

The key insight was to focus on Self-SNPs, defined as all the SNPs obtained by comparison of shotgun sequencing reads from a specific individual. These SNPs have a simple ascertainment as they are obtained by comparing two chromosomes from the same ancestral background (an individuals two haplotypes). Our initial analysis focused on self-SNPs from seven individuals: 2 European Americans, 2 East Asians, 2 African Americans, and a Mbuti Pygmy. We studied the frequency distribution of each Self-SNP set (tens to hundreds of thousands of SNPs) in the 420 worldwide chromosomes genotyped in HapMap.

Initial analysis of the unbiased frequency distributions focused on comparing the European Americans with the East Asians, two populations that are commonly assumed to share a very similar demographic history. Our rigorous full-likelihood analysis points to a significantly more severe out-of-Africa population bottleneck in the history of East Asians. We further estimated the intensities and timing of the main demographic events experienced by these two populations.

Serotonin Transporter mRNA Levels are associated with the Methylation of an CpG Island. *A. Madan, R. Philibert, A. Anderson, R. Cadoret, H. Packer, H. Sandhu* Neurosurgery, University of Iowa, Iowa City, IA.

The serotonin reuptake transporter (5HTT) is thought to be the principal regulator of serotonergic activity and epigenetic effects at this locus are thought to be important moderators of vulnerability to neuropsychiatric illness. In attempt to understand the basis of this regulation, several gene polymorphisms that affect 5HTT mRNA levels have been described. But to date, no clear mechanism linking these polymorphisms to vulnerability to epigenetic effects have been described. We describe a CpG island in the 5 region of the 5HTT gene that contains an alternative exon 1 and possible promoter for 5HTT. We then confirm the existence of this transcript and ascertain the methylation status of this CpG island in 49 lymphoblast cell lines and analyze the relationship between methylation and 5HTT mRNA levels. We demonstrate that methylation at this CpG island is associated with decreased levels of 5HTT mRNA, but that this effect is evident only when 5HTTLPR genotype is taken into account. We suggest that these findings have significant implications for the understanding of the role of this locus in behavioral illness.

Metabolic polymorphisms as markers for pediatric cancer risk following genotoxic exposure. *H.E.J. Kendall, P.M. Vacek, B.A. Finette* University of Vermont, Departments of Pediatrics, Microbiology and Molecular Genetics, and Medical Biostatistics, Burlington, VT.

Polymorphisms in metabolic enzymes can affect the detoxification of chemicals by varying rates of biotransformation. This can increase internal doses of mutagenic substances, susceptibility for acquiring mutations, and cancer risk. We are investigating whether the prevalence of polymorphisms in metabolic enzymes are associated with pediatric cancer risk following exposure to genotoxic chemicals. Our focus is on children from a pediatric cancer cluster in Toms River, NJ from 1979-1995, who were shown to have a 70% higher incidence of cancer as a consequence of consuming contaminated drinking water. Specific aim: Determine the frequency of 26 polymorphisms in 13 metabolic genes, in (A) children from Toms River, who developed cancer and were exposed to contaminated drinking water, (B) exposed siblings who did not develop cancer, (C) children with no known genotoxic exposure who developed cancer, and (D) healthy children with no known genotoxic exposure who did not develop cancer. Polymorphism analysis was performed using TaqMan allelic discrimination, PCR, restriction analysis and single-nucleotide extension. To date, we have analyzed 7 polymorphisms from 121 subjects. Analysis at the *EPHX1* (T/C: Tyr113His) locus revealed a statistically significant association with the number of copies of the C allele in the exposed Toms River cancer cases. Although the remaining polymorphisms showed no statistically significant difference in prevalence, likely due to limited power, there are a number of potentially important observations. The Neg/Neg genotype for the *GSTT1* deletion polymorphism occurred in 27.6% of the exposed cancer cases, compared to 18.4% in the siblings and 16.3% in healthy controls. The Pos/Pos *GSTT1* genotype occurred in 34.5% of the exposed cancer cases, compared to 46.9% of siblings. *NAT2* polymorphisms (T341C C/C; A803G G/G; G857A G/A) were all higher in the exposed cancer cases compared to exposed non-cancer siblings. Continued analysis will determine the utility of metabolic polymorphisms as biomarkers for pediatric cancer risk following genotoxic exposure.

Investigation of age-related cognitive decline and memory loss among *FMR1* premutation allele carriers. *J.E. Hunter[1], E.G. Allen[1], A. Abramowitz[2], M. Rusin[3], R. Letz[4], M. Leslie[1], L. Shubeck[1], G. Novak[1], S. Sherman[1]* 1) Department of Human Genetics, Emory University, Atlanta, GA; 2) Department of Psychology, Emory University, Atlanta, GA; 3) Department of Rehabilitation Medicine, Emory University, Atlanta, GA; 4) Department of Behavioral Sciences and Health Education, Rollins School of Public Health, Emory University, Atlanta, GA.

Fragile X mental retardation gene (*FMR1*) alleles with long unmethylated CGG repeats in the range of ~59-199, termed premutation alleles, have recently been associated with a tremor/ataxia syndrome (FXTAS) in older males. This condition is unrelated to the fragile X syndrome phenotype associated with highly methylated alleles with greater than 200 repeats. Cognitive decline and short-term memory loss are well-documented symptoms of FXTAS. To examine the progression of symptoms associated with FXTAS, we investigated the age-related decline of memory and cognition in a cross-section study of 131 males and 364 females age 18-84 with varying repeat size. The Wechsler Adult Intelligence Scale (WAIS-III) provided cognition measures including full-scale IQ (FSIQ), verbal IQ (VIQ), and performance IQ (PIQ) as well as a working memory index (WMI). The Wechsler Memory Scale (WMS-III) provided visual and logical memory measures for immediate and delayed recall as well as delayed recognition. Among cognitive measures, only VIQ showed a significant negative association with repeat size ($p<0.05$). For memory, WMI was significantly lower for premutation carriers compared with noncarriers ($p<0.05$) only when repeat size groups were used. When repeat size was defined as continuous, logical memory immediate and delayed recall scores were positively associated with repeat size ($p=0.05$ and $p=0.03$, respectively), but only explained about 1% of the variance. Age and gender by repeat size interaction were not significant for any measures. Results indicate that there is not a detectable difference in the age-related rate of memory or cognitive decline between premutation carriers and noncarriers. However, independent of age, premutation carriers seem to have relative deficits in working memory and verbal IQ.

Association of Progerin-Interactive Partners with Lamina Proteins. *W. Ju, G. Radu, W.T. Brown, N. Zhong* Dept Human Genetics, New York State Inst Basic Res, Staten Island, NY.

The Hutchinson-Gilford progeria syndrome (HGPS or progeria) is a premature aging disorder of childhood. It results from a dominant negative effect of progerin, which is the truncated protein encoded by a mutant LMNA gene carrying G608G mutation. The detailed pathogenic mechanism of progerin involved in HGPS is yet unclear. To study this unknown mechanism, we have earlier identified four novel progerin-interactive partner proteins (PIPs), including hnRNP E1, EGF, Mel18, and UBC9. These PIPs were found to have no differential interaction with normal lamin A/C when compared to progerin. To further investigate whether there is any abnormality that PIPs associate with lamina proteins, we performed co-immunoprecipitations in both normal and HGPS cells. Our results showed that (1) all PIPs but Mel18 associate with lamin B1, (2) all PIPs but UBC9 associate with emerin, (3) UBC9, EGF, and hnRNP E1 associate with SREBP, and (4) UBC9 and hnRNP E1 associate with LAP2a. The co-immunoprecipitations showed that UBC9 was able to pull down Mel18, EGF, and hnRNP E1; EGF pulled down UBC9 and hnRNP E1; and Mel18 pulled down hnRNP E1. Although our earlier results demonstrated that PIPs were co-immunoprecipitated with lamin A/C and progerin, the current study showed that progerin only pulls down hnRNP E1 but not EGF, nor UBC9. No significant difference could be noticed between normal and HGPS conditions. We also investigated if PIPs change their subcellular localization in the HGPS condition. Immunostaining of PIPs with nuclear markers, including progerin, showed that immuno-signals of all PIPs were within nuclei and there was no difference between normal and HGPS cells. Our results indicated that PIPs associate with lamina proteins; however, they are unlikely involved in the pathogenesis of HGPS.

Clinical Predictors of risk of Optic Pathway Glioma in Neurofibromatosis Type-1 patients. *A. Mian[1], J. Perentesis[1], E. Schorry[2], H.S. Lee[3], R. Chakraborty[3], B. Chakraborty[4]* 1) Div of Hematology-Oncology, Cincinnati Children's Hospital, Cincinnati, OH; 2) Div. of Human Genetics, Cincinnati Children's Hospital, Cincinnati, OH; 3) Center for Genome Info, University of Cincinnati, Cincinnati, OH; 4) Dpt Env Health, University of Cincinnati, Cincinnati, OH.

Neurofibromatosis type-1 (NF-1), an autosomal dominant disease, is characterized by widely variable clinical features. These patients are at an increased risk of developing wide variety of benign and malignant neoplasia. Optic pathway glioma (OPG) occurs in almost 20% of NF-1 patients. There is poor genotype-phenotype correlation and limited data to quantify the risk of tumor development when preceded by specific clinical features. We hypothesized that presence of specific clinical features in NF-1 patients can predict their risk for subsequent OPG development. Based on logistic regression modeling of data on 75 cases (NF-1 patients with subsequent development of OPG) and 215 controls (NF-1 patients without OPG or co-existing neoplasia), we observed that patients with ages 4 years or less at diagnosis of NF-1 (odd ratio, OR=3.8, p=0.002), presence of T-2 hyperintense lesions (UBOs) on imaging (OR=3.3, p=0.005), developmental delay (OR=1.8, p=0.04) and seizures (OR=13.0, p=0.07) appear to be at an increased risk of developing subsequent OPG compared to the patients without these clinical features. Race (Caucasian) appears to have a disease modifying effect on progression. Early age of diagnosis of NF-1 and presence of seizures also have an interaction effect (OR=5.3, p=0.029). In an attempt to define the high risk group of NF-1 patients who may develop OPG subsequently, a composite score with these risk factors was computed, based on the estimated coefficients of the logistic regression model. This score provides an appreciable sensitivity (82%), but modest (47%) specificity in predicting subsequent development of OPG in NF-1 patients. These findings indicate that combinations of clinical features of NF-1 may be used to define the high risk group of NF-1 patients who are prone to develop OPG. Consequently, such high risk patients may benefit from frequent screening and follow up for early detection of OPG.

A Practical View of Genome-Wide Association Studies. *M. Li[1], W. Guan[2], R.S. Spielman[1], C. Li[3]* 1) Univ Pennsylvania; 2) Univ Michigan; 3) Vanderbilt Univ.

Genome-wide association studies (GWAS) are a powerful approach to identify common genetic variants that predispose to complex diseases. Its success will depend on many factors, including the effect size of disease genes, the degree of LD between tagSNPs and disease loci, and the sample size. In this project, we examine the impact of these factors on power of GWAS. Based on the phased HapMap CEU sample, we simulated 300K SNP genotype data using SNPs of Illuminas HumanHap300. We also selected 100K and 500K SNPs from the HapMap by deleting or adding SNPs to the 300K set. These three SNP sets represent the current available commercial SNP panels that most investigators will use. We simulated case-control data assuming a model with 6 unlinked disease loci with different levels of effect on disease risk and placed them in regions with various levels of LD. The simulated data show similar patterns of LD as the HapMap sample. We evaluated power using both 1- and 2-stage designs. Our results indicate that: 1) High LD coverage is crucial for GWAS. The power is dramatically reduced if the disease locus is in a region of moderate LD as compared to a region of strong LD (61% vs 90% for 2000 cases and 2000 controls and s=1.02 using 300K SNPs), indicating the importance of selecting more tags in regions of moderate/weak LD as it is unknown where the disease genes reside; 2) Power generally increases as the number of markers increases; however, if the disease locus is in a region of strong LD, then power can decrease due to the correction for an increased number of tests; 3) As noted by others, for a study with a 2-stage design, if a smaller proportion of subjects are used in stage 1, then a larger proportion of markers should be followed up in stage 2 in order to have power comparable to that of a 1-stage study. Many GWAS will rely on the HapMap for tagSNP selection. However, its limited sample size may lead to over-estimation of LD for some regions and an under-powered study if the disease genes fall in such regions. We therefore evaluated the extent of power loss when tags are selected based on such a small sample. Our results shall give researchers a practical view of GWAS and provide useful guidance when designing such large-scale studies.

Mach 1.0: Rapid Haplotype Reconstruction and Missing Genotype Inference. *Y. Li, J. Ding, G.R. Abecasis* Center for Statistical Genetics, Department of Biostatistics, School of Public Health, University of Michigan, Ann Arbor, MI.

Missing genotype imputation and haplotype reconstruction are valuable for many analyses in revealing disease genetic mechanisms. We present a fast and flexible markov chain haplotyping method that resolves haplotypes, and thus missing genotypes, from unphased genotypes in samples of genetically unrelated individuals. The method is motivated by coalescent theory, particularly the observation that only a limited number of similar haplotypes are observed over short regions of the genome. As in other haplotyping methods motivated by the coalescent, our method captures the block-like pattern of haplotypes and the local nature of recombination by making each individual haplotype a mosaic of the currently resolved haplotypes of other individuals. Our method has been demonstrated to be faster and more accurate than existing methods in both missing genotype imputation and haplotype inference when applied to simulated or real datasets. In addition to the most likely genotype at each position, we provide two additional summaries that reflect model uncertainty: (1) a quality score for each inferred genotype and (2) an estimated allele dosage, that reflects estimated counts for a reference allele and which can be used in subsequent statistical analyses. The method is implemented in our MACH (MArkov Chain Haplotyping) software and freely available with C++ source code.

Demography and selection affection human prtoein coding genes. *R. Nielsen[1], M. Hubisz[2], C. Bustmante[2], A. Andres[3], S. Williamson[2], A.G. Clark[3]* 1) Dept of Biology, University of Copenhagen, Kbh Ø, Denmark; 2) Dept. of BSCB, Cornell University, Ithaca, NY; 3) Dept. of Mol. Biol. Genet., Cornell University, Ithaca, NY.

We analyze directly sequenced data from more than 10,000 loci from 20 European-American and 19 African-American individuals. We establish a nd fit a demographic model to the data which includes admixture, divergence, migration and changes in population size - and show that this model adequately fits the observed allele frequency distributions. We then scan the data for loci targeted by natural selection and identify a number of genes with variants targeted by natural selection that have not previously been identified by studies of natural selection in humans. These genes include a number of genes that have been shown directly to affect higher cognitive functions and several genes relating to human disease. Our approach for detecting natural selection in humans differ from previous approaches in adequately accounting for demography while not being challenged by ascertainment biases influencing many other studies based on publicly available SNP genotyping data.

SLOS like phenotype in two sisters with low serum cholesterol and normal enzymatic activities of cholesterol pathway, a new cholesterol disorder? *M. Michelson[1,2], C. Vinkler[1,2], T. Lerman-Sagie[2,3], D. Lev[1,2]* 1) Genetics Institute, Wolfson Medical Center, Holon, Israel; 2) Metabolic- Neurogenetic Clinic,Wolfson Medical Center, Holon, Israel; 3) Pediatric Neurology Unit,Wolfson Medical Center, Holon, Israel.

Recent publications point at an increasing importance of the role of cholesterol and cholesterol intermediates in the development of the fetus. Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive, multiple malformation/mental retardation syndrome with typical dysmorphic features. The metabolic basis is a reduction in the activity of 7-dehydrocholesterol reductase. We present two sisters with SLOS-like features, low serum cholesterol and normal levels of intermediates in the cholesterol pathway. These sisters, aged 6 and 4 years old, had normal birth weight and developed severe FTT thereafter. They had similar dysmorphic features: microcephaly, small nose, anterverted nostrils, depressed nasal bridge, low set ears, prominent metopic suture, epicantal folds, light and sparse hair, micrognathia, short neck and syndactyly of the II and III toes. They both had hypotonia and developmental delay. Extensive metabolic, cytogenetic and molecular tests were normal. Brain imaging studies were unremarkable in both sisters. Plasma bile acids by GC-MS were normal. Sterol pathway studies were normal except for a slight elevation of lathosterol relative to cholesterol. These findings might point out an increased rate of cholesterol synthesis or a relative block in intracellular trafficking of precursor sterols. Based on the distinct dysmorphic features with low levels of cholesterol we suggest that this is a new autosomal recessive syndrome involving cholesterol metabolism. Further assays evaluating cholesterol transport are being done.

Discovery of DNA methylation sites in MYOD1, MLH1, and RB1 in heterogeneous DNA samples with a modified quantitative sequencing method. *A. Lakdawalla, V. Boyd, K. Hunkapiller, C. Brown, E. Gerber, S. Chen* Applied Biosystems, Foster City, CA.

DNA methylation of cytosine in promoter regions has been shown to produce transcriptional silencing and resultant changes in phenotype. Conversely, phenotype can be associated with a unique DNA methylation signature. Therefore, significant effort is underway to discover specific C residues in DNA sequences that are associated with methylation and gene silencing. Bisulfite treatment of gDNA is used to convert non-methylated Cs to uracil (U). 5methyl-C is resistant to conversion therefore a change from Cytosine to Thymine indicates that the C residue was not methylated. As methylation signatures can be different due to cell - to - cell heterogeneity, discovery of DNA methylation requires extensive cloning and sequencing to discover rare methylation events in a mixed DNA population.

To circumvent the laborious cloning step, we have modified standard PCR-DNA sequencing to directly discover and quantitate putative DNA methylation sites in mixed samples. The modified method was utilized to effectively discover DNA methylation sites associated with differential expression of MYOD1, MLH1 and RB1 in normal and abnormal tissue samples.

Evaluation of the effect of the $Pl^{A1/A2}$ dimorphism, of the $P2Y_1$ receptor gene 1622A/G mutation and of the COX-1 gene 22C/T mutation on platelet response to aspirin. *M. Lordkipanidzé[1,2], C. Pharand[1,2], J.G. Diodati[1,2], D.A. Palisaitis[1,2], F. Bélanger[2], Y.K. Sia[1], E. Schampaert[1,2], J. Turgeon[2]* 1) Hôpital du Sacré-Coeur de Montréal, Québec, Canada; 2) Université de Montréal, Québec, Canada.

Background: The antiplatelet drug aspirin is widely used to prevent acute ischemic events. However, platelet response to aspirin is highly variable, which may be partly explained by genetic variability affecting platelet enzymes or receptors. Objective: To evaluate the impact of the dimorphism on the 3 allele of the 2b3 integrin (PlA1/A2), of the P2Y1 receptor gene mutation (1622A/G) and of the COX-1 gene mutation (22C/T) on platelet response to aspirin. Method: One hundred and ninety-two stable CAD patients under daily aspirin therapy were recruited. Platelet aggregation was measured by light transmission aggregometry (LTA) with arachidonic acid at 1.6 mM as the agonist. Genotyping was performed by standard PCR method. Results: The genotype frequency of PlA1A1:PlA1A2:PlA2A2 was 72.8%:25.6%:1.6%. The genotype frequency AA:AG:GG of the P2Y1 receptor gene at the 1622 position was 70.7%:25.5%:3.6%. The genotype frequency CC:CT:TT of the COX-1 gene at the 20 position was 84.9%:14.6%:0.5%. Mean platelet aggregation (%) with the PlA1A1:PlA1A2:PlA2A2 genotypes was 5.4, 4.8, and 6.5%, respectively ($p=0.95$); with the AA:AG:GG genotypes of the P2Y1 receptor gene, it was 5.0, 3.0, and 25.6% ($p<0.0001$); with CC:CT:TT of the COX-1 gene, it was 5.7, 2.5, and 3.0% ($p=0.46$). Aspirin resistance, defined as LTA > 20%, was associated with the 1622A/G polymorphism of the P2Y1 receptor gene ($p=0.002$); neither PlA1/A2 nor the polymorphism of the COX-1 gene was linked to aspirin resistance ($p=0.933$ and $p=0.476$, respectively). Conclusion: In our population, the mutation affecting the P2Y1 receptor gene was linked to aspirin resistance.

Utility of genetic markers used in human identification to detect genetic structure of global populations. *W. Niu, H.S. Lee, R. Chakraborty* Ctr Genome Information, Univ Cincinnati, Cincinnati, OH.

Hypervariable short tandem repeat loci used in DNA forensics and for human identification were originally chosen for their ubiquitous polymorphic status in global populations. High level of polymorphisms at these loci, caused by their high rate of mutations, produce a small coefficient of gene differentiation across populations (generally less than 1% in cosmopolitan populations within continents, and around 3-5% between isolated populations within geographic regions). Nonetheless, multilocus DNA profiles generated by these loci aids in human identification, since the chance of observing the same profile given that it is observed in another person is often quite small. This research attempts to determine the utility of these forensic markers in detecting structure in global populations. Using data on 1,981 13-locus DNA profiles from 10 worldwide populations (representing Africans, Asians, Europeans, Native Americans, as well as admixed Africans, such as Jamaicans and Brazilian Blacks, and Hispanics), we used the STRUCTURE software to determine the genetic structure revealed by these loci. We observe that a four-component structure model fits these DNA profiles adequately. The continental populations are well separated, and the individuals from the admixed populations show a remarkable consistency of contributions from the component structures. These observations imply that even though the coefficient of gene differentiation at these forensic loci is small, their high degree of polymorphism still allows for a rather good degree of geographic separation of profiles, with admixed individuals showing the structure of their admixture history at least as a group level. Of course lesser polymorphic ancestry-informative markers (AIMs) are better suited for such purposes, specifically for admixture estimation at individual level, though the ability of the AIMs for identification purposes may be compromised. (Research supported by US Public Health Service Research Grant GM 41399).

Knowledge among universitary women about folic acid and its importance during pregnancy: a survey in the Pontificia Universidad Javeriana Colombia. *A. Ordoñez, F. Suárez* Intituto de Genética Humana, Pontificia Universidad Javeriana, Bogota D.C.

Neural tube defects (NTDs) affect about 7,28 of every 10.000 deliveries in Colombia. Periconceptional folic acid supplementation is recommended to prevent NTDs. To reduce the risk for NTDs women are encouraged to supplement with 400 mcg folic acid daily during their reproductive years. This study examines folic acid knowledge, and supplementation practices among women of childbearing (WCBA) age in one of the principal private Universities of Colombia. We conducted a survey in 390 women over a calculated random sample of 356 women. We calculated the relative frequencies for the answers with the respective confidence intervals. The model of the survey was based in the Pregnancy Risk Assessment Monitoring System of the CDC (centers for disease control and prevention) at the end of the survey every women received patient education materials. Mean age of women was 21 years old. We found that 189 women (49%, CI, 95%: 43.02 - 52.98) had listened or read some information about folic acid, but are not sure about the benefits. 197 women (51%, CI, 95%: 46.01 - 55.99) did not have any information about the vitamin in relation to pregnancy and birth defects, but have heard about the vitamin. 128 women (33.2%, CI, 95%: 28.32 - 37.69) know something about the benefits in pregnancy and 258 women (66,8%, CI, 95%: 61.27 - 70.73) did not have information and know nothing about the benefits in pregnancy. None of the women actually consume folic acid at the recommended doses. 96 women (24.9%, CI, 95%: 19.74 - 28.26) consume some other kind of multivitamin supplementation. The women with less knowledge of folic acid had a greater consumption of multivitamin and folate-rich fruits and vegetables (p 0.0005). In Colombia, there is not information about periconceptional folic acid, as we demonstrate in this particularly educated women population. A major educational campaign and fortification of grain with folic acid may be the best practical solution. Fortification of food may need to be used to increase folate consumption in those with unplanned pregnancy.

Heritability of plasma amino acid levels in different nutritional states. *K.L. McBride[1], J.W. Belmont[2], W.E. O'Brien[2], T.J. Amin[2], S. Carter[2], B.H. Lee[2]* 1) Dept Molecular & Human Gen, Columbus Child Res Inst, Columbus, OH; 2) Dept of Molecular & Human Gen, Baylor College of Medicine, Houston TX.

Plasma amino acid (PAA) levels are impressively regulated to maintain a tight range of values that are consistent across ethnic groups. Ranges do vary dependent on age, sex, fasting status and protein intake. Previous studies have indicated a genetic component to PAA homeostasis for several amino acids, but they have been hampered by a combination of mixed fed and fasting samples or variability in the preceding protein intake. We had the unique opportunity to study the heritability of PAA levels before and during a steady-state of low protein intake, from data obtained for nitrogen flux stable isotope studies. Subjects were fed frequent, small meals totaling 0.4 grams of protein /kg of body weight per day, for 3 days. A total of 71 individuals in 21 families from that study were included in this analysis, consisting of 11 with a urea cycle defect (UCD), 10 UCD heterozygotes, 13 OTC heterozygous females, 26 unknown status, and 13 unaffected or controls. Samples were drawn on hospital admission (regular diet), after 2 days on the steady-state diet after overnight fast, and while on the low protein steady-state diet (fed every 2 hours). The program SOLAR was used for Heritability (h2) estimates, with covariates sex, weight and age. PAAs with high h2 on a regular diet were Ser, Thr, Gly, and Pro (h2=0.82, 0.53, 0.66, 0.78); while fasting were Cys, Gly, Ala, Pro, Asn, and Tyr (0.75, 0.83, 0.83, 0.74, 0.84, 0.83); and on steady-state were Tyr, Phe, and Trp (0.75, 0.79, 0.55), which utilize the same large neutral amino acid transporters. Heritability of PAAs varied by nutritional state and could be grouped by common metabolic fates or transporters. This confirms previous work on fasting subjects, and expands this to include additional PAAs whose homeostasis in different nutritional states demonstrates significant genetic control. We speculate genetic and environmental influences on individual PAA homeostasis vary throughout the feed-fast cycle, are influenced by previous and current amount of protein, and cannot be appreciated without careful controlled study.

The function of BLOC-2 in lysosome-related organelle biogenesis. *A. Helip Wooley[1], W. Westbroek[1], H. Dorward[1], P. Held[1], M. Ayub[1], R. Boissy[2], M. Huizing[1], W.A. Gahl[1]* 1) Medical Genetics Branch, NHGRI, NIH, Bethesda, MD; 2) Department of Dermatology, University of Cincinnati College of Medicine, Cincinnati, OH.

Hermansky-Pudlak syndrome (HPS) is a disorder of oculocutaneous albinism and prolonged bleeding due to defects in the formation of lysosome-related organelles (LROs), specifically melanosomes in melanocytes and dense bodies in platelets. Eight genes are associated with different types of HPS in humans. HPS3, HPS5 and HPS6 proteins interact with one another in the Biogenesis of Lysosome-related Organelles Complex-2 or BLOC-2. The function of BLOC-2 is unknown. Ultrastructural studies of BLOC-2 deficient melanocytes revealed predominantly immature melanosomes. Tyrosinase and TYRP1 reside mainly on small vesicles, not on melanosomes, as in normal melanocytes. The abundance of TYRP1, a melanosomal protein, is markedly reduced in HPS-5 melanocytes. We previously demonstrated that HPS3 interacts with clathrin, and that GFP-HPS3 localizes to small vesicles and endosomal structures in normal melanocytes. We have now performed subcellular fractionation of human melanoma cells (MNT1) to study the localization of endogenous BLOC-2 components on the different organelles. Differential centrifugation fractions were characterized by immunoblotting and electron microscopy. BLOC-2 components were enriched on a single high-density fraction that contained larger endosomal structures as well as small vesicles; the fraction was positive for EEA1, an early endosomal marker. Markers of early and late melanosomes were absent from this fraction. We hypothesize that BLOC-2 functions at a sorting endosome-like structure and is necessary for the proper sorting of a subset of melanosomal proteins into transport vesicles. BLOC-2 may also play a role in the subsequent trafficking of these vesicles to maturing melanosomes. In the absence of BLOC-2, these melanosomal proteins may be randomly incorporated into vesicles that are incapable of efficient trafficking to early melanosomes. Alternatively, they may remain in an endosomal structure and follow a default pathway toward degradation.

Androgen Receptor (AR) and Estrogen Receptor alpha (ER) Variants Have Minimal Impact upon Cancer Susceptibility in BRCA1 Mutation Carriers. *S. Harbord[1], C.J. Brown[1], D. Horsman[1,2], S. Young[2], W.P. Robinson[1]*
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A mutation in the BRCA1 gene dramatically increases a womans chance of developing breast and/or ovarian cancer, however, penetrance is incomplete. Epidemiological data implicates hormones in both the presence and the severity of breast and ovarian cancers, and hormone receptor activity has been linked to BRCA1 protein activity. Polymorphisms in hormone receptors may act as genetic modifiers and influence risk of breast and/or ovarian cancer in BRCA1 mutation carriers. Androgen Receptor (AR) and Estrogen Receptor alpha (ER) contain polymorphisms that have been associated with breast cancer, though results have been inconsistent. Here we have studied AR CAG repeat length, ER TA repeat length and two ER SNP polymorphisms at -397 and -351 in 42 affected BRCA1 mutation carriers, 15 unaffected BRCA1 mutation carriers, 24 BRCA2 mutation carriers, 24 familial controls and 152 population controls. Repeat length was determined using a conventional PCR-based assay, while ER SNP polymorphisms were analyzed by allelic discrimination with Real Time PCR. Despite previous reports, no patient group was significantly different from controls for any hormone receptor polymorphism considered. Neither affected BRCA1 mutation carriers nor affected non-BRCA1 mutation carriers had an increase in long AR alleles when compared to pooled familial and population controls (BRCA1: 7/42, non-BRCA1: 4/33, controls: 30/186). Affected BRCA1 mutation carriers had a non-significant trend towards a decrease in long AR alleles when compared to combined controls (BRCA1: 12/42, controls: 33/186). Long ER TA repeats were also not increased in affected BRCA1 mutation carriers (29/42) or affected non-BRCA1 mutation carriers (20/33) compared to combined controls (80/128). In addition, no ER SNP polymorphism is overabundant in either affected BRCA1 mutation carriers or affected non-BRCA1 mutation carriers compared to controls. Though sample size is small, it seems that androgen receptor and estrogen receptor a variants do not largely affect breast and/or ovarian cancer risk in BRCA1 mutation carriers.

Evaluation of the effect of the H2 haplotype and CYP3A5 polymorphisms on the antiplatelet response to clopidogrel given before elective percutaneous coronary intervention. *J. Turgeon[2], M. Lordkipanidzé[1,2], C. Pharand[1,2], J. G. Diodati[1,2], T. A. Nguyen[1], D. A. Palisaitis[1,2], F. Bélanger[2], Y. K. Sia[1], E. Schampaert[1,2]* 1) Hôpital du Sacré-Coeur de Montréal, Montréal, Québec, Canada; 2) Université de Montréal, Montréal, Québec, Canada.

Clopidogrel needs to be metabolized by the CYP450 3As enzymes to be active. CYP3A5*1 confers higher levels of expression while CYP3A5*3 confers low expression. The active metabolite prevents ADP-induced platelet aggregation by irreversibly binding the ADP P2Y12 receptor. Four polymorphisms (including the i-T744C SNP) in the P2Y12 gene define two haplotypes: H1 and H2. H2 is associated with higher maximal platelet aggregation in response to ADP. Objective: To evaluate the impact of the presence of the H2 haplotype and of the CYP3A5*1 and *3 polymorphisms on the antiplatelet response to clopidogrel. Method: A total of 116 patients with stable CAD were recruited prior to elective angiography. They received clopidogrel for 1 to 7 days prior to the procedure. Blood samples were obtained before clopidogrel initiation and at the time of angiography. Platelet aggregation was measured by light transmission aggregometry with ADP 20 M as the agonist. Genotyping was performed by standard PCR method. Genetic screening was performed to identify active (*1/*1 or *1/*3) or inactive (*3/*3) CYP3A5. Genotyping of P2Y12 H2 haplotype was performed by targeting the i-T744C SNP. Results: The genotype frequency of CYP 3A5 *1*1:*1*3:*3*3 was 0%:9.5%:90.5%, giving a frequency of the non-expressor (*3) and expressor (*1) alleles of 95.3% and 4.7% respectively. The genotype frequency of the ADP i-T744C wild-type WT/WT:WT/variant:variant/variant was 72.4%:24.1%:3.4%, giving a frequency of the WT allele of 84.5%. Mean absolute decreases in % platelet aggregation with clopidogrel were 23.7 and 27.7% (p=0.56) with the CYP 3A5 *1*3 and *3*3, respectively, and 28.7% and 23.3% (p=0.23) with the ADP i-T744C WT/WT and WT/variant (or variant/variant), respectively. Conclusion: In our population, the presence of the i-T744C and CYP3A5 polymorphisms does not appear to affect the antiplatelet efficacy of clopidogrel.

QTL-ALL: software for QTL linkage analysis using score statistics and other new approaches. *N. Mukhopadhyay, S. Bhattacharjee, C-L. Kuo, D.E. Weeks, E. Feingold* Dept Human Genetics, Univ Pittsburgh/Sch Pub Health, Pittsburgh, PA.

There has been extensive development of new statistics for QTL mapping over the last few years, but a number of the most promising new methods are not yet available in end-user software. This is particularly true of methods such as score statistics that are important for studies using selected (non-population) samples. Our new "QTL Analysis and Linkage Library" (QTL-ALL) is a software package designed to make as many as possible of these new statistics widely available. The software consists of a Mega2-like interface for data analysis preparation and a library of R routines that computes linkage statistics. QTL-ALL reads in input data, creates re-formatted output data files, calls external IBD-generation software such as MERLIN or SimWalk2, then computes statistics using our R library, and finally produces tables and plots of statistics and p-values. This entire sequence is highly automated, requiring minimal user-intervention. The initial release of the software computes a number of newer QTL-mapping statistics, including several score statistic variants, and can handle nuclear family data, including specialty designs such as discordant and concordant (affected) pairs. QTL-ALL will be available from http://watson.hgen.pitt.edu/register/.

A case of Whistling face autosomal dominant. *P.M. Hurtado, I. Zarante* Instituto de Genetica Humana, Pontificia Universiadad Javeriana, Bogota, Colombia.

Introduction. Freeman-Sheldon syndrome is a rare disorder, and the diagnosis is clinical by unusual facial features and skeletal abnormalities. It was first described by Freeman and Sheldon in 1938; since then, cases of Freeman-Sheldon syndrome have been reviewed extensively. There is genetic heterogeneity, but most cases are thought to be sporadic. Case report. We reported a mother of 32 year-old, a son (2 year-old) and a daughter (4 year old), with physical features and with a peculiar aspect of "whistling face". Both siblings had deep-set eyes, prominent supraorbitary edge, epicanthus, blepharophimosis, microstomia, mask-like face, high-arched palate, nasal speech, long philtrum. The Mother had almost the same physical findings, especially in her face. They had also thumb adduction contracture. We found in both sibs a history of global retardation of development. Discussion. The Freeman-Sheldon syndrome is heterogeneous not only in its clinical presentation but also in its genetic transmission. It is very important to be informed about the existence of more than one form of hereditary transmission of this syndrome, since genetic counseling should take into consideration all possibilities. Freeman-Sheldon syndrome, also called "whistling-face syndrome," is a very rare genetic condition, occurring both sporadically and by transmission through autosomal dominant or recessive mode, which affects primarily the face and skeleton. Characteristics include microstomia of the mouth, which gives the person a whistling appearance, a flat face, club feet, contracted joint muscles of the fingers and hands, and underdeveloped nose cartilage. In our case, clearly it corresponds to an Autosomal Dominat presentation of Freeman Sheldon Syndrome.

Methamphetamine-dependence whole genome association identifies genes including WDR1. *A. Hishimoto[1], T. Drgon[1], C. Johnson[1], Q.R. Liu[1], J. Drgonova[1], D. Walther[1], H. Ujike[2], T. Komiyama[2], M. Harano[2], Y. Sekine[2], T. Inada[2], N. Ozaki[2], M. Iyo[2], N. Iwata[2], M. Yamada[2], I. Sora[2], G.R. Uhl[1]* 1) Molecular Neurobiology Branch, NIDA/IRP/NIH, Baltimore, MD; 2) Japanese Genetics Initiative on Drug Abuse (JGIDA), Japan.

Dependence on methamphetamine and other substances displays substantial complex genetic underpinnings, including many that are shared by multiple addictive substances and some that are substance-specific. To identify SNP markers and genes whose alleles distinguish methamphetamine-dependent from control individuals, we used 100k SNP arrays and DNA from 200 Japanese methamphetamine-dependent and matched control individuals to assess allele frequencies at 98,398 autosomal SNPs. SNPs clustered in 47 small genomic regions that contain both genes and positive results from other abuser *vs* control comparisons display nominally-significant abuser/control allele frequency differences that are very unlikely to be due to chance. One of these genes, WDR1, is expressed in brain, encodes common missense and alternative splicing variants, and contains SNPs that display significant ($p = 3 \times 10^{-6}$- 6×10^{-4}) individual abuser vs control genotypes. A haplotype tagged by *Val*185*Ile* missense rs13441 displays association with methamphetamine abuse and also correlates with levels of amphetamine abuse in two separate NIDA samples ($r^2 = 0.29 - 0.44$; $p < 0.02 - 0.005$). By contrast, this haplotype does not predict significant allele specific expression in mRNAs from postmortem brains. While many of the genomic variants identified here are likely to contribute to vulnerability to various abused substances, WDR1 variation marked by *Val*185*Ile*/ rs13441 is a strong candidate variant for selective predisposition to develop or resist methamphetamine dependence *(Support: DHSS/NIH/IRP/NIDA)*.

Community accessibility of family health history tools. *J. O'Leary[1], J. Williams[2], M. Taualii[3], P. Kyler[4], S. Terry[1]* 1) Genetic Alliance, Washington, DC; 2) Clinical Genetics Institute, Intermountain Healthcare, Salt Lake City, UT; 3) Urban Indian Health Institute, Seattle Indian Health Board, Seattle, WA; 4) Genetic Services Branch, MCHB, HRSA/DHHS, Rockville, MD.

We hypothesize that engaging families in the collection of family health history may well be the ideal tool to promote positive health behavior and basic genetics education. Family health history is ideal because it combines clinical utility (as a predictor of health) with accessibility (by featuring folklore and genealogy). However, the amount of diversity and number of individuals in any community make creating a one-size-fits-all family health history tool nearly impossible. The varied needs of different cultures require a flexible approach, one best vetted by the organizations that already serve those communities directly. Of course, existing tools are an important resource, but they must be paired with complementary materials. The Coalition for Accessible Family Health History Tools was formed in order to promote family health history within all types of communities via the creation and dissemination of *Using Family Health History Tools: A Guide to Making Tools Accessible to Your Community*. The Guide is aimed at national, community, and disease-specific advocacy organizations interested in promoting the collection of family health history among their members and in their locality. The goal of the Guide is to help organizations use existing family health history tools and create complementary materials that will increase the accessibility of those tools. It provides techniques for developing new materials and tools while simultaneously highlighting dissemination techniques and promoting the distribution of existing materials. Finally, the Guide outlines how to lead a successful family health history initiative, from assessment of community knowledge to targeted dissemination of materials.

TRISOMY 8 RESCUE IN A PRENATAL CASE WITH MOSAIC STRUCTURAL CHROMOSOME REARRANGEMENTS. *A. Hajianpour, A. Zhang, K. Mac, J. Cary, B. Diaz, D. Burkhardt, M. D'addario, L. Dong, R. Habibian* Genzyme Genetics 655 East Huntington Drive Monrovia, CA 91016.

Amniocentesis was performed on a 23 year old woman at a gestational age of 18.6 weeks due to abnormal maternal serum screen and increased risk for chromosome abnormality. There were no abnormal ultrasound findings. Cytogenetic analysis revealed a mosaic karyotype consisting of two cell lines, one as a normal cell line and the second cell line containing a supernumerary marker chromosome as well as additional material attached to the short arm of one chromosome 8. Fluorescence in situ hybridization (FISH) using whole chromosome paint 8 probe revealed that both the marker chromosome and the additional material on the abnormal chromosome 8 were derived from chromosome 8: 47,XX,add(8)(p22),+mar[19].ish der(8)t(8;8)(p23.1;q21.2)(wcp8+),+r(8)(wcp8+)/46,XX[2]. It is speculated that the initial event leading to the formation of both the derivative and the marker chromosome 8 was the presence of trisomy 8 in the zygote or early embryo. A phenomenon known as trisomy rescue presumably followed in which all or part of one of the trisomic chromosome is lost. The resulting chromosome imbalance is therefore less severe than a full trisomy due to the selective survival of the rescued cells, hence the fetus may consequently survive to term. Rather than full trisomy 8, the net imbalance of the abnormal cell line in this case is monosomy 8p23.1-pter, trisomy 8q21.2-qter, and partial trisomy of the pericentromeric region of chromosome 8 (present as very small ring chromosome). Further studies are in progress to better understand the origin of the abnormal cell line, including re-examination of the slides to search for trisomy 8 and additional FISH studies. The pregnancy is still ongoing.

Identifying transcriptional targets in cancer. *K. Johanson, A. Sidhu, A. Hollenbach* Department of Genetics, LSU Health Sciences Center, New Orleans, LA.

Changes in gene expression are an integral part of a cells transition into a malignant state and are often brought about by alterations in the expression or binding capacity of transcription factors. This fact is illustrated by Alveolar Rhabdomyosarcoma (ARMS), which is characterized by a t(2;13)(q35;q14) chromosomal translocation that results in the fusion of two myogenic transcription factors Pax3 and FKHR (FOX01a). ARMS is an aggressive solid muscle tumor in children that has a event-free four-year survival rate of only 17%. Despite the identification and characterization of the oncogenic fusion protein Pax3-FKHR, little is known about the genes directly regulated by Pax3 or FKHR, and how their expression may be altered by Pax3-FKHR. While many techniques exist to investigate the possible gene targets and binding specificity of different transcription factors, the majority of these are too labor-intensive to be adapted for use in a genomic screen. The ability to easily and quickly screen an entire genome for potential transcription factor targets would provide valuable information about some of the molecular mechanisms behind cancer development. Therefore, we developed a modified yeast one-hybrid assay using Pax3, FKHR, and Pax3-FKHR as a model system. This assay provides a method of identifying gene targets of specific transcription factors by testing their ability to directly bind genomic elements in vivo. This method has a distinct advantage over other current screening applications in that it involves an assay that is directly dependent on the binding of Pax3, FKHR, or Pax3-FKHR to promoter elements and therefore allows for a full examination of the natural in vivo substrates of all three proteins. The identification of genes directly regulated by Pax3 and FKHR will help elucidate the role that these factors play in myogenic differentiation. In addition, by comparing how the expression of these genes is altered by Pax3-FKHR and identifying possible novel targets of the fusion protein, we will be able to develop a mechanism for Pax3-FKHR aids in the development of ARMS. This project also establishes a general method that could be adapted for use in identifying the targets of any known transcription factor.

Prioritized subset analysis in genome-wide association studies. *C. Li[1], M. Li[2], E.M. Lange[3], R.M. Watanabe[4]* 1) Ctr Human Genetics Research, Dept Biostatistics, Vanderbilt Univ, Nashville, TN; 2) Dept Biostatistics, Univ Pennsylvania, Philadelphia, PA; 3) Dept Genetics, Univ North Carolina, Chapel Hill, NC; 4) Dept Preventive Medicine, Keck School of Medicine, Univ Southern California, CA.

Genome-wide association (GWA) studies are a popular approach to study the genetics underlying complex diseases. Since GWA studies generate large volumes of data, it would be desirable to prioritize these data for analyses. Often a list of candidate genes or candidate regions (e.g. determined through linkage studies) exists such that it may be more efficient to use such information to prioritize the genome in data analysis. Traditional approaches to GWA such as Bonferroni and the false discovery rate (FDR) ignore such information, inherently treating all markers equally. We evaluate a prioritized subset analysis (PSA), in which markers are partitioned and prioritized into subsets based on supplemental data and FDR is then applied to each subset. We simulated 300K SNP genotypes from Illuminas HumanHap300 set, based on a phased HapMap CEU sample. The datasets we simulated show similar patterns of LD as the HapMap sample. We simulated case-control datasets assuming a disease model with six unlinked disease loci across the genome with various levels of effect on disease risk (s=1.01 to 1.05) and placed them in regions with different levels of LD. We evaluated power using both whole-genome and PSA approaches. Our results indicate that: 1) When the priority subset contains a higher fraction of disease genes than the genome, PSA significantly increases power to detect these genes compared to the whole-genome approach; 2) The higher the fraction of disease genes in the priority subset, the higher the power to detect them; 3) If a disease gene is not included in the priority subset, power is similar for both approaches. This is an advantage of FDR over the Bonferroni approach, for which power will decrease for disease genes not included in the priority subset to control the overall false positive rate. We also note that the overall false discovery rate is practically under control if the genome is partitioned into a few subsets (5), but can inflate as the number of subsets increases.

Limited instability of fragile X full mutation alleles. *S.L. Nolin, X. Ding, G.E. Houck, W.T. Brown, C. Dobkin* Human Genetics Dept, NYS Insttitute for Basic Research in Developmental Disabilities, Staten Island, NY.

Fragile X full mutation alleles are thought to be highly unstable during early embryonic development. This hypothesis is based on the heterogeneous molecular pattern, often referred to as a smear, that is typically observed in Southern analyses of full mutation alleles. Studies in different tissues of affected fetuses and also in affected monozygotic twins suggests that the heterogeneity occurs in early development (Devys et al., AJMG 43:208, 1992; Malmgren et al., Eur J Hum Genet 2:103,1994; Kruyer et al., AJHG 54:437, 1994). Additional studies demonstrating clonal stability of full mutation alleles in cultured somatic cells further support this conclusion (Worhle et al., Nat Genet 4:140, 1993).

We have re-examined the issue of somatic heterogeneity in DNA from 52 males and 29 females with full mutation alleles. In a modification of Southern analysis procedures, the samples were digested with EcoR I/Eag I and separated by agarose gel electrophoresis without the inclusion of ethidium bromide or other DNA intercalating dye during or following electrophoresis. The resulting molecular patterns were remarkably different from the heterogeneous pattern for full mutation alleles seen in standard Southern analysis. Nearly all samples exhibited 1 to 5 discrete fragments for full mutations with an average of 2.5 fragments/sample. Approximately 15% of the 81 samples analyzed also included a faint heterogeneous pattern. Parallel analyses of selected samples showed a smear in the presence of ethidium bromide and a few discrete fragments in its absence.

The use of ethidium bromide appears to alter the migration of the fragile X CG-rich fragments during electrophoresis giving an exaggerated impression of heterogeneity. These studies suggest that most full mutation alleles have only limited instability in early embryogenesis and other developmental periods, consistent with the clonal stability observed in cell culture. Finally, eliminating ethidium bromide during Southern analysis makes the diagnosis of females with full mutation alleles more reliable.

Bardet-Biedl syndrome mice have defective hippocampal development associated with mislocalization of neuronal primary cilia receptors. *K. Mykytyn[1,2], N.F. Berbari[1], L.M. Bohn[1], G.A. Bishop[3]* 1) Department of Pharmacology, Ohio State Univ, Columbus, OH; 2) Department of Internal Medicine Division of Human Genetics, Ohio State Univ, Columbus, OH; 3) Department of Neuroscience, Ohio State Univ, Columbus, OH.

Cilia are hair-like appendages that extend from the basal bodies of cells and are classified as either motile or primary. Motile cilia and flagella are responsible for generating flow or movement. Primary cilia are generally immotile, are present on almost all human cell types, and provide important cellular sensory and signaling functions. Defects in primary cilia formation or function have been implicated in many diseases. Although neurons throughout the brain possess primary cilia, their functions are unknown. We have found that hippocampal development is profoundly altered in mouse models of Bardet-Biedl syndrome (BBS), a human genetic disorder whose etiology has been linked to cilia dysfunction. The primary features of BBS include obesity, retinal dystrophy, renal abnormalities, polydactyly, cognitive deficits, and mood disorders. We find that mice lacking Bbs4 display progressive hippocampal dysplasia concomitant with an increase in neuronal cell death. We demonstrate that hippocampal neurons are ciliated during development and ciliary membranes contain functional receptors. Strikingly, neurons from *Bbs4*$^{-/-}$ mice have defective localization of neuronal ciliary receptors, suggesting that neuronal ciliary signaling is necessary for normal hippocampal development. Our findings may explain the pathophysiology of the cognitive and behavioral features of BBS patients, and thereby implicate defective cilia-mediated neuronal signaling as a basis for the development of cognitive and neuropsychiatric diseases.

Family Functioning, Age, & Coping Mechanisms in Smith-Magenis Syndrome (SMS). *R.S. Morse, R.A. Bernert, W. Introne, A.C.M. Smith* OCD, NHGRI/NIH/HHS, Bethesda, MD.

Introduction: A paucity of research has investigated family coping strategies and communication skills within rare genetic syndromes, including SMS. Few studies have evaluated child age and sex according to these areas of psychosocial functioning. **Methods:** Baseline data was collected among 39 parents with a cytogenetically-confirmed SMS (del 17p11.2) child. Administered measures: *Family Crisis-Oriented Personal Evaluation Scales* (F-COPES) & *Family Assessment Device* (FAD). **Results:** A one-way ANOVA revealed no significant mean sex differences on FAD & F-COPES ($p > .05$). Two multiple linear regressions were conducted to test associations between age and F-COPES & FAD subscale and total scores; age was the predictor variable. F-COPES analysis dependent variables (DV) included *Social Support Seeking, Spiritual Support Seeking, Passive Appraisal, Reframing*. When all 4 DVs were entered into the regression, as a set, a nonsignificant trend emerged (R^2=.17, F4,34=1.73, P=.167). With regard to the unique contribution of each DV to the prediction of age, *Seeking Spiritual Support* was significantly linked with age (=.37, t=2.08, P<.05); the link between age & *Acquiring Social Support* emerged as a ns trend (=-.35, t=-1.94, P=.06). *Passive Appraisal* (=-.21, t=-1.27, P>.05) & *Reframing* (=.14, t=.84, P>.05) subscales did not contribute to the variance beyond that contributed by the first two DVs. FAD analysis DVs included the *Global Functioning Scale* (GFS) & the *Communication Scale* (CS). When DVs were entered into the regression as a set, a ns trend emerged for the model (R^2=.09, F2,36=1.8, P=.18). With regard to the unique contribution of each DV to the prediction, the link between age & CS emerged as a ns trend (B=-.39, t=-1.89, P=.066), but not with GFS (=.22, t= 1.12, P=.27). **Conclusions:** Cross-sectional results indicated that increasing age was associated with decreasing levels of social support seeking, yet increasing levels of spiritual support seeking. Results also revealed that greater age was associated with less effective familial communication. Research is needed to clarify whether this is mediated by increased syndrome severity or age at diagnosis.

The Pompe Registry: Observations of diagnostic practices around the world. *P. Kishnani[1], L. Merlini[2], B. Byrne[3], W. Mueller-Felber[4], A. van der Ploeg[5]* 1) Duke Univ Medical Ctr, Durham, NC, USA; 2) University of Ferrara, Ferrara, Italy; 3) University of Florida, Gainesville, FL, USA; 4) Friedrich-Baur-Institut, Munich, Germany; 5) Sophia Childrens Hospital, Rotterdam, The Netherlands.

Background: Pompe disease is a rare, and often fatal muscle disease caused by a deficiency of lysosomal acid alpha-glucosidase (GAA). Patients span a spectrum of phenotypes ranging from infantile (cardiomyopathy, skeletal/respiratory muscle weakness, death within first year of life) through late onset (little/no cardiac involvement, progressive skeletal/respiratory muscle weakness, longer survival). Methods: To better understand disease natural course, an international Registry has been developed to collect anonymous data on patients with a confirmed diagnosis of Pompe disease. Participation is voluntary. Preliminary Data Overview: The Pompe Registry was launched September 2004 and contained 202 natural history patients from 16 countries as of April 18, 2006. Preliminary data indicates regional differences in diagnostic methods. Relative to ROW, participating North American physicians were at least three times less likely to use DBS [Dried Blood Spot] (5% vs. 14%), two times less likely to use muscle biopsy (18% vs. 31%) and four times (5% vs. 20%) less likely to use DNA analysis. In North America the most common tissues for enzymatic assay was via skin fibroblasts and blood lymphocytes. Summary: Early registry data indicates that regional differences may exist in the diagnostic algorithms used for Pompe diagnosis. With the availability of less invasive techniques such as DBS in diagnostic laboratories in the US it is likely that these tests will be used more frequently as the first tier approach to help identify new cases, followed by fibroblasts for confirmation. The Pompe Registry is sponsored by Genzyme Corporation with oversight provided by an independent committee of disease experts.

Strong Evidence for a Prostate Cancer Susceptibility Gene at Chromosome 17q22. *E. Lange[1], C. Robbins[2], E. Gillanders[3], S. Zheng[4], J. Xu[4], Y. Wang[1], K. White[5], B. Chang[4], L. Ho[1], J. Trent[2], J. Carpten[2], W. Isaacs[6], K. Cooney[5]*
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Prostate cancer linkage studies have previously suggested the existence of a prostate cancer susceptibility gene on chromosome 17q21-22. Herein, we report multipoint nonparametric linkage results from 453 prostate cancer pedigrees from the University of Michigan Prostate Cancer Genetics Project and Johns Hopkins University using 24 chromosome 17 microsatellite markers. This study includes 95 new multiplex prostate cancer families and 9 additional microsatellite markers not previously reported. The maximum LOD score for all 453 pedigrees was found to be 2.99 at approximately 81 cM. The evidence for linkage at 81cM was similar in the 34 African-American (LOD = 1.90) and 415 European-American (LOD = 1.86) families. The European-American pedigrees had a second peak (absent in the African-Americans) at 53 cM (LOD = 2.75). An approach that has been widely used in linkage studies of complex traits is to focus on subsets of pedigrees that are more likely to segregate highly penetrant susceptibility alleles. Thus restricting the analysis to the subset of 147 families that had four or more prostate cancer cases and that had an average age of prostate cancer diagnosis < 65 years resulted in a maximum LOD score of 5.49 at 78 cM, including a LOD of 1.70 in the 27 new families belonging to this subset, with a corresponding 1-LOD support interval of 10 cM. These results represent one of the strongest reported linkage findings for prostate cancer to date and lead to a substantial reduction of the 17q21-22 candidate region. This large set of pedigrees with four more prostate cancer cases characterized by early-onset disease will serve as a useful resource for identifying the 17q21-22 prostate cancer susceptibility gene.

Utilization of whole genome SNP panels for efficient genetic mapping in the mouse. *J.L. Moran[1], A. Bolton[1], J. Moore[2], D. Mirel[2], S. Gabriel[2], D.R. Beier[1]* 1) Genetics Div., Brigham and Women's Hospital/Harvard Medical School, Boston, MA; 2) Center for Genotyping and Analysis, The Broad Institute of MIT and Harvard, Cambridge, MA.

We have previously described a whole genome panel of 394 mouse SNPs that we used to successfully map 44 loci, including ENU-induced and spontaneous mutations, modifier loci, quantitative trait loci and loci that demonstrate loss-of-heterozygosity (Moran et al., Genome Research 2006). The average number of affected mice genotyped for 22 monogenic mutations mapped using a wide variety of strain combinations was 8, and map locations have been obtained by genotyping as few as 4 affected mice. The average recombinant interval was 43 Mb. Thus, for monogenic mutations the 394 SNP panel proved useful for moderate resolution genetic localization with small numbers of mice in a high-throughput manner. The major utility of a whole genome SNP panel is that complete genome haplotype characterization is obtained in a single analysis. This facilitates the efficient discrimination between true and false positive association, as well as the discovery of unexpected modifier effects. We have now developed a new whole genome panel of 768 SNPs that is analyzed using the Illumina genotyping platform. SNP density averages 3 Mb across autosomes and 7 Mb across Chr. X. The panel was designed to maximize the number of markers that would be informative for crosses made using C57BL/6J, such that the average number of informative SNPs between C57BL/6J and common inbred strains is 550. However, to insure that the panel is suitable for many strain combinations, a haplotype binning strategy was used to maintain informativeness across the genome between other strains. The new panel and analysis protocol allows for genetic mapping at higher resolution and higher throughput. Initial results demonstrate this panel is extremely robust with respect to both informativeness and allele discrimination, and we have successfully mapped several loci that were not detected using our 394-marker panel. Cost-effective, high through-put, whole genome SNP analysis should have wide applicability for genetic investigation in model systems.

Power considerations for the Affymetrix 100K and Illumina 300K platforms. *L. Ho, F. Zou, H. Huang, Y. Wang, F. Wright, E. Lange* University of North Carolina.

Genome-wide association (GWA) analyses using commercially available highly-multiplexed genotype arrays are becoming increasingly popular for genetic association studies of complex traits. One of the first and most important steps in planning a GWA study is the accurate estimation of the power under a proposed study design and sample size. A critical element of these calculations is the specification of the significance threshold. Typically, this threshold is chosen to ensure a desired overall family-wise error rate (FWER) after accounting for uninformative markers and correlated tests between markers in linkage disequilibrium. We have performed a simulation study, based on bootstrap resampling, to empirically estimate the appropriate significance threshold when analyzing genotype data from the Affymetrix GeneChip Mapping 100K Assay and the Illumina HumanMap300 BeadChip platforms to ensure an overall FWER of 0.05. Specifically, we have performed 5000 simulations where we have randomly sampled from 120 phased autosomal haploid genomes containing the SNPs in the Affymetrix and Illumina platforms, using the HapMap Phase I/II CEU data, under the null hypothesis of no association and determined the significance threshold to achieve a desired FWER. These simulations were performed for both 1- and 2-stage genotyping designs, samples based on 1000 cases/controls and 2500 cases/controls and when analyzing all markers versus restricting the analyses to markers with a minor allele frequency >0.10. For each simulation condition, we estimated the ratio of the Bonferroni threshold p-value over the threshold p-value value obtained via the simulation. These ratios varied considerably over the different scenarios. Using a 1-stage design, the ratios ranged from 0.53 for the Affy 100K panel, 1,000 cases/controls, and no SNP removal to 0.90 for the Illumina 300K panel, 2,500 cases/controls, with removal of SNPs with minor allele frequency <0.10. Some 2-stage designs had a 10% further ratio reduction. Simulations performed under alternative models suggest that the significance threshold can impact the estimated power substantially when the underlying effect size is modest.

GENZ-112638: A novel orally available ceramide-based inhibitor of glucosylceramide synthase for treating Gaucher disease. *K. McEachern[1], C. Siegel[1], J. Fung[1], S. Komarnitsky[1], W.L. Chuang[1], J. Johnson[1], E. Hutto[1], K. Zhang[1], C. Sung[1], L. Blankstein[1], M.J. Peterschmitt[1], G.A. Grabowski[2], J. Marshall[1], D.P. Copeland[1], S.H. Cheng[1]* 1) Genzyme Corp., Framingham, MA; 2) Cincinnati Children's Hospital Research Foundation, Cincinnati, OH.

Inhibitors of glucosylceramide synthase (GCS) are being evaluated for substrate inhibition therapy of Gaucher disease. We have developed a novel ceramide-based inhibitor of GCS (GENZ-112638), and assessed its activity and safety in an animal model of Gaucher disease as well as in healthy volunteers in Phase I clinical trials. In vitro characterization of GENZ-112638 showed significantly increased potency and specificity in comparison to sugar-based inhibitors of GCS. GENZ-112638 demonstrated up to 3500-fold greater potency (IC50) for inhibition of glucosylceramide (GL-1) synthesis and specific inhibition of GCS. Using a Gaucher mouse model (D409V/null), we demonstrated that treatment with GENZ-112638 was efficacious at well-tolerated oral doses in young mice prior to substrate accumulation and in older mice with established disease. In young mice treated with GENZ-112638 for 10 weeks, Gaucher cell formation was greatly reduced, and GL-1 accumulation significantly inhibited in the spleen (25%), lung (60%), and liver (45%). In older mice with established disease, treatment with GENZ-112638 resulted in a quantitative reduction of Gaucher cells in spleen, lung, and liver (~80%) and reduced tissue levels of GL-1 by 40-60%. In a clinical Phase Ib trial in normal volunteers, exposure levels at and above the in vitro IC50, which are expected to be in the efficacious range, were achieved with doses of 50 and 200mg BID and were well-tolerated. Plasma GL-1 reductions were observed after 4-10 days of dosing. These pre-clinical and Phase I data suggest that GENZ-112638 may be a promising orally available inhibitor of GCS for the treatment of Gaucher disease. A Phase II study is underway to investigate the pharmacokinetics, long-term safety, and efficacy of GENZ-112638 in type 1 Gaucher patients.

The genetics of Hermansky-Pudlak Syndrome. *R. Hess, A. Helip-Wooley, R. Kleta, M. Huizing, W.A. Gahl* MGB, NHGRI, NIH, Bethesda, MD.

Hermansky-Pudlak syndrome (HPS) comprises a group of autosomal recessive disorders of lysosome-related organelle (LRO) biogenesis. Eight HPS-causing genes (HPS1-HPS8) have been reported in humans and are associated with eight clinical subtypes. Oculocutaneous albinism, due to abnormal melanosome formation, and prolonged bleeding, due to the absence of platelet dense bodies, define all HPS subtypes. We have studied 168 HPS patients at the NIH Clinical Center. These studies have provided insights into genotype-phenotype characteristics. An accurate diagnosis of each subtype has important prognostic and therapeutic implications and also provides insights into the cell biology of LROs. HPS-1 and HPS-4 patients are at increased risk for developing pulmonary fibrosis and granulomatous colitis. A diagnosis of HPS-1 or HPS-4 also allows us to enter patients into our clinical trial of pirfenidone, an antifibrotic agent previously shown to slow the decline in pulmonary function in HPS-1 patients. HPS-1 (110 patients, 13 mutations) comprises the largest group due to a founder mutation in NW Puerto Rico. We also identified 10 HPS-4 patients (8 mutations). HPS-2 results from mutations in AP3B1, a gene encoding the beta3A subunit of adaptor complex-3, a coat protein that mediates vesicle formation. We identified 3 HPS-2 patients, harboring 4 different mutations. Our study of HPS-3 (20 patients, 10 mutations), HPS-5 (5 patients, 7 mutations), and HPS-6 (3 patients, 5 mutations) concluded that these subtypes are clinically milder, with no apparent pulmonary involvement. HPS3 has founder mutations in Central Puerto Rico and in Ashkenazi Jews, along with other scattered mutations. We have not identified any HPS-7 or HPS-8 patients and only one patient/family of each subtype has been reported. Our 17 unclassified HPS patients provide opportunities to identify new HPS-causing genes. Several genes, some corresponding to HPS mouse models that manifest both hypopigmentation and a platelet storage pool deficiency, are good candidates. Any new genetic causes of HPS will aid in elucidating the mechanism by which melanosomes, dense bodies, and lysosomes are created.

Preimplantation Genetic Diagnosis (PGD) for numerical and structural chromosome abnormalities: a laboratory experience. *R. Habibian[1], A. Hajianpour[1], S.Y. Kou[1], Q.Q. Huang[1], L. Drugan[1], L. Dong[1], D. Drugan[2]* 1) Genzyme Genetics, Monrovia, CA; 2) Occidental College, LA, Ca.

Genzyme Genetics has been one of the leading centers to offer PGD by fluorescence in situ hybridization (FISH) for chromosomal aneuploidy/imbalances since 2002 (then Alfigen). A retrospective PGD data analysis was performed herein on 8001 blastomeres from 800 consecutive patients. FISH was performed using Vysis probe mixtures including: X & Y, MultiVysion PGT (X/Y/13/18/21), MultiVysion Custom 5A (8/9/15/16/22), and subtelomere probes for the detection of chromosomal rearrangements. In one case, the STS probe for the detection of X-linked ichtyosis was used. Table 1 shows the number of patients/blastomeres tested for different probe mixtures according to indications.

	PGT+5A-prob	PGT-probe	Gender det.	Transloc.	STS	Total
Patients	257 (32.1%)	425 (53.1%)	100 (12.5%)	17* (2.1%)	1 (0.1%)	800
Blastomeres	2398 (30%)	4417(55.2%)	1009(12.6%)	166 (2.1%)	11 (0.1%)	8001

* One patient with 22 blastomeres was tested for translocation and 5-probe panel. The average turn around time was 0.93 days. The mean patient age was 37 (range 20.8-53). The average number of blastomeres tested was 10 per patient (range 1-41). Table 2 shows the PGD FISH results on 747 of 800 patients with known age. The abnormality rates were 50.5%, 54%, and 59% for the age groups <35, 35-39, and 39, respectively. Of 8001 blastomeres tested overall, 2793 (34.9%) were normal, 4313 (53.9%) were abnormal, 286 (3.6%) showed no signals, 162 (2%) had inconclusive results, and in 448 (5.6%) no nuclei were found. Of 800 patients tested, 115(14.4%) with mean age of 38.5 years showed no normal blastomeres. The average number of blastomeres tested in this group was 6.1 (range 1-17). More data, detailed description of data, and follow up information will be presented.

Paternal uniparental disomy (UPD) of chromosome 1 results in both Gaucher disease type 3 and hereditary sensory motor neuropathy. *K.S. Hruska[1,3], W.S. Benko[2,3], M.J. Eblan[1], O. Goker-Alpan[1], P.S. Hart[1], R. Schiffmann[2], E. Sidransky[1]* 1) Medical Genetics Branch, NHGRI/NIH, Bethesda, MD; 2) Developmental and Metabolic Neurology Branch, NINDS/NIH, Bethesda, MD; 3) These authors contributed equally to this work.

We report the first identified case of Gaucher disease (GD) caused by uniparental isodisomy. A 3½-year-old child presented with congenital sensorineural deafness, persistent eczema, tonic pupils, generalized hypotonia and lower extremity areflexia. This child had been diagnosed with GD type 3 at 22 months with hepatosplenomegaly, nystagmus, and Gaucher cells on bone marrow biopsy. Molecular diagnosis at that time revealed homozygosity for the c.1448T>C (L444P) allele of glucocerebrosidase (GBA), the enzyme deficient in GD. The family history was negative for consanguinity, but was remarkable for demyelinating Charcot-Marie-Tooth (CMT) disease in the probands father, paternal grandfather and great-grandfather. This history, coupled with the hearing loss and pupillary abnormalities, led to evaluation of the myelin protein zero (MPZ) gene, which causes CMT2J, as well as CMT1B and other polyneuropathies. Both GBA and MPZ are located on chromosome 1q21-22. Mutational analysis of the proband demonstrated homozygosity for the c.263C>T (S78L) allele of MPZ and confirmed homozygosity for the GBA mutation. The probands father was found to be heterozygous for mutations in both genes, while neither was identified in the mother. Further genotyping of the parent:child trio for five microsatellite markers spanning chromosome 1 showed no maternal contribution for three, while two were inconclusive. The proband was homozygous at all marker loci. Heterozygosity for the MPZ S78L allele is associated with a late-onset, mild form of CMT, so this early atypical presentation may reflect a gene dosage effect or be attributable to the influence of homozygosity for other loci on chromosome 1. While presumably rare, this case indicates that UPD should be considered in GD when the proband is homozygous for a disease allele in a non-consanguineous family and underscores the importance of obtaining parental genotypes whenever possible.

MOSAIC STRUCTURAL REARRANGEMENTS AND THE CHALLENGES THEY POSE TO CYTOGENETIC LABORATORY. *K. Mac, A. Zang, J. Cary, M. Samy, L. Drugan, R. Habibian, A. Hajianpour*
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Mosaic structural rearrangements can be easily overlooked at the microscope, often detected only when extra effort and closer scrutiny are applied. We present three cases with mosaic structural rearrangements found at later stages of analysis. Case 1: Blood chromosome analysis was performed on a newborn girl for unspecified congenital abnormalities. Two cell lines were detected, one normal, and a second that was only detected when questioning an overlapped chromosome 2 in one of the karyotypes. Examining of additional cells revealed an interstitial deletion in the short arm of one chromosome 2 in 12 of 23 cells examined, resulting in a mosaic karyotype: 46,XX,del(2)(p21p22)de novo[12]/46,XX[11]. Parental chromosomes were normal. Case 2: Blood chromosome analysis indicated for family history of undefined chromosome abnormality was performed on a 44 year old female and revealed two cell lines, one normal, and a second that was first seen while analyzing the twentieth cell. It showed a paracentric inversion in one chromosome 13. Reexamination of the slides revealed three additional cells with the same inversion, and the karyotype was reported as 46,XX,inv(13)(p12q22.1)[4]/46,XX[19]. Inverted and recombinant chromosome 13s had been reported in this individuals daughter and grandchild, respectively. Case 3: Chromosome analysis, indicated for a history of missed abortions was performed on a POC specimen from a 40 year old female. Two cell lines were discovered. Five cells showed an apparent deletion in the short arm of one chromosome 5, and 15 cells were normal. The karyotype was reported as: 46,XY,del(5)(p14)[5]/46,XY[15]. Parental chromosome analysis was requested, but since the karyotype in this male fetus is mosaic, it is unlikely that a balanced rearrangement will be found in one of the parents. These cases each show how mosaic structural rearrangements challenge the technologists to avoid dismissing single abnormal cells as artifacts, and encourage them to check additional cells while heightening their awareness of the significance of family history.

The Role of MMP2 and COL1A1 Genes in High Myopia in Young Taiwanese Men. *H.Y. Hsieh[1], W.S. Zhang[1], K.S. Hung[2], H.S. Wang[2], C.L. Liang[2,3], S.H. Juo[1]* 1) Kaohsiung Medical University, Kaohsiung, Taiwan; 2) Chang Gung University, Taiwan; 3) Bright-Eye Clinic, Kaohsiung, Taiwan.

Purpose: Sclera contains approximately 90% collagen and the majority is type I collagen. The matrix metalloproteinase-2 (MMP2) is responsible for degradation of sclera extracellular matrix, which may contribute to myopia. This study was aimed to investigate the relationship between high myopia and polymorphisms of MMP2 and type I collagen, alpha 1 (COL1A1) genes.

Design: A case-control study

Methods: The study subjects were recruited from military conscripts in Taiwan. A case was defined as refraction less than -6 D as well as control greater than -1.5 D. A total of 382 cases and 358 controls were genotyped for the MMP2 gene. Besides, there are 307 cases and 678 controls were genotyped for the COL1A1 gene. Ten tagging single nucleotide polymorphisms (tSNPs) at COL1A1 gene, and 17 SNPs at MMP2 gene were genotyped.Statistical analyses included Hardy-Weinberg equilibrium (HWE) test, chi-squared test to evaluate the genotypic effect.

Results: All SNPs are in HWE. Three SNPs at MMP2 had significance ($p<0.05$). Among them, a functional promoter SNP is particularly interesting. Using more stringent cutoff points to re-define high myopia, we found this SNP remains significant. The most significant result was the CT genotype of the promoter SNP had an OR of 0.53 ($p=0.006$) compared with the reference CC genotype. The risk C allele has a higher prevalence in Chinese (~90%) than in Caucasians (~70%). The results for the other two SNPs were not consistent across different cutoff points to re-define high myopia. For the COL1A1 gene, all 10 tSNPs did not yield promising results.

Conclusions: This is the first human study to comprehensively study the MMP2 and COL1A1 genes for high myopia. Our study indicated that the functional promoter polymorphism of MMP2 may play a role for the status of high myopia in human subjects. This promoter SNP may partially account for a higher susceptibility to myopia in Chinese.

SOS1 mutation alteres cell cycle progression in hereditary gingival fibromatosis. *S.I. Jang[1], E.J. Lee[1], P.S. Hart[2], M. Ramasawmi[1], D. Pallos[3], T.C. Hart[1]* 1) Section of Human and Craniofacial Genetics, National Institute of Dental and Craniofacial Research, NIH, Bethesda, MD; 2) Office of the Clinical Director, NHGRI, National Institutes of Health, Bethesda, MD 20892; 3) Periodontics Research and Graduate Studies Division, Department of Dentistry, University of Taubate, Sao Paulo, Brazil.

Hereditary gingival fibromatosis (HGF) type 1 is a rare autosomal dominant form of gingival overgrowth with some cases due to mutation of the son of sevenless-1 (SOS1). A single base insertion results in a frame shift that leads to premature termination and abolishes multiple SH3 binding domains. Cell proliferation studies show that fibroblasts of HGF1 have higher proliferation rates compared to normal control fibroblasts and the presence of mutant SOS1 leads to constitutive activation of ERK signaling which showed higher magnitude and longer duration in HGF1 fibroblasts. To further understand how the sustained ERK signaling affects the expression of cell cycle regulators, total RNA were obtained from cultured HGF1 fibroblasts and controls and cRNA probes were synthesized. Using focus oligoarray membranes containing more than 100 key genes involved in cell cycle regulation, the expression profiles of transcripts were analyzed and compared between normal control and HGF1. The results reveal that a significant increase on several cell cycle regulators including cyclin C (7-fold) which regulating S phase transition; cyclin D (4-fold) which regulating early stage of G1 phase; cyclin E1 (4-fold) and E2 (5-fold) which only elevates markedly in tumor-derived cells and functions in late G1 and early S phase. Also, while there is a modest increase of E2F1, E2F2 transcription factors, the expression of DP1 and DP2 show 6- and 11-fold increase, respectively. In addition, Rb and retinoblastoma binding protein 8 (RBBP8) show about 4- and 15-fold increase in HGF1. Taken together, these data support that mutant SOS1 of HGF1 fibroblasts causes continuous activation of MAP kinase signaling pathway which then results in up-regulation of cell cycle regulators that play a major role in cell cycle progression from G1 phase to S phase.

A sequence variation of the Myelin Oligodendrocyte Glycoprotein (MOG) gene in the HLA-Class I region is involved in susceptibility to Multiple Sclerosis (MS). *P. Momigliano-Richiardi[1], N. Barizzone[1], F. Guerini[2], P. Naldi[3], S. Calzoni[3], M. Trojano[4], S. D'Alfonso[1]* 1) Dept. Medical Sciences and IRCAD, Eastern Piedmont University, Novara, Italy; 2) Laboratorio di Biologia, Fondazione Don Gnocchi, IRCCS , Milano; 3) Clinica Neurologica, Ospedale Maggiore della Carità, Novara; 4) Dipartimento di Scienze Neurologiche e Psichiatriche, Bari.

Several studies suggest that the HLA class I region harbours genes modulating MS susceptibility independently from the effect of class II alleles. A candidate gene in this region is MOG, encoding the Myelin Oligodendrocyte Glycoprotein. A significant association with the missense variation V142L, in the transmembrane region, was previously reported in a small sample of 50 Italian MS patients. We confirmed this result both by a case-control test (L142 allele frequency in 617 Italian MS patients vs 573 matched controls: 0.15 vs 0.20, p=0.003, OR: 0.73) and TDT in 255 trio families (44T : 79NT L142 alleles, p=0.002). Combining allele frequencies from the two sample sets L142 had an OR = 0.71 (p=0.0003). This result was independent of DR15. We further tested by TDT in 199 trio families the involvement of additional MOG polymorphisms, namely 7 previously described SNPs in the 5 flanking (positions -1077, -910, 875, -93) and coding region (S5S, L22del, V145I), 4 newly detected SNPs in the 3UTR, and 2 microsatellites in the 5 flanking and 3UTR, respectively. None of these alleles or their haplotypic combinations showed a significant transmission distortion, in the absence of the V142L marker. To test whether the association observed for V142L extended outside the MOG gene, we analysed in the same 199 trio families the association with MS of 4 microsatellites (MIB, D6S265, D6S1683 and D6S2239) localized in the HLA-class I region from HLA-B to HFE. None of the microsatellite alleles showed a significant transmission distortion indicating that there is no further detectable effect on MS susceptibility. These data suggest the presence of a second MS susceptibility factor in the Class I region, independent from the effect of class II alleles. It is located in the MOG gene and it likely corresponds to the V142L variation.

Analysis of *IC140*: An IDA intermediate chain dynein as a candidate gene for Primary Ciliary Dyskinesia (PCD).

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Primary ciliary dyskinesia (PCD) is caused by abnormalities in the structure and function of cilia and flagella of sperm. It is inherited as an autosomal recessive trait and characterized by oto-sino-pulmonary disease. Half the patients have situs inversus (Kartagener syndrome). It is a genetically heterogeneous disorder which complicates identification of disease-causing mutations. The ultrastructural defects identified in ~90% of PCD patients involves either the outer dynein arm (ODA), inner dynein arms (IDA) or both. Thus far, disease-causing mutations have been identified in *DNAI1* and *DNAH5* (both encoding ODA proteins) and account for 38% of PCD patients; however, no disease-causing mutations have been detected in IDA candidate genes. The flagella of biflagellate protozoa, *Chlamydomonas*, shares a high degree of structural homology with human cilia; therefore, gene mutations identified in motility defective mutant *Chlamydomonas* are likely candidates for PCD. We have identified the human orthologue of *IC140* gene from the human genome database designated as NYD-SP29. *IC140* is an intermediate chain of IDA which is paralogue of *DNAI1*of ODA. We carried out mutation analysis by sequencing all of the 22 coding exons and intron/exon junctions of human *IC140* in 126 unrelated well-characterized PCD patients (n=63 had ODA defect and n=51 had isolated IDA defects). Almost 70% of the sequence analysis is completed thus far, but no disease-causing mutations have been identified. Our results currently suggest that *IC140* is not frequently mutated in human PCD. This abstract was funded by UNC/URC, GCRC#00046, MO1 RR00046-42, 1 RO1 HL071798, 5 U54 RR019480.

Whole genome association study of chronic inflammatory arthritis. *S. John[1], A. Hinks[1], N. Shephard[1], M. Cargill[2], Y. Turpaz[2], E. Wang[2], A. Barton[1], S. Eyre[1], W. Thomson[1], J. Worthington[1], G. Kennedy[2]* 1) ARC Epidemiology Unit, Univ Manchester, Manchester, United Kingdom; 2) Affymetrix, Santa Clara, USA.

Rheumatoid arthritis (RA) and juvenile idiopathic arthritis (JIA) are both chronic inflammatory conditions with an autoimmune pathology. In common with other autoimmune diseases, both RA and JIA are heritable and are associated with genes in the MHC. The recent association of the PTPN22 R620W SNP with several autoimmune diseases confirms the hypothesis that there is an underlying common genetic basis in autoimmunity. Aim: To identify novel inflammatory arthritis susceptibility loci. Methods: 184 healthy adult controls and 449 cases with a chronic autoimmune arthritis and an age at onset <40 years were available for study. Of the cases 168 had a clinical diagnosis of RA and 281 a diagnosis of JIA. Genotyping was performed using the Affymetrix GeneChip mapping 100K array. The Armitage test for trend was employed to test for association with disease and the false discovery rate (FDR) q-value method was applied to account for the multiple comparisons performed. Results: 82,500 SNPs with a MAF>5%, a HWE p>0.0001 in controls and a >80% call rate were analysed for evidence of association to disease. Applying a FDR of 5%, the q-value method generated 118 SNPs that remained significantly associated after correction for multiple testing. An additional 65 SNPs were selected after applying more stringent quality control; p<0.001 and with a call rate >95% in both cases and controls and 32 SNPs that mapped to peaks of linkage. These SNPs (n=215), which included two within the HLA region and four within the PTPN22 gene region, were genotyped in a second cohort of 553 (405 RA and 128 JIA) cases and 426 controls. To date eleven SNPs have evidence of association with inflammatory arthritis (p<0.05) in both cohorts and will be investigated further. Discussion: Detection of known disease susceptibility loci (HLA and PTPN22) in this study is a proof of principle that whole genome SNP association studies have the potential to identify novel arthritis genes.

Extending Mendelian Mutation Prediction Models to Account for Medical Interventions: Application to Incorporating Oophorectomy into BRCAPRO. *H.A. Katki* Divison of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, MD.

Mendelian models for predicting who may carry an inherited deleterious mutation of known disease genes based on family history are used in a variety of clinical and research activities. Family members who carry the mutation or are otherwise at high risk for disease may undergo medical interventions to reduce their disease risk. For example, oophorectomy, the removal of the ovaries, is a drastic intervention that is being increasingly offered to women at high risk of breast or ovarian cancer, and so oophorectomy is increasingly being reported in family histories of breast and ovarian cancer. Mendelian models should account for medical interventions because interventions modify the mutation penetrances and thus also the carrier probability estimate. In this work, we extend Mendelian models to account for medical interventions. We show that ignoring medical interventions undergone by a family member can seriously affect the mutation carrier probability estimate, especially when the family member has lived many years post-intervention. Incorporating medical interventions into Mendelian models requires only an extra factor accounting for post-intervention disease history. This post-intervention factor can be estimated from published studies of the effects of interventions. We apply our results to the BRCAPRO model that predicts a woman's risk of carrying mutations in *BRCA1* and *BRCA2* based on her family history of breast and ovarian cancer. We apply this methodology to incorporate oophorectomy into BRCAPRO, and this extension is available for clinical use at: http://www.cancerbiostats.onc.jhmi.edu/BayesMendel/.

A Case Report of Perinatal Lethal Gaucher Disease. *J. Oliver[1], S. Bhatt[1], M. Bocian[2], N. Narula[2], J. Bonadio[2]* 1) Genzyme Genetics, Orange, CA; 2) University of California, Irvine Medical Center, Orange, California.

We present a case of a 25-year-old G2P1 woman of Palestinian ancestry, referred at 19 weeks gestation for a positive triple marker screen with a Down syndrome risk of 1/80 and Smith Lemli Opitz syndrome risk of 1/16. The patient and her husband are consanguineous and this pregnancy was achieved using a frozen embryo transfer from eggs harvested at age 21. Fetal ultrasound was abnormal with increased nuchal thickness of 8 mm, abdominal ascites, absent stomach bubble, and bilateral club feet. Fetal karyotype was normal, 46,XY; AF-AFP and 7-dehydrocholesterol were also normal. Worsening of ultrasound findings led the patient to elect pregnancy termination. The most significant finding on fetal autopsy was the identification of typical Gaucher cells in brain, spleen, liver, thymus, lymph nodes, and placenta. Enzyme analysis on cultured cells revealed absent beta-glucosidase activity, confirming the diagnosis of perinatal lethal Gaucher disease. Gene sequencing revealed that the fetus appears to be homozygous for the rare C342Y mutation; although DNA testing of the parents will be required before the possibility of compound heterozygosity for a large deletion and the C342Y mutation can be excluded. Family history was significant for four female family members with significant developmental problems and decreased life span. Their neonatal course was characterized by low or high blood sugar, seizures, and brain damage. Three who survived the neonatal period had severe developmental delay and remained curled up in a ball; two died at ages 13 and 19, respectively, while one is reportedly still alive. It is possible that some of these individuals may have been compound heterozygotes resulting in a subacute course with death before age 20. This case shows the importance of offering the fetal autopsy to arrive at a diagnosis, especially in the presence of unexplained family histories and non-specific ultrasound abnormalities. The diagnosis of perinatal lethal Gaucher in this pregnancy, confirmed by molecular methods, will allow for prenatal diagnosis in this couples future pregnancies and identification of other at-risk family members.

A Complex Chromosomal Rearrangement of Chromosome 14- a prenatal case. *C. Lee, A. Hajianpour, S.Y. Kou, P.C. Ng, R. Habibian* Genzyme Genetics, Monrovia, CA.

We present a prenatal case of stable non-Robertsonian dic(14;15) found in all metaphase cells examined from an amniotic fluid specimen on a 35 year old G3, P2 female at 16.1 weeks gestation due to omphalocele finding. Cytogenetic analysis revealed trisomy 18 as well as an abnormal chromosome 14 with a classic acrocentric short arm, an unusual banding pattern below the centromere, and additional chromatin material on the long arm. FISH using whole chromosome paint 14 probe revealed an unexpected result. Hybridization of the probe was not observed either on the short arm nor on the terminal long arm of the abnormal chromosome 14, might suggesting that another acrocentric chromosome be involved. Additional FISH performed utilizing probes specific for the centromeres of acrocentric chromosomes, subtelomere 14q and Prader-Willi/Angelman syndrome chromosome region (SNRPN). It showed that the primary constriction and the short arm initially assumed to be of chromosome 14 is of chromosome 15 origin with breakpoint at 15q11.2 and lacking SNRPN region. The telomere 14q was juxtaposed to 15q11.2. The 14/22 probe hybridized to the opposite end (non-constricted end) of the abnormal chromosome 14, representing an inactive centromere 14. The abnormal chromosome 14 was subsequently re-interpreted as a pseudodicentric chromosome comprised of chromosome 14 and 15 material with breaks and reunion at bands 14q32.3 and 15q11.2. The resulting net imbalance in addition to trisomy 18 is partial trisomy 15pter-15q11.2. The karyotype was determined as follows: 47,XY,der(14),+18.ish psu dic(15;14)(q11.2;q32.3)(wcp14-,D15Z4+,SNRPN-;wcp14+,14qter+,D14Z1/D22Z1+). The pregnancy was terminated due to the presence of trisomy 18. Parental chromosome studies are pending. The presence of three active centromeres 15 and an inactive centromere 14 is most likely clinically insignificant as they are reported in phenotypically normal individuals with a gain of marker chromosome 15 or Robertsonian translocation involving chromosome 14. This case may represent a rare trisomy rescue mechanism. However, re-examination of the slides did not reveal the presence of a trisomy 15 cell line.

PRELIMINARY RESULTS OF AN OPHTHALMOLOGICAL EVALUATION AND MOLECULAR STUDIES IN A DEAF COLOMBIAN POPULATION. *M. Olarte[1, 2], N. Gelvez[1], G. Mariluz[1], S. Florez[2], D. Medina[2], M. Tamayo[1, 2]* 1) Inst de Genetica Humana, Univ Javeriana, Bogota , Colombia; 2) Fundación Oftalmológica Nacional, Bogotá, Colombia.

We performed a preliminary study for non-syndromic deafness in Bogotá, Colombia. The Connexin 26 gene was sequencing in a total of 112 individuals with non-syndromic deafness. The inclusion criterias for the selection were: individuals with non-syndromic deafness with familiar history of deafness and sporadic cases with unknown cause. The first step of the selection of our population was the ophthalmological examination as important criteria. From 276 deaf individuals, the 74.3% (205/276) showed normal fundoscopy, while in the 19.9% (55/276) we found salt and pepper retinopathy and the remaining 5.8% (16/276) presented other ocular alterations. A complete medical history, prenatal antecedents and clinical examination were performed in the 205 selected deaf individuals. All the confirmed acquired deafness were excluded. In total, 112 deaf individuals were studied, which were classified in two groups: the 59.9% (67/112) as recessive nonsyndromic hearing loss (RNSHL) and, the remaining 40.1% (45/112) as sporadic cases without known cause. We identified three mutations and two polymorphisms in Cx26 gene: mutations 35delG, S199F and 167delT reported in the literature and, the polymorphisms V27I and M34T. Among the total of 224 alleles in the studied population, S199F was the most frequent (17.9%), follow by 35delG (17.0%) and 167delT in 0.4%. We identified some mutation in the GJB2 gene in 50.7% of the group with genetic etiology, and in the 33.3% of the sporadic cases with unknown cause. The frequency of S199F mutation in our nonsyndromic deaf population is higher than other reported studies, while the frequency of the 35delG mutation was similar to reported in Caucasian population. This frequency reported in Bogotá is a preliminary data in Colombia.

False evidence for imprinting using linkage data. *M.C. Monti[1,2], J. Zhang[1], L.J. Strug[1], B. Feenstra[1,3], D.A. Greenberg[1]* 1) Div Stat Genet, Dept Biostat, Columbia Univ, NY, NY; 2) Dept. Applied Health Sci, Pavia Univ, Italy; 3) Royal Vet & Agricult Univ, Dept Natural Sci, Denmark.

We previously studied two methods to detect imprinting: lod score maximization with respect to parent-of-origin-based penetrances (PP) and lod score maximization with respect to independent male-female recombination fractions (RF). We found that the PP method had more power to detect imprinting than the RF method; however, under sex-specific penetrance, the PP method could falsely indicate imprinting. To better understand when either method provides false evidence for imprinting, we studied the distribution of the likelihood ratio test statistic under the null hypothesis, for the PP and RF method. We simulated nuclear family and extended pedigree data with and without imprinting, which we defined as the parental-origin-dependent penetrance in offspring. We maximized two-point lod scores over parent-of-origin penetrances (PP) and, in separate experiments, over combinations of male-female recombination fraction (RF). In both cases, we examined the power to detect, and the probability of falsely detecting, imprinting. We tested a range of data set sizes as well as determined the effects of reduced penetrance, heterogeneity, and ascertainment criteria. When there is no imprinting, the type I error rate is strongly affected by locus heterogeneity and the ascertainment criteria. Without heterogeneity or reduced penetrance in the generating model, the likelihood ratio test was quite conservative for both PP and RF methods. However, the type 1 error rate exceeded the 5% level when genetic heterogeneity was present in the data, and worsened with increasing heterogeneity. Families selected for having a high density of affected members also resulted in an increased Type I error rate. Thus, ascertainment criteria and heterogeneity are important confounders in detecting imprinting, whether via the RF or PP method, in linkage analysis.

A loss of function mutation in the fibulin-4 gene causes a severe form of recessive cutis laxa. *V. Hucthagowder[1], N. Sausgruber[1], K.H. Kim[2], B. Angle[2], L.Y. Marmorstein[3], Z. Urban[1,4]* 1) Department of Pediatrics, Washington University School of Medicine, St.Louis, MO, USA; 2) Childrens Memorial Hospital, Chicago, IL, USA; 3) Department of Ophtalmology and Vision Science, University of Arizona, Tucson, AZ, USA; 4) Department of Genetics, Washington University School of Medicine, St.Louis, MO, USA.

Cutis laxa (CL) is a heterogeneous group of inherited or acquired disorders characterized by pendulous and redundant skin with lack of elasticity. We have begun the mutational evaluation of fibulins, a recently recognized family of extracellular matrix proteins, as candidate genes for CL. A collection of 65 unrelated CL patients were analyzed for mutations in the fibulin-4 gene by direct DNA sequencing of genomic amplimers. We identified a homozygous missence mutation (169G>A) E57K in a patient. Both of her parents were heterozygous, suggesting autosomal recessive inheritance. The patient had multiple fractures at birth and was diagnosed with vascular tortuosity, ascending aortic aneurysm, developmental emphysema, inguinal and diaphragmatic hernia, joint laxity and pectus excavatum by age 2 years. Histological evaluation of her skin revealed smaller than normal collagen bundles, increased vascularization of the upper dermis and severely underdeveloped elastic fibers. RT-PCR analysis showed comparable expression of fibulin-4 mRNA in skin fibroblasts from the patient to healthy controls and sequencing confirmed the presence of mutation E57K in the patient. Immunoblots showed normal abundance of fibulin-4 in patient cell lysates, but extracellular matrix extracts of patient fibroblast cultures showed greatly reduced fibulin-4 content. Pulse-chase immunoprecipitation studies further confirmed that E57K mutant fibulin-4 was not secreted and degraded inside the cell, suggesting that this mutation results in loss of fibulin-4 function. Our results demonstrate for the first time, that a homozygous mutation in fibulin-4 is associated with a new recessive cutis laxa syndrome characterized by severe systemic connective tissue abnormalities.

CARD8 is associated with Crohns disease in the Puerto Rico population. *L. Mei[1], E.A. Torres[2], Y. Picornell[1], X. Su[1], F. Gregory[2], R. Mera[2], P.J. Nieves[2], D. Dutridge[1], K. Taylor[1], H. Yang[1], J.I. Rotter[1]* 1) Medical Genetics, Cedars-Sinai Medical Center, Los Angeles, CA; 2) University of Puerto Rico, San Juan, Puerto Rico.

Background: Caspase-associated recruitment domains (CARD) are key modules in proteins that play important roles in the NFB signaling pathway, which is important in the activation of genes involved in immunity, inflammation, and apoptosis. CARD15/NOD2, the first susceptibility gene identified in Crohns Disease (CD), indicated the role of a disturbed innate immune response in CD. Recently a new member of CARD-containing family, CARD8 located in the IBD6 region (19p13), was reported in abstract to be associated with CD in adults and Ulcerative Colitis (UC) in children. Aim: To investigate the association between CD and CARD8 variant in the Puerto Rican (PR) population. Methods: 38 trio families with one affected offspring, 128 unrelated CD cases and 110 healthy controls were ascertained from Puerto Rico (PR). The SNP (23192A/T) at codon 10 which the variant codes for a stop codon in CARD8 was genotyped using the TaqMan MGB platform (ABI). The transmission disequilibrium test (TDT) was employed to test association with CD using Haploview 3.2. Multiple logistic regression was carried out to analyze the case-control sample. Results: There was significant distortion of transmission of the CARD8 A allele, the common allele, in CD parent-offspring trios (T: U=22:9, P=0.02). The A allele also had a higher frequency in cases than in controls (77% vs 69%, p=0.05). Multivariable analysis showed that the AA genotype was associated with increased likelihood of CD (AA vs. AT+TT: p=0.04, OR 1.8, 95% CI: 1.03-3.1) after controlling for age and gender. Conclusions: This is the first report of a CARD8 association with CD in a Hispanic population. Recent studies have suggested that CARD8, like other CARD family proteins, is involved in apoptosis and NFB activation. The data herein further underscore the existence of a genetic basis for alteration in the innate immune response pathway in the pathogenesis of CD.

Summary of clinical implementation of Chromosomal Microarray Analysis (CMA) in 2668 cases. *X.Y. Lu, J. Li, A. Patel, M.L. Cooper, W.R. Wells, C.M. Sullivan, T. Sahoo, S.A. Yatsenko, Z. Ou, P. Stankiewicz, J.R. Lupski, C. Chinault, A.L. Beaudet, S.W. Cheung, P.A. Ward* Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.

Array CGH is a powerful molecular cytogenetic technology that enables detection of genomic imbalances with higher resolution than current clinical methods. We report the outcome of array CGH studies, termed Chromosomal Microarray Analysis (CMA), performed clinically in 2668 postnatal patients referred with variable clinical phenotypes. The initial 930 samples were studied using CMA version 4.0 (V4.0, 362 BAC/PAC clone coverage) and the remaining 1738 samples used CMA version 5.0 (V5.0, 830 BAC/PAC clone coverage). Overall, CMA identified clinically relevant genomic imbalances in 215 patients (8.1%) [V4.0, 66/930 (7.1%) and V5.0, 149/1738 (8.6%)]. Abnormal CMA findings were seen in 95 of 1975 patients (4.8%) with normal karyotypes, and there was a 44.7% higher detection rate among samples studied on V5.0 versus V 4.0. Among 121 cases referred for additional investigation of a known cytogenetic rearrangement, CMA identified genomic imbalances in 72 cases (59%); the remaining 41% had normal results. For 572 cases with unavailable cytogenetic results, 48 (8.4%) had abnormal CMA results. Improved diagnostic potential of CMA is illustrated by 89 cases identified with cryptic microdeletions (57) and the predicted reciprocal microduplications (32) within 12 specific chromosomal regions associated with known genomic disorders; conventional cytogenetics failed to detect 75% of microdeletions and 91% of microduplications in a subset of 68 patients in this group. Overall, CMA identified copy number variations (CNVs) of uncertain significance in 276 probands, requiring parental studies. Of these, 192 (70%) were interpreted as familial variants and 11 (4%) were de novo; the remaining 73 (26%) await parental study to resolve clinical significance. Our experience using CMA in a large clinical cohort demonstrates the significantly improved sensitivity of this technology for diagnosis of genomic imbalances and highlights the need for comprehensive genetic counseling to facilitate accurate clinical correlation.

Minimal Phenotype Resulting from Mosaic Deletion of 11p12p14 in a Patient with Primary Dopamine beta-Hydroxylase Deficiency: Identification and Characterization by High-Resolution Array-CGH. *J. Li[1], M.L. Cooper[1], M.T. Geraghty[2], D.E. Mensing[1], K.D. Vonalt[1], Z. Ou[1], A.N. Pursley[1], A.C. Chinault[1], A. Patel[1], S.W. Cheung[1], T. Sahoo[1]* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Childrens Hospital of Eastern Ontario, ON, Ca.

Deletions involving the short arm of chromosome 11 have been well characterized in the context of WAGR syndrome (Wilms tumor, Aniridia, genitourinary abnormalities and mental retardation) involving 11p13, and the Potocki-Schaffer syndrome resulting from more proximal deletions at 11p11.2. Mosaic deletions of 11p13 in WAGR syndrome are extremely rare. Large deletions involving contiguous segments between 11p12 and 11p14, in a mosaic form, have not been reported earlier in the absence of WAGR phenotype. We report here the characterization of the cytogenetic abnormality and genotype-phenotype correlations in a 16 year-old female with primary dopamine beta-hydroxylase (DBH) deficiency, bilateral iris coloboma, short hands and short, high-arched feet and having normal intelligence and development. High-resolution array-CGH revealed a loss for a segment of at least 1 Mb across 11p13 that included the PAX6 and WT1 genes within the WAGR syndrome critical region. The array-CGH results were suggestive of mosaicism for the deletion. Analysis of G-banded chromosomes and fluorescence in situ hybridization revealed a deletion of the region between 11p12 and 11p14.3 in only 28% of cells analyzed. Utilizing a genome-wide oligonucleotide array, the deletion segment was estimated to encompass approximately 11 Mb including the 11p13 segment seen by array-CGH. Since the patient is known to be homozygous for two disease causing mutations in the DBH gene that maps to 9q34.2, the 11p12p14 deletion is unlikely influence her manifestations from DBH deficiency. It is possible that her bilateral iris colobomata might be a manifestation, albeit abbreviated, of the haploinsufficiency for PAX6. This unusual combination of genetic and cytogenetic abnormalities is probably coincidental and helps explain some components of her phenotype, and further characterization is in progress to elucidate a stronger genotype-phenotype correlation.*PAX6 WT1 DBH*.

Prevalence of somatic alterations in sporadic prostate cancer genomes. *N. Makridakis[1], T. Phipps[1], J. Reichardt[2]* 1) Biochem/Mol Biol/Inst Gen Med, Univ Southern California, Los Angeles, CA; 2) Medical Foundation Building (K25), University of Sydney, Sydney, NSW, Australia.

Little is known about the prevalence of somatic alterations in most human cancers. In order to assess global mutation rates in the prostate cancer genome, we analyzed 100 kb of total sequence using DNA from blood lymphocytes (constitutional), normal prostate and tumor prostate taken from 6 men with prostate cancer. Specifically, each genomic DNA was cut with AluI, subcloned, and then 50-100 colonies were sequenced from each sample. Comparison of the sequencing results with the nr-database in Genbank identified a total of 62 variations in the tumors, 32 in the normal prostates, and 31 in the blood DNAs, using approximately equal sequence lengths of 34 kb. This PCR-free method resulted in a significant over-representation of variants in the tumor compared with the normal prostate ($p= 0.002$). Extrapolation of this difference in variation rate between the normal and prostate tumor DNA suggests that there are 1-6 million somatic alterations per tumor cell genome in most (5 out of 6) patients. These results are in contrast to previously published results obtained in colorectal cancer by PCR-based methods. Further analysis demonstrated that 71% of the tumor variations occur in the sequence motif we previously identified (THEMIS motif; Makridakis et al., submitted), while only 39% of the normal prostate variations occur in this motif (with one mismatch allowed; $p= 0.009$), and 40% of the blood variations occur in the motif. Thus, many of the somatic alterations in the prostate tumor genome may have similar etiology. These results are consistent with the mutator phenotype model for prostate tumorigenesis and have implications for the cancer genome project.

Genotype-phenotype correlations at the MKS1 and MKS3 loci in Meckel syndrome and related phenotypes. *R. KHADDOUR[1], L. BAALA[1], U.M. SMITH[2], C. OZILOU[1], J. MARTINOVIC[1], S. AUDOLLENT[1], C. ESCULPAVIT[1], N. KADHOM[1], M. KYTALLA[3], A. MUNNICH[1], F. RAZAVI[1], E. GENIN[4], J. ROUME[5], M.C. GUBLER[6], M. VEKEMANS[1], C.A. JOHNSON[2], T. ATTIE-BITACH[1]* 1) Genetics and INSERM U781, Hopital Necker, Paris, France; 2) Medical and Molecular Genetics, University of Birmingham Medical School, Birmingham; 3) Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland; 4) INSERM U535, Hôpital Paul Brousse, Villejuif; 5) Génétique Médicale, CHI Poissy, Saint Germain en Laye; 6) INSERM U574, Hopital Necker, Paris.

Meckel syndrome (MKS) is a lethal autosomal recessive disease characterized by cystic kidneys, a brain malformation (usually occipital encephalocele), polydactyly, and hepatic developmental defects. Recently, two genes have been identified: *MKS1* on 17q in Finnish kindreds and *MKS3* on 8q in families from Pakistan and Oman, encoding ciliary proteins. We report on the sequence analysis of the *MKS1* and *MKS3* genes in a large multhiethnic cohort of 56 cases of MKS as defined by Salonen and 45 related phenotype or Meckel-like. *MKS1* recessive mutations were identified in 6 families with classical MKS phenotype. Most mutations abolished the B9 conserved domain of the protein. One case had a situs inversus. Two cases, from families of French origin, carried the Finnish major mutation allowing us to estimate its age to 4350 years. Seven fetuses carried MKS3 mutations or a large intragenic deletion. All missense mutations lied in the extracellular domain of the protein. Polydactyly was significantly less frequent when carrying *MKS3* (2/12) than *MKS1* mutations (8/10). All had bile duct proliferation of liver. In contrast to *MKS1*, brain phenotype varied from occipital encephalocele, isolated Dandy-Walker malformation to normal brain in one case. Two sibs presented vermis agenesis and small cysts in renal medulla, a phenotype not usually observed in MKS. Our results indicate that *MKS1* and *MKS3* genes are each responsible for at least 10 % of MKS cases with various mutations in different populations. A strong phenotype/genotype correlation was observed concerning the polydactyly, brain and kidney anomalies.

Mutational analysis of mitochondrial tRNALeu(UUR) gene in 740 Chinese subjects with type 2 diabetes. *J. Lu[1], D. Wang[1], WeL. Wei[1], J. Ji[1], W. Ye[1], J. Zhao[1], R. LI[2], M. Guan[1,2]* 1) Medical Genetics, Wenzhou Medical College, Wenzhou, Zhejinag, China; 2) Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio.

Mutations in mitochondrial DNA, especially in the tRNA-Leu(UUR) gene, have been associated with type-2 diabetes. With the aim of investigating the prevalence and clinical features of Chinese subjects with type-2 diabetes, a systematic and extended mutational screening of mitochondrial DNA has been initiated in the large clinical population at the Wenzhou Medical College, China. In the current study, we performed the mutational screening of tRNA-Leu(UUR) gene in 740 Chinese subjects with type 2 diabetes. Of these subjects, one subject carried the A3243G mutation, one individual harbored the T3253G mutation, 5 patients had T3258G mutation, one subject carried the G3277A mutation, 10 individuals contained T3290C mutation and one subject has T3300C mutation. The prevalence of A323G mutation in Chinese type-2 diabetes seems to be lower than other ethnic populations. Further molecular analysis of the mitochondrial DNA in the Chinese pedigree carrying the A3243G mutation revealed the presence of the A3243G mutation and 37 other variants, belonging to the Asian haplogroup D5. The A3243G mutation is present at heteroplasmy in matrilineal relatives of this Chinese family. None of other variants showed evolutionarily conservation and functional significance. These suggest that mutations in tRNA-Leu(UUR) gene are one of molecular basis in type-2 diabetes in Chinese population.

Neanderthal Genomics. *J.P. Noonan[1,2], G. Coop[3], S. Kudaravalli[3], D. Smith[2], J. Krause[4], J. Alessi[2], F. Chen[2], D. Platt[2], S. Pääbo[4], J.K. Pritchard[3], E.M. Rubin[1,2]* 1) Genomics Division, Lawrence Berkeley National Laboratory, Berkeley, CA; 2) US DOE Joint Genome Institute, Walnut Creek, CA; 3) Department of Human Genetics, University of Chicago, Chicago, IL; 4) Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany.

Our knowledge of Neanderthals is based on a very limited number of remains and associated artifacts from which we must make tenuous inferences concerning their biology, behavior and relationship to ourselves. Here we describe a new dataset with significant potential for characterizing these extinct hominids from a new perspective based on the development, high-throughput sequencing and analysis of Neandertal metagenomic libraries. The first library studied was created from the remains of a 45,000-year old Neanderthal. Multiple lines of evidence indicate the vast majority of the 66,643 bp of hominid sequence so far identified in the library are of Neanderthal origin, the strongest being the identification of sequence changes in humans at sites where Neanderthal and chimpanzee genomic sequences are identical. These findings have enabled us to calculate the human-Neandertal divergence time based on multiple, randomly distributed autosomal loci, in contrast to previous studies that relied on only a few mitochondrial loci. Our analyses suggest that the Neanderthal genomic sequence we obtained and the reference human genome sequence diverged ~730,000 years ago. We also estimate that the ancestral populations that gave rise to the modern human and Neanderthal lineages diverged 370,000-470,000 years ago, prior to the emergence of anatomically modern humans. Based on these results, we are able to exclude a substantial Neandertal contribution to modern human genetic diversity. In order to identify Neanderthal orthologs of human sequences of specific interest without having to sequence the entire genome, we have also developed a directed genomic selection method that we have successfully used to recover targeted sequences from Pleistocene metagenomic libraries. These studies advance our understanding of the evolutionary relationship of *Homo sapiens* and *Homo neanderthalensis* and signify the beginning of Neanderthal genomics.

Genetic Tools: An online resource to help primary care faculty integrate genetics into primary care training.

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Genetics is increasingly relevant to primary care, but genetics content in primary care training remains limited. Building on the work of the HRSA-funded Genetics in Primary Care (GPC) project, an online resource was developed to help primary care faculty integrate genetics content into their teaching. The Genetic Tools website (www.genetictools.org) contains 41 case-based modules on 25 different topics and a section on general genetics concepts and skills. All materials underwent external review by experts in both genetics and primary care. The Genetic Tools website was advertised to GPC participants and advisory committee members, the American Academy of Family Physicians, and the National Coalition for Health Professional Education in Genetics. Visitors to the website are asked to answer an exit survey about the content and format of the website. Preliminary results are available (n=30). Respondents include family physicians(11), pediatricians(1), internists(4), ob/gyn(1), genetics professionals(8), and others(5). The majority of users found the website easy to use (87%), clear and easy to understand (90%), and relevant for primary care providers (90%). Of the 25 case topics, breast cancer (50%), colorectal cancer (27%), hearing loss (27%), and developmental delay (27%) were appreciated the most. The multiple ways of indexing cases (by disease, mode of inheritance, or age group) were found to be very useful (77%). Among the subjects covered in the genetics concepts and skills section, respondents preferred the modules on family history and red flags (indicators of a genetic diagnosis) (43% and 40% respectively). The majority of respondents would definitely use this website for teaching (70%), but 37% anticipate barriers to its use, such as lack of time or online access. The majority (77%) would definitely recommend this website to their colleagues. The Genetic Tools website seems to fulfill a need for an easily accessible and up-to-date genetic teaching resource for primary care faculty. Further evaluation is needed to identify ways to continue to improve the website.

A Comparison of genomewide linkage results for autism spectrum disorders using different diagnostic criteria.

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Background: Genetic studies for susceptibility genes for autism typically use two well-known diagnostic instruments, the Autism Diagnostic Interview-Revised (ADI-R) and the Autism Diagnostic Observation Schedule (ADOS), to define affected individuals. However, a diagnostic scheme that combines the ADI, ADOS and other instruments may produce a more reliable diagnostic trait for genetic analysis of children with the broader category of autism spectrum disorders (ASD). Objectives: To compare the effects of different ASD diagnostic definitions on linkage results. Methods: Non-parametric linkage analyses, using multiplex families from the Autism Genetic Resource Exchange (AGRE) data, were performed on ASD defined using 3 different criteria: ADI (broad definition); ADOS (definition of ASD); and the combination of ADI and ADOS (combined definition). There were 292, 113 and 104 informative families in each group, respectively. Results: A locus on chromosome 5p13.3 reached suggestive linkage (LOD score = 2.34, p = 0.0005) for ASD using the ADOS definition, while the LOD scores were 0.25 (p = 0.14) and 1.23 (p = 0.009) at this locus using the ADI broad and combined definitions, respectively. Conclusion: Linkage analysis results varied dramatically when different diagnostic schemes were used to derive ASD affection status. Finding a trait that optimizes the genetic component in ASD is important for future genetic analysis.

A genome-wide scan for quantitative trait loci of serum lactate dehydrogenase -- the Framingham Offspring Study. *J-P. Lin*[1], *L.A. Cupples*[2], *C.J. O'Donnell*[3] 1) Office of Biostatistics/DECA/National Heart, Lung and Blood Institute, Bethesda, MD; 2) Dept. of Biostatistics, Boston University School of Public Health, Boston, MA; 3) Framingham Heart Study/DECA/National Heart, Lung and Blood Institute, Framingham, MD.

Serum lactate dehydrogenase (LDH) is widely 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 ranging from 40-60%. Four isoforms have been previously mapped to chromosome 11q15, 12p12 and 16q22. To date, no linkage analysis on serum LDH levels has been reported. We carried out a 10 cM genome-wide scan for quantitative trait loci of LDH in a community-based Caucasian cohort, the Framingham Heart Study. LDH was measured in the first examination of the offspring cohort. Our study population consisted of 330 families with 1260 individuals being both genotyped and phenotyped, including 1329 full-sibling pairs, 52 half-sibling pairs, 669 cousin pairs, and 89 avuncular pairs. Using variance-component linkage methods implemented in SOLAR, the heritability was estimated as 40% after age and gender adjustment. The genome-wide linkage analysis yielded several chromosomal regions with LOD scores between 1-2: LOD scores of 1.51, 1.10, 1.54, 1.63, 1.18, 1.27, 1.02 and 1.16 on chromosomes 4, 7, 8, 9, 14 (three peaks) and 19, respectively. Our study suggests that 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 reside in chromosomal regions that differ from those containing the genes encoding LDH isoforms.

Clinical, cytogenetic and molecular analysis of a female with partial trisomy X and a de novo Xp;Yq translocation. *L. Miravalle, P.R. Delk, W. Torres-Martinez, D.D. Weaver, R. Stohler, V.C. Thurston, G.H. Vance*
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We report detailed clinical, cytogenetic, and molecular studies on a 7-month-old female with a partial trisomy X associated with a de novo unbalanced X;Y translocation. The proband was born at 36 weeks gestation to a 21-year-old G4P3 Caucasian female. Ultrasound studies at 16 weeks gestation identified an echogenic lesion located in the left ventricle, hypoplastic right ventricle, hypoplasia of the middle segment of the fifth digit, and a hypoplastic nasal bone. Conventional cytogenetic analysis on amniotic fluid cells and cultured peripheral blood lymphocytes demonstrated a karyotype of 47,XX,+der(X)t(X;Y)(p22.3;q12). FISH analysis showed that the abnormal chromosome X was negative for the Y- satellite centromeric probe, the LSI SRY probe, and the Yp subtelomeric region probe, but was positive for the heterochromatic region of the Y chromosome probe at Yq12. In addition, it was negative for the LSI STS probe at Xp22 and the Xp subtelomeric region probe, but was positive for the LSI KAL probe at Xp22. FISH analysis for the *SHOX* and *VCX-A* genes and X-inactivation studies are currently in progress. At birth, the patient presented with pulmonary valve atresia with intact ventricular septum, hypoplastic right ventricle, severe aortic stenosis, hypertrophied left ventricle, atrial septal defect, and other dysmorphic features. Birth measurements were below the 3rd percentile for both weight and length, and her head size was below -2 SD. At age 7 months her weight and length were still below the 3rd percentile and her head size below -2 SD. At this time she had developmental delay and was receiving physical and occupational therapies. The clinical phenotype resembles the phenotype found in individuals carrying deletions in chromosome Xp. Clinical findings compatible with the triple-X syndrome were not observed. Translocations t(X;Y)(p22.3;q11.2) have been described in the literature in patients with a modal number of 46 chromosomes. We present here the first case with partial trisomy X coupled with an unbalanced X;Y(p22.3;q11.2) translocation.

Splicing products of dystrophin pre-mRNA in 49 cases with intraexonic small mutations in the dystrophin gene.
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Splicing is the maturation process of pre-mRNA by which introns are removed. Mutations at splicing consensus sequence located at intron / exon boundaries result in splicing errors. Recently exonic mutations are attracting much attention as a cause of splicing errors. In the dystrophin gene, a limited number of intraexonic small mutations have been shown to induce splicing errors. In order to further characterize splicing errors caused by intraexonic small mutations in the dystrophin gene, dystrophin mRNA in lymphocytes was analyzed in dystrophinopathy cases with intraexonic small mutations by RT-nested PCR. Forty nine cases were enrolled in this study: 36 had a single nucleotide substitution leading to a nonsense mutation and 13 had one or a few nucleotides deletion / insetion mutations, creating a premature termination codon. In 10 cases, skipping of the exon encoding the mutation was disclosed in a fraction of the PCR product, but not in the other 39 cases. Particularly enough, two nonsense mutations was found to induced not only exon skipping but aberrant splicing due to creation of new splice site. Dystrophin mRNA in muscles was further analyzed in 3 of these 10 cases. Unlike in lymphocytes, splicing error was disclosed only in one case. This suggested the difference of splicing reaction between lymphocytes and muscles. In conclusion, 20% of intraexonic small mutations in the dystrophin gene induced splicing errors in lymphocyte mRNA. Further detail splicing analysis will make it possible to clarify the function of exonic sequence for splicing reaction.

CYP8B1 gene associated with gallstone disease in Chinese population. *T. Han[1], J. Qin[1], Z. Niu[2], K. Zhang[2], Z. Jiang[1], Z. Jiang[1], S. Zhang[1], W. Huang[1, 2]* 1) Ruijin Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai, China; 2) Chinese National Human Genome Center at Shanghai, Shanghai, China.

In the previous study, we identified the association between the locus D3S1266 within 3p22-21.3 on chromosome 3 and the gallstone disease in Chinese population. In this study, we re-sequenced the two genes in this region, cholecystokinin (CCK) and sterol 12-hydroxylase (CYP8B1), in 24 patients and 24 normal individuals. Totally, we discovered 11 SNPs including 5 SNPs in promoter, 1 in coding region and 5 in 3UTR of CYP8B1, in which 6 SNPs allelic frequency exceeded 5%. Therefore, a further large scale association study for these 6 SNPs was carried out in 190 patients and 189 normal subjects. One SNP E/3310(A/G)polymorphism in 3UTR region of CYP8B1 gene showed a significant difference of allelic frequency between patients and controls (p=0.022). The allelic frequency of G allele was higher in control group than that in case group. However, there was no significant difference in the distribution of the SNPs of CCK gene between cases and controls. This preliminary study implicated a strong correlation between the polymorphism of E/3310(A/G)of CYP8B1 gene and gallstone disease, and G allele may play a protective role in the gallstone formation though the further related investigation should be needed.

Ring chromosome 14: characterization of the phenotype with a novel finding, CGH delineation, long-term follow-up and literature review. *I. Kalampokis[1], A. Iglesias[1], M. Macera[2], J. Breshin[2], A. Babu[2]* 1) Division of Genetics, Department of Pediatrics, Beth Israel Medical Center, New York, NY; 2) Division of Molecular Medicine and Genetics, Department of Medicine, Wyckoff Heights Medical Center, Brooklyn, NY.

A Hispanic male with ring chromosome 14 is described. The patient is a 4 year old male with micro/dolicocephaly, downslanting palpebral fissures, blepharophimosis, epichanthal folds, corneal opacities, micrognathia, downturned upper lip, high-arched narrow palate, bilateral clinodactyly of the 5th fingers, joint hyperextensibility, hypertrichosis, generalized hypotonia, mental retardation, global developmental delay, failure to thrive, gastro-esophageal reflux, asthma, tracheomalacia, multiple episodes of otitis media and pneumonia as well as massive adenoid hyperplasia resulting in a cyanotic episode secondary to upper airway obstruction. Incidentally, he was found to have an elevation of aPTT which was attributed to coagulation factor XI deficiency. He had exhibited seizures since age 3½ months. Following 24-hour video EEG recording, the patient was diagnosed with atypical infantile spasms. Brain MRI revealed hypoplastic corpus callosum. Cytogenetic analysis (GTW banding technique) of peripheral blood chromosomes revealed a 46,XY,r(14)(p11.2q32.3) karyotype. Due to the nature and size of the ring 14 chromosome, CGH analysis was used and a deletion was detected at 14q31q32.3. The patient is monosomic for the chromosome segment 14q31-qter. The karyotype was revised to 46,XY,r(14).ish cgh r(14)(?p11q31). In summary, we describe a patient with ring chromosome 14 exhibiting several phenotypic features commonly associated with this chromosomal condition. This is the second case of ring chromosome 14 in the literature with hypoplastic corpus callosum to our knowledge. An extensive literature review summarizing the most commonly described phenotypic features of ring chromosome 14 on a table format is included as an attempt to further delineate the basic phenotype and its variants. Knowledge of the phenotypic spectrum and its natural history will help clinicians in the long term care of these patients.

SDHB and SDHD Mutations in Malignant Pheochromocytomas. *R. Klein, L. Jin, K. Rumilla, R. Lloyd* Dept Lab Medicine & Pathology, Mayo Clinic, Rochester, MN.

Background: Germline mutations in the genes encoding the B (SDHB) and D (SDHD) subunits of the heterotetrameric protein succinate dehydrogenase (mitochondrial complex II) are important causes of inherited and apparently sporadic paragangliomas. Mutations in SDHB appear to be found more frequently in association with extra-adrenal and malignant pheochromocytomas, while mutations in SDHD are more often identified in benign head and neck paragangliomas. In an effort to further investigate the role of SDHB and SDHD in apparently sporadic malignant intra- and extra-adrenal pheochromocytomas, we screened a series of tumors for mutations in the SDHD and SDHB genes. Materials and Methods: Mutation testing was performed on DNA extracted from formalin fixed, paraffin embedded tumor and adjacent normal tissue by PCR amplification and direct sequencing of the coding regions and intron-exon junctions of the SDHB and SDHD genes. Results: Among 17 extra-adrenal pheochromocytomas with proven metastatic disease, 4 nonsense, 1 splice site, 1 insertion causing a frameshift, and 3 probable missense mutations were found in SDHB. No mutations were detected in SDHD. Mutations in SDHD and SDHB were not identified in 9 malignant intra-adrenal pheochromocytomas. The identical SDHB mutation was detected in DNA extracted from accompanying normal tissue for each of the 7 cases on which this analysis was performed. Conclusions: Germline mutations in SDHB are common in patients with malignant extra-adrenal pheochromocytomas, while SDHD mutations rarely occur. The disparate mutational spectra in malignant intra- and extra-adrenal pheochromocytomas may reflect differences in tumor biology.

Idiopathic Mental Retardation:A Brazilian study. *M. Mulatinho[1,4], L. Moraes[2], T. Reis[2], L. Carvalho[2], M. Camilo[2], V. Moura[2], J. Santos[3], R. Boy[3], D. Horovitz[2], R. Zlot[2], M. Pimentel[3], H. Ramos[2], N.P. Rao[4], J. Llerena[1,2]* 1) Genetics,Universidade Federal do Rio de Janeiro,Brazil; 2) Instituto Fernandes Figueira; 3) Universidade do Estado do Rio de Janeiro; 4) Univ. of California of Los Angeles,CA.

In order to set up a clinical strategy to study Idiopathic Mental Retardation (IMR) in a Brazilian population, 121 patients with dysmorphism to behavioral disorders, attending two institutions in Rio de Janeiro were selected. The common clinically recognizable syndromes were excluded. Cytogenetic analysis at 400-bands and screening for FRAXA and FRAXE loci were done. They were further studied with 550 bands-HR-GTG and subtelomeric FISH. 42 individuals were analyzed so far. Of these 6 had an abnormal karyotype. Two had features characteristic of chromosomal syndromes. Case A appeared to have the Potocki-Shaffer syndrome, 46,XX,del(11)(p11.2p11.2). Case B had a deletion of 1p36 [46,XX,del(1)(p36)]and features of Monosomy 1p36 syndrome. FISH confirmed the deletion. There were four other cases, all de novo, with the following karyotypes:C)46, XX, add(13)(q34); D)46, XX,t (5;6)(q35.1;p22.2); E) 46,XX,ins(2)(pter->q12::?::q14->qter), F) 46, XY, add(3)(p25). Although Case E had an apparently balanced translocation, the patient had large and malpositioned central incisors, partial cutaneous syndactyly in hands, small hands and feet, centripetal obesity, IUGR, myopia, genu valgum. FISH with the subtelomeric probes did not identify any aberrations in 17 of the 42 cases. The remaining are currently being screened by Subtelomere FISH, and the complete results will be presented. Genetic counseling was provided to the 6 families involved. Samples from patients that are normal by HR-GTG and FISH will be subjected to Oligonucleotide Array-CGH technology to determine the frequency of submicrocipic aberrations in this select group of IMR patients. With the methodologies used we could identify 6 cases. These data reinforces the suggestion of studying all MR with HR-GTG banding. While the yield may be limited, chromosomal abnormalities can be found regardless of the severity of MR, and the number of abnormal physical findings.

A genetic network in age-related macular degeneration. *R.J. Klein[1], M. Othman[2], J.-Y. Tsai[3], S. Zareparsi[2], M. Campos[2], P. Atmaca-Sonmez[2], K.H. Branham[2], A. DeWan[4], A.K. Henning[5], E.Y. Chew[3], F.L. Ferris[3], H. Grossniklaus[6], G. Abecasis[7], C. Barnstable[8], A. Swaroop[2,9], J. Hoh[4,8]* 1) Laboratory of Statistical Genetics, Rockefeller Univ, New York, NY; 2) Dept of Ophthalmology & Visual Sciences, Univ of Michigan, W.K. Kellogg Eye Center, Ann Arbor, MI; 3) National Eye Institute, Bethesda, MD; 4) Dept of Epidemiology and Public Health, Yale Univ School of Medicine, New Haven, CT; 5) The EMMES Corporation, Rockville MD; 6) Dept of Ophthalmology, Emory Univ School of Medicine, Atlanta, GA; 7) Center for Statistical Genetics, Dept of Biostatistics, School of Public Health, Univ of Michigan, Ann Arbor, MI; 8) Dept of Ophthalmology and Visual Science, Yale Univ School of Medicine, New Haven, CT; 9) Dept of Human Genetics, Univ of Michigan, Ann Arbor, MI.

Age-related macular degeneration (AMD) is the leading cause of blindness in the elderly in the developed world. The risk for developing AMD is determined by the complex interplay of genetic variants, many of which are as yet unidentified. Several chromosomal regions have been repeatedly linked with AMD in family-based linkage studies. SNPs in two of these regions - in the genes CFH and LOC387715 - have been previously found to be associated with AMD.

To understand more fully the network of genes influencing AMD, we looked at the region around hypothetical gene LOC387715 in our genome-wide association data set of AMD. We confirm an association between LOC387715 and AMD. Using a new method to test for interactions, we identify two SNPs in our genome-wide data that show a statistically significant genetic interaction with LOC387715. While our screen could have identified SNPs anywhere in the genome, these two SNPs are found within two additional regions that show strong evidence for linkage to AMD. The interaction of these three loci, along with CFH, appears to account for considerable genetic risk for AMD.

Tachyon: A new frontier for exact multipoint likelihood calculations on large pedigrees. *J.R. O'Connell* Div Endo/Diabetes/Nutrition, Univ Maryland, Baltimore, MD.

Exact multipoint likelihood calculations are the core of pedigree-based gene mapping methods. Current exact methods are limited to only a few markers on many relatively small pedigrees. We introduce a new approach to exact multipoint likelihood calculations whose complexity scales linearly in the number of loci that can tackle pedigrees significantly beyond the maxbits limit of the Lander-Green-Kruglyak algorithm. Conceptually the approach decomposes the pedigree into two types of overlapping components: G components with substantial genotype data and M components with none except on overlaps. The likelihood calculation is then a joint sum over the intersection of the components with summand the products of the likelihood of the components, creating a potentially large sum of smaller problems. The M component is in general the source of intractability, however, gene flow in this component contains rich symmetry that we exploit to eliminate its complexity. To illustrate our approach we create a pedigree with G component a fully genotyped CEPH pedigree with 5 offspring and M component the four CEPH grandparents connected as N^{th} cousins. The pedigree has four loops, maxbits equal to 4N+20, and intractable if N > 5. The M component has maxbits 4N+6. Using a novel algebra on grandparent-to-founder gene flow vectors we can write the M component transition probabilities between two loci as a hidden Markov model in closed form as a function of N. Thus for N large enough the exact likelihood can be computed faster manually than by any current program. The G component transition probabilities can be computed locus by locus using current methods. The likelihood of the full pedigree is the sum over joint genotypes of the grandparents of products of conditionally independent G and M component probabilities. We can compute the exact likelihood of the full pedigree using 250 markers in less than two minutes independent of N. We present examples of currently intractable real data pedigrees that this approach easily solves. We discuss limitations and the potential of TACHYON to exploit the power of larger pedigrees for linkage and association mapping of complex diseases.

Comparative analysis of serum proteomes for the discovery of biomarkers in Wilson disease. *S.H. Heo, G-H. Kim, S-W. Park, H-W. Yoo* Genome Research Center for Birth Defects & Genetic Disorders, Asan Institute for Life Sciences, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea.

Wilson Disease(WD) is an autosomal recessive inherited disorder leading to impaired intrahepatic trafficking and biliary excretion of copper, resulting in the accumulation of copper in various organs including the liver, cornea, and brain. The excess copper and oxidative stress maybe produces several proteins different from wild type. To date, no diagnostic biomarker for WD has been identified except ceruloplamsin. In order to identify diagnostic biomarkers for WD, efforts have been made to correlate the serum protein profiles of three asymptomatic WD patients with two normal controls, whose age and sex were matched. Fractionated serum proteins were displayed on two-dimensional electrophoresis (2-DE) gel using multiple affinity removal columns (MARC) to remove six high-abundant proteins. Analyses of these gels allowed us to identify two differentially expressed proteins that were remarkably reduced in the group of WD patients as compared to the control group. These reduced spots were analyzed by matrix assisted laser desorption - ionization time of flight/ ionization-time of flight (MALDI-TOF/TOF). The reduced proteins were complement component C3c (C3c), carbonic anhydrase 1 (CAH1), peroxiredoxin-2 (thiol-specific antioxidant), dihydropyrimidinase-like 1 variant (DPYSL2), and complement factor B (BF). It indicated that enhanced oxidative stress caused by excessive copper accumulation might down regulate the expression of thiol-specific antioxidant, C3c, and BF, which were known to be associated with oxidative stress. In addition, six of the protein spots were significantly down regulated in the 2-D gel of whole serum of nine WD patients. These spots were identified as haptoglobin-1 precursor. And further identification of the spots at different pathological grades was confirmed by western blotting using polyclonal anti-heptoglobin antibody. Our strategy using comparative analysis of parallel protein quantification on 2-D gels will provide us with cues to accelerate the discovery of novel serum protein biomarkers for the diagnosis of WD.

Identification of early gene expression changes in two mouse models of motor neuron degeneration using whole genome microarrays. *L.C. Kudo, M. Wiedau-Pazos, S.L. Karsten* UCLA Department of Neurology, Los Angeles, CA 90095.

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease selectively affecting motor neurons in the central nervous system (CNS). A subset of familial ALS patients carries a mutation of the superoxide dismutase1 gene (SOD1). The crucial question of how and why only motor neurons degenerate in the presence of the ubiquitously expressed SOD1 mutation remains to be answered. Factors that may contribute to motor neuron degeneration have been identified, including but not limited to, disruption of axonal transport, glutamate metabolism, oxidative stress, copper metabolism, and the availability of growth factors. Yet, it is still not clear how these factors may act together in the etiology of ALS. In order to characterize the molecular events involved in initiation of neurodegeneration in ALS, we studied motor neuron-specific gene expression in two mouse models of ALS: familial ALS linked to SOD1 (G93A-SOD1) and frontotemporal dementia with ALS linked to mutant TAU (P301L-TAU). DNA microarray technology in combination with laser-capture microdissection was used to discover gene expression changes at the early stages of the disease prior to neurodegeneration. Spinal cords were dissected from 3 months old female transgenic mice and their non-transgenic littermates. Axial cryostat sections from the lumbar spinal cords of transgenic and non-transgenic control animals were fixed in ethanol and stained with Cresyl violet. Motor neurons from the ventral horns were microdissected on a PixCell Arcturus LCM device. RNA extracted from the collected motor neurons was subjected to microarray experiments using Agilents Mouse Whole Genome Oligonucleotide Microarray. In addition to gene expression changes found in both mouse strains, we have identified a set of genes specific for a particular genotype. Here, we discuss the relevance of these findings with respect to the mechanisms of motor neuron dysfunction and death. Supported by MDA grant #20050289.

Physician Awareness of genetics (PHAGEN) among primary care physicians in Israel. *G. Hirsch-Yechezkel[1], R. Grossman-Yahalom[2], Y. Shachar[1], E. Friedman[3]* 1) Gertner Institute of Public health, Tel-Hashomer, Israel; 2) Clalit HMO center Rehovot, Israel; 3) The Susanne Levy Oncogenetics Unit, Sheba Medical center, Tel Hashomer, Isarel.

In order to provide adequate patient care, primary care physicians (PCP) are required to have a basic understanding in genetics. This trend of using modern genetics tools is forecasted to increase in the near future. In order to define the subset of PCP who would benefit most from an intervention program, we wanted to assess the baseline level of knowledge of genetics among PCP) in Israel. To that end, a 10-point, multiple-choice questionnaire was administered and completed in person by 120 (of 298 eligible) PCP. Recruitment was during semi annual conferences. The questions ranged from classical genetics scenarios to cancer genetic counseling issues, with the maximal score 40 points. The majority of participants were women (n=70-58.3%), age range of 41-50 years (n=64 - 53.3%), and graduated from medical schools 20+ years prior to questioning (n= 66- 55%). The mean score was 29+ 5.4 (range 14-38), there was an inverse correlation between age and high score (34/40 -85%): 4/26 (15.4%) PCP age 56+ years scored 85% compared with 9/21 (42.3%) PCP aged 32-40 years (OR 0.24 95% CI 0.05-1.13; P for trend <0.05). In addition, an inverse correlation was noted between time from graduation from medical school and score: 6/10 (60%) PCP who graduated up to 9 years prior to questioning scored 85% or higher compared with 3/24 (12.5%) that graduated 30+ years (OR 0.1 -95% CI 0.01-0.71; p<0.05). There were no gender differences. We conclude that older physicians, who graduated from medical school before 1985, should be targeted for intervention in order to enhance their knowledge in genetics, in order to facilitate better patient care and rational use of genetic tools.

Parent of Origin Effects and HLA-DRB1 Risk in Systemic Lupus Erythematosus. *L.K. Komorowski[1], L.F. Barcellos[1], P.P. Ramsay[1], R.R. Graham[2], G. Artim[1], M.F. Seldin[3], J.B. Harley[4], T.W. Behrens[5], L.A. Criswell[6]* 1) University of CA, Berkeley, CA; 2) Broad Institute of M.I.T. and Harvard, Boston, MA; 3) University of CA, Davis, CA; 4) Oklahoma Medical Research Foundation, Oklahoma City, OK; 5) University of Minnesota, Minneapolis, MN; 6) University of CA, San Francisco, CA.

Genetic susceptibility for systemic lupus erythematosus (SLE) has been associated with HLA class II loci within the MHC, yet the contribution of this region explains only part of the conferred genetic risk. To date, HLA-DRB1*0301, *1501 and *0801 alleles have demonstrated the strongest evidence for association with SLE. Parent-of-origin effects operating at DRB1, such as imprinting, may contribute to SLE, and if so, may result from underlying epigenetic mechanisms. The role of microchimerism due to maternal-child cell transfer during pregnancy has also been linked to risk for autoimmune disease, and is mediated by parent-of-origin HLA effects. We examined parent-of-origin effects for DRB1 and disease risk in a large SLE family dataset. Paternal and maternal transmission frequencies of DRB1 alleles to affected offspring in 714 Caucasian SLE families were compared to identify evidence for imprinting. Maternal-child histocompatibility relationships in SLE cases and unaffected siblings were also examined. Discordant sibpairs from families were grouped by maternal-child DRB1 profiles as compatible (mother-child, child-mother, bidirectional) or not compatible. Differences would provide evidence to support a role for microchimerism mediated by HLA in SLE. No difference between paternal and maternal transmission frequencies for any DRB1 allele was observed, including for known risk alleles, DRB1*0301, *1501 and *0801. TDT results confirmed that maternal and paternal transmissions for the known DRB1 risk alleles were very similar. Furthermore, no evidence was observed for SLE risk due to maternal-child histocompatibility relationships based upon DRB1 genotypes. These results indicate that novel mechanisms such as imprinting and maternal-child histocompatibility relationships involving DRB1 are not contributing a major role to SLE risk.

Analysis of Iranian patients with hearing loss For the second most prevalent locus DFNB4. *M. Mohseni[1], K. Kahrizi[1], F. Azizi[4], C. Nishimura[2], N. Bazazzadegan[1], G. Asaadi Tehrani[1], M. Sayfati[1], M. Taghdiri[1], P. Jamali[1], A. Daneshi[3], R.J.H. Smith[2], H. Najmabadi[1]* 1) Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran (Islamic Republic of); 2) Molecular Otolaryngology Research Laboratories, Department of Otolaryngology Head and Neck Surgery, University of Iowa, Iowa, IA, United States; 3) Research Center of Ear, Nose, Throat, and Head and Neck Surgery, Tehran, Iran(Islamic Republic of); 4) Endocrine Research Center , Taleghani hospital. Tehran, Iran.

Background: Mutations in the SLC26A4 gene in DFNB4 locus is responsible for syndromic (Pendred syndrome) and non-syndromic hereditary hearing loss(HHL). In many populations mutations in this gene have been reported as a second cause of HHL. The objective of our study was to investigate the prevalence of SLC26A4 mutations in our HHL consanguineous families. Materials and methods: After complete clinical examination the consent form was taken from each family. We included 87 families with two or more than two affected individuals, who have been referred to our center. All families had previously been tested negative for the DFNB1 locus were candidates for homozygosity mapping using STRs for DFNB4 locus. Families localized to this region were subjected complete DNA sequencing. Results: Twelve out of eighty seven families were mapped to DFNB4. Sequence analysis of six linked families revealed seven mutations (T420I, 1197delT, G334Y, R409H, T721M, R79X, S448L). The T420I , G334V and R79X were novel mutations. Mutations detection for the other families is being performined. Conclusion: We have been able to localize total of 12 families (13.8%) to the DFNB4 locus. Nine families had thyroid dysfunction(pendred syndrome) and in three families we couldn't find any symptoms of thyroid impairment. This investigation, demonstrated that the SLC26A4 gene mutation is the second most prevalent cause of HHL in Iran. This result is in accordance with reports from other countries. Key words: DFNB4, SLC26A4 gene, hereditary hearing loss.

Glucocorticoid-induced granzyme A expression can be used as a marker of glucocorticoid sensitivity for acute lymphoblastic leukemia therapy. *A. Myoumoto[1], K. Nakatani[1], T. Koshimizu[1], H. Matsubara[2], S. Adachi[2], G. Tsujimoto[1]* 1) Dept. Genomic Drug Discovery Sci., Grad. Sch. Pharmaceu. Sci., Kyoto Univ; 2) Dept. Pediatrics, Grad. Sch. Med., Kyoto Univ.

Glucocorticoids (GC) sensitivity is an important prognostic factor in the treatment of patients with acute lymphoblastic leukemia (ALL). However, the clinical assessment for GC sensitivity is very time-consuming. We have recently found that granzyme A (GZMA) mediates GC-induced apoptosis in human ALL-derived cell line 697 (FASEB J. M. Yamada *et.al.*). In this study, we examined whether GC-induced GZMA expression could predict the GC sensitivity leading to apoptosis of seven established cell lines derived from ALL patients.

We have treated seven established ALL cell lines derived from pre-B ALL patients with GC (100 nM DEX or 800 nM prednisolone) or vehicle for 24-h. The 697 and 697Bcl2 cell lines were used as a positive and negative control, respectively. The 697Bcl2 cells stably express Bcl2 and thus were protected from GC-induced apoptosis. We have analyzed cellular indicators of apoptosis, such as cell death and mitochondrial transmembrane potential depolarization using flowcytometry in pre-, vehicle- and GC-treated groups. GZMA mRNA expression was analyzed by quantitative RT-PCR in each group.

The apoptosis assay showed four cell lines were GC-sensitive, while three cell lines were GC-resistant. GC treatment markedly enhanced GZMA mRNA expression only in GC-sensitive cell lines, but not in GC-resistant cell lines. Moreover, GC-induced GZMA mRNA expression correlated well with the extent of GC-induced apoptosis.

We found a highly significant correlation between the GC-induced GZMA expression and apoptosis at 24-h by using seven cell lines established from ALL patients. Therefore, we propose that GC-induced GZMA mRNA expression at 24-h could be such a biomarker to asses GC sensitivity. Further studies will be required to establish 24-h GC-induced GZMA expression as an early biomarker for personalized ALL therapy.

Reconsideration of the inheritance mode of febrile seizures. *Y. Nagao*[1, 2] 1) Department of Pediatrics, Social Health Insurance Medical Center, Shinjuku, Tokyo, Japan; 2) Department of Pediatrics, School of Medicine, The University of Tokyo.

Febrile seizures (FS) are rather common disorders. Especially prevalence of FS in Japanese population is high and is reported 0.06 - 0.09. The inheritance mode of FS had been discussed, and FS are now accepted as multi-factorial diseases. Efforts to identify a single gene locus for FS have been performed utilizing non-parametric linkage analysis, because this method can be applied for multi-factorial diseases, and an apparent disease-sensitive gene for FS has not yet been identified. But is there no possibility that FS are disorders caused by a single major gene? In this paper I show the calculation of the recurrence risk of the sibling of a FS patient. With assumption that penetrance is less than 1, the recurrence risk of a sibling is calculated both by autosomal recessive model and by autosomal dominant model, and the calculated risk was significantly consistent with the reported incidence of a sibling. But when the incidence of a parent is considered, the autosomal recessive model is more consistent. The result of calculation does not exclude the multi-factorial inheritance of FS, but when the single major gene hypothesis is applied, the parametric linkage analysis can be performed because the frequency of mutant allele and the penetrance are also calculated. Therefore the calculation of these parameters based on a single major gene hypothesis might simplify the linkage analysis of the possible major gene for FS.

Not sequence, but structure: palindrome-mediated translocation. *H. Inagaki[1], T. Ohye[1], H. Kogo[1], B.S. Emanuel[2], H. Kurahashi[1]* 1) Division of Molecular Genetics, Fujita Health University ICMS, Toyoake, Aichi, Japan; 2) Division of 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 palindromic AT-rich repeats (PATRRs). We propose that the PATRRs form cruciform structures in vivo and induce double-strand-breaks (DSBs) that are illegitimately repaired through non-homologous end joining (NHEJ). However, there is to date no evidence for cruciform formation in living cells. To elucidate how the PATRRs induce DSBs, we established a plasmid-based model system using a mammalian cell line as host. We independently cloned the PATRRs from 11q23 and 22q11 into plasmids. Plasmids were manipulated topologically so as to extrude or not extrude the cruciform structure and then transfected into HEK293 cells. Using translocation-specific PCR and a fluorescence-based reporter system, we demonstrated that the cruciform-extruded plasmids introduced into cells were cleaved and rejoined at the PATRRs. Sequence analysis demonstrated that the breakpoint sequences were quite similar to those of human t(11;22) junction fragments. By contrast, no rearrangement was observed with cruciform-negative plasmids. Southern analysis indicated that this rearrangement is initiated by diagonal cleavage of the cruciform DNA prior to the subsequent cleavage of hairpin tips at the ends of the broken DNA. This is then followed by NHEJ. Our results strongly suggest DNA secondary structure-specific cleavage as a mechanism leading to palindrome-mediated translocations.

Why do de novo t(11;22)s arise only in sperm?: Analysis using a yeast model system. *T. Ohye[1], H. Inagaki[1], H. Kogo[1], B.S. Emanuel[2], H. Kurahashi[1]* 1) Div Molecular Genetics, Inst Compre Med Sci, Fujita Health Univ, Aichi, Japan; 2) Div Human Genetics, Childrens Hosp Philadelphia, Philadelphia, PA.

?The constitutional t(11;22)(q23;q11) is the most frequently occurring non-Robertsonian translocation in humans. The breakpoints of the t(11;22) were identified within palindromic AT-rich repeats on chromosomes 11 (PATRR11) and 22 (PATRR22), suggesting that cruciform structures mediate double-strand-breaks (DSBs) leading to this recurrent translocation. De novo t(11;22)s are detected in sperm from healthy men at a frequency of $1/10^4$-10^5, but never in lymphocytes or fibroblasts. To investigate the mechanism of sperm-specific generation of the t(11;22), we utilized a yeast model system for the t(11;22). We created a diploid yeast harboring the 445bp PATRR11 at the TRP1 locus on chromosome IV and the 595bp PATRR22 at the LEU2 locus on chromosome III. We isolated genomic DNA and performed translocation-specific PCR using primer pairs flanking the PATRR both on chromosomes IV and III. As a result, we successfully detected de novo translocation-specific PCR products. Based on our previous observation that the PATRRs prefer to adopt a cruciform conformation in a temperature-dependent manner, and the fact that the temperature of the testis is lower than that of other tissues, we examined translocation frequency at various temperatures in yeast cultures. As a result of this analysis we determined that the translocation frequency was significantly higher at 25C than at 34C. Next, we determined the translocation frequency either in vegetative or meiotic yeast, finding a higher translocation frequency during meiosis. Our results suggest that complex factors such as temperature-dependent DNA conformation and meiosis-specific recombination machinery during spermatogenesis are likely to be involved in sperm-specific generation of the t(11;22) rearrangement. These results begin to elucidate the factors that influence the formation of structural chromosomal aberrations that are primarily derived during male gametogenesis.

Neonatal screening for Pompe disease: result from the Taiwan screening program. *W.-L. Hwu[1], J. Keutzer[2], S.-C. Chiang[1], X. Zhang[2], Y.-H. Chien[1], N.-C. Lee[1], S. Young[3], D. Millington[3], M. Fietz[4]* 1) Department of Pediatrics and Medical Genetics, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan; 2) Genzyme Corporation, Cambridge, MA, USA; 3) Department of Pediatrics, Duke University Medical Center, Research Triangle Park, NC, USA; 4) Department of Chemical Pathology, Women's and Children's Hospital, North Adelaide, Australia.

Pompe disease is caused by the deficiency of acid-alpha-glucosidase (GAA). Recently, recombinant human GAA has been used to treat infantile-onset Pompe disease (IOPD) with good success, resulting in prolonged survival, reversal of cardiomyopathy, and growth and motor gains, although not all patients achieve ambulation. Best motor outcomes are reached when treatment is initiated early. A neonatal screening program for Pompe disease started in Oct. 2005. Blood spots were obtained from babies around 3 days of age, and GAA activities were measured using 4-MU-glucoside as the substrate, with acarbose as an inhibitor of maltase-glucoamylase (MGA). The assay employed an initial screen for GAA activity and a retest assay measuring the GAA to neutral maltase ratio and percent inhibition. The recall rate was below one percent. After screening 55,897 newborns, one case was found to have IOPD, and the other case either the late infantile or juvenile form of Pompe disease. In 36 other babies who received confirm blood testing, 7 had GAA activities between 5 and 10% of the normal mean. However, none became symptomatic after a follow up period of 1 to 4 months. Low GAA activities in their blood spot were further confirmed by the immune-capture enzyme activity assay, but none of them showed elevation of urine glucose tetrasaccharide, a biomarker for the disease. Molecular analysis showed that 5 of the 36 cases were heterozygous carriers of a common mutation, with all mutant alleles inherited from their parents. During this period, two cases of IOPD were diagnosed clinically at the ages of 4 and 5 months, respectively. They were not screened, but their newborn bloodspot GAA activity was in the deficiency range when assayed retrospectively.

DigiTag2 assay for multiplex SNP typing. *N. Nishida[1, 2], T. Tanabe[2], M. Takasu[1], A. Suyama[3], K. Tokunaga[1]* 1) Dept Human Genetics, University of Tokyo, Tokyo, Japan; 2) Biomedical Business Incubation Division, Olympus Corporation, Tokyo, Japan; 3) Dept Life Sciences, University of Tokyo, Tokyo, Japan.

There are a number of single nucleotide polymorphisms (SNPs) to be candidate susceptibility or resistance genetic factors for multifactorial disease. Genome-wide search for disease susceptibility regions followed by high-resolution mapping of primary genes requires high-throughput, cost-effective and highly reliable technology. At present, a variety of SNP genotyping applications exist, however, many applications need to select relevant SNPs for their assay by in silico assay design, and then a part of candidate SNPs will be excluded from investigation. To accomplish successful typing for all candidate SNPs at a low cost, new technologies must be developed.

We developed a new multiplex SNP typing method, named DigiTag assay. In the DigiTag assay, all of the SNP genotypes are encoded to the well-designed oligonucleotides, named DNA coded numbers (DCNs). The assignment of the DCNs to the SNPs is unconstrained, therefore, the DNA chips prepared to read out the types of DCNs are universally available for any types of SNPs. We have reported in the ASHG2005 that the DigiTag assay have the potential to analyze almost all kind of the SNPs with high accuracy and reproducibility. However, the DigiTag assay needs the washing step with magnetic beads, which is laborious step in manipulation. And also, the biotinylated probes, which are necessary for the washing step, are expensive.

For the next version of the assay, we improved the protocol to exclude the washing step and named DigiTag2 assay. The DigiTag2 assay uses non-labeled primers and probes, which lead to save the cost of the assay. We investigated the feasibility of the DigiTag2 assay using the SNPs located in the 500 kb genomic region including *IL-4* and *IL-13* genes. The success rate, which is defined by the proportion of successfully genotyped SNPs in the total number of SNPs examined, was revealed to be over 90%, and the typing result was 100% identical to the result from direct sequencing.

Knockdown of frataxin causes loss of aconitase activity, induction of oxidative stress and induction of heme transcripts. *C. Lu, G.C. Cortopassi* department of molecular biosciences, University of California, Davis, Davis, CA.

Friedreichs ataxia (FRDA) is an autosomal recessive disease characterized by neurodegeneration and cardiomyopathy. It is caused by the mutations in the frataxin gene which decrease frataxin protein expression. Frataxin is expressed in the cytoplasm and mitochondria. In order to isolate the primary consequences of frataxin deficiency, we generated a cell model using a frataxin-specific siRNA driven by a tetracycline inducible promoter in HEK293 cells. By immunoblot assay of frataxin and mitochondrial marker proteins, we demonstrated that the weight of cytoplasmic frataxin is equal to or exceeds mitochondrial frataxin. Half-depletion of cytoplasmic frataxin upon tetracycline induction occurred at 1.5 days, 3 days earlier than that of the mitochondrial frataxin. Thus cytoplasmic frataxin turns over three times faster than mitochondrial frataxin. In parallel with cytoplasmic frataxin depletion, the activity and amount of the cytoplasmic iron-sulfur cluster proteins aconitase and ISU1 declined, respectively, at 2 days. The antioxidant cytoplasmic CuZnSOD protein was induced from day 2 onward, and increased protein oxidative damage was observed from day 4 onward, consistent with the induction of unfolded protein response transcription factors ATF4 and CHOP. By contrast, mitochondrial aconitase activity started to decline only at 7 days, i.e. after frataxin protein levels were depleted in the mitochondria by 60%. The heme-dependent transcripts ALAS1 and MAOA were induced only after day 8, coincident with the decrease in heme-containing cytochrome c protein. Overall, these results suggest that the earliest consequences of frataxin deficiency occur in cytoplasmic iron-sulfur proteins and result in oxidative damage and stress, and trigger the unfolded protein response, and that the deficiency of mitochondrial cytochrome c and induction of ALAS1 occur only after mitochondrial frataxin depletion. These data suggest that the consequences of extra-mitochondrial frataxin deficiency could be as important as the mitochondrial ones and should be taken account of in the Friedreichs ataxia pathophysiological mechanism.

Human-specific differences in the rates of peroxisomal lipid metabolism relative to the great apes. *J. Hacia[1], A. Moser[2], K. Ramaswamy[1], M. Karaman[1], O. Ryder[3], P. Watkins[2]* 1) Department of Biochemistry and Molecular Biology, University of Southern California, Los Angeles, CA; 2) Kennedy Krieger Institute, Baltimore, MD; 3) Zoological Society of San Diego, San Diego, CA.

Meat, fish, and dairy consumption have resulted in significant differences in the lipid composition of human and great ape (chimpanzee, bonobo, gorilla, and orangutan) diets. In order to identify adaptive biochemical changes occurring in the human lineage in response to dietary lipids, we analyzed rates of fatty acid metabolism in primary cultures of human and great ape fibroblasts. Relative to those of the great apes, human cells had approximately two-fold higher catabolic rates of phytanic acid, a branched chain fatty acid present in ruminant fats unique to historical human diets. These catabolic rates are consistent with the species-specific message levels of PHYH, a peroxisomal enzyme involved in the initial step of phytanic acid -oxidation prior to complete catabolism via -oxidation. Interestingly, inactivation of the *PHYH* gene in humans leads to Refsums Disease, an autosomal recessive disorder involving peripheral sensory neuropathies as well as cardiovascular and skeletal abnormalities. Remarkably, the rates of other peroxisomal lipid metabolic processes, including pristanic acid catabolism and plasmalogen biosynthesis, did not show significant interspecies differences. Likewise, rates of mitochondrial -oxidation of long chain (LCFA) and very long chain (VLCFA) fatty acids were similar across all species. Overall, this indicates that specific adaptive changes in peroxisomal metabolic pathways have occurred in human cells that may be linked to increased levels of potentially toxic lipids unique to human diets. In turn, this may have influenced the evolution of human phenotypes involving the nervous, cardiovascular, and skeletal systems.

MITOCHONDRIAL DNA HAPLOGROUP J ALTERS THE SUSCEPTIBILITY TO INHERITED TYPE 2 DIABETES AND COMPLICATIONS. *D. Mishmar[1], J. Feder[1], I. Blech[2], O. Ovadia[1], J. Wainstein[3], I. Raz[4], S. Dadon[1], B. Glaser[2]* 1) Dept of Life Sciences, Ben-Gurion University, Beer-Sheva, Israel; 2) Endocrinology and Metabolism Service, Internal Medicine Department, Hadassah-Hebrew University Medical Center, Jerusalem, Israel; 3) Israel Diabetes Research Group (IDRG), Wolfson Hospital, Holon, Israel; 4) Diabetes Unit, Internal Medicine Department, Hadassah-Hebrew University Medical Center, Jerusalem, Israel.

Type 2 Diabetes Mellitus (T2DM) has a well-established genetic component, yet linkage to specific genetic loci is apparent only in few studies. Therefore, combinations of predisposing common genetic variants may underlie T2DM. Evidence from numerous human, animal and in-vitro studies show dysfunction of mitochondrial bioenergetics in T2DM. It has been proposed that certain mitochondrial DNA (mtDNA) common genetic variants are associated with prolonged aging, adaptation to different climatic conditions and with the susceptibility to certain age-related disorders. Nevertheless the association of such mtDNA common variants with T2DM is yet to be deciphered. To assess the contribution of mtDNA to the etiology of T2DM we analyzed the mtDNA genetic variability in 765 T2DM Ashkenazi Jewish patients. Strikingly, haplogroup J1, was underrepresented in sporadic T2DM patients (11/249, 4.4%) as compared to offspring of T2DM parents (41/425, 9.6%) (G test, P=0.01), suggesting that mutations defining haplogroup J1 alter the susceptibility to T2DM depending on additional inherited factors. Interestingly haplogroup J1 was over-represented among patients with nephropathy (33/308, 10.7%, P = 0.007) and retinopathy (14/118, 11.9%, P=0.05) as compared to patients without these complications (23/431, 5.7%; 40/614, 6.5%, respectively) implying that this haplogroup alters the susceptibility to these diabetic complications. Since haplogroup J1 is defined by mutations altering highly conserved positions in the mtDNA, to our knowledge this is the first support for mtDNA functional involvement in the etiology of T2DM. As haplogroup J alters the susceptibility to Parkinsons disease, and supports longevity, our results strongly suggest its general involvement in aging.

Genetic Investigation of Hearing loss in Iranian Population (a seven year survey). *H. Najmabadi[1], N. Meyer[2], K. Kahrizi[1], Y. Riazalhosseini[1], A. Daneshi[3], M. Farhadi[3], M. Mohseni[1], N. Bazazzadegan[1], F. Esteghamat[1], M. Avenarius[2], C. Nishimura[2], M. Malekpour[1], S. Arzhangi[1], R.J.H. Smith[2]* 1) Genetics Research Center, University of the Social Welfare and Rehabilitation Sciences, Tehran,Iran; 2) Molecular Otolaryngology Research Laboratories, Department of Otolaryngology, University of Iowa, Iowa City, IA, United States; 3) Rasoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran.

Since 1999, total of 1711 families with hearing loss have been referred to our center for genetic testing. Thirty eight families had syndromic hearing loss. Majority of families had autosomal recessive non syndromic hearing loss (ARNSHL) pattern of inheritance. In 135 probands, no other affected relative could be identified - we classified these as simplex cases. GJB2 mutation screening was complete in 1523 patients with presumed ARNSHL; initially we tested for 35delG mutation. Persons either negative or heterozygous for this mutation were analyzed by denaturing high performance liquid chromatography and direct sequencing. We found GJB2-related hearing loss in 244 of 1523 (16.02%) ARNSHL cases. Identified deafness-causing allele variants included 22 mutations which 507insAACG, 329delA, 363delC and Q80L are the novel mutations and they have not been reported in other populations. The objective of this study is to identify the gene(s) involve in ARNSHL in Iranian Populations. As the first step the 14 known loci of ARNSHL with three and more affected individuals are being investigated by homozygosity mapping using STRs Markers. Whole Genome Screening using SNP typing has been performed for the families which can not be localized to the known loci. So far over 7 years, we have been able to collect over 772 families with two or more affected deaf and our linkage result have identified 3 new Loci which have not been mapped previously.

A critical evaluation of phenotypes associated with mutations in the TGF receptor genes. *B. Loeys[1], U. Schwarze[2], T. Holm[3], B. Callewaert[1], G. Thomas[3], J. De Backer[1], P. Coucke[1], A. Braverman[4], A. De Paepe[1], H. Dietz[3,5]* 1) Ghent Univ Hosp, Ghent; 2) Univ of Wash, Seattle; 3) Johns Hopkins Univ, Baltimore; 4) Wash Univ, St Louis; 5) HHMI.

TGFBR1/2 mutations are associated with Loeys-Dietz syndrome (LDS), a pleiotropic connective tissue disorder with particularly agressive vascular disease characterized by aortic root dissections at small dimensions and in early childhood and a predisposition for aneurysms throughout the arterial tree. Others have suggested that a subset of individuals with Marfan syndrome (MFS) or familial thoracic aortic aneurysm (FTAA), conditions usually associated with less aggressive and isolated involvement of the ascending aorta, can also be caused by TGFBR2 mutations. If individuals with TGFBR mutations cannot be distinguished clinically, the practical implication is that all patients with MFS and FTAA require genotyping to identify those at extreme vascular risk. To address this issue, we performed clinical and molecular analyses of diverse patient groups. We found no TGFBR mutations in 90 patients with ascending aortic aneurysms without specific connective tissue disorder and in 143 consecutive unrelated patients with classic MFS. In the latter, we found FBN1 mutations in 132 patients including 3 large intragenic deletions/duplications (identified by MLPA) that were missed by standard screening procedures. Of the 90 LDS patients from 52 families with TGFBR mutations, virtually all had clinical features that were distinguishing from either MFS or FTAA, including hypertelorism, cleft palate/bifid uvula, arterial tortuosity, aneurysms beyond the aortic root, craniosynostosis, club foot, easy bruising, atrophic scarring, translucent skin and visceral rupture. Natural history studies confirmed the aggressive nature of vascular disease. Mean age at death, due to thoracic (66.6%), abdominal (22.2%) and cerebral (7.4%) vascular events, was 26.0 yrs (0.5-47). For one third of patients, surgery or death occurred under age 19, a clear distinction from MFS or FTAA. Taken together, our data suggest TGFBR mutations underlie a clinically distinct phenotype requiring specialized management and counseling.

Contiguous hemizygous deletion of TBX5, TBX3, and RBM19 resulting in a combined phenotype of Holt-Oram and ulnar-mammary syndrome. *W. Borozdin[1], A.M. Bravo Ferrer Acosta[1], E. Seemanova[2], M. Leipoldt[1], M. Bamshad[3], S. Unger[1], J. Kohlhase[1]* 1) Institute for Human Genetics and Anthropology, Freiburg University, Freiburg, Germany; 2) Institute for Biology and Medical Genetics, 2nd Medical Faculty, Charles University, Prague, Czech Republic; 3) Dept. of Pediatrics, Division of Genetics & Developmental Medicine, University of Washington School of Medicine, Seattle, Washington.

Heterozygous mutations in the gene *TBX5* cause Holt-Oram syndrome (HOS), an autosomal dominant disorder characterized by radial ray upper limb malformations in combination with congenital heart defects and/ or cardiac conduction anomalies. Mutations in the gene *TBX3* result in ulnar-mammary syndrome (UMS). HOS and UMS are thought to result from haploinsufficiency of *TBX5* and *TBX3*, respectively. Although both genes are closely linked on chromosome 12, no contiguous deletion of both had yet been described. While performing deletion screening for *TBX5* by quantitative Real Time PCR we detected a heterozygous deletion in a family diagnosed with HOS. Further mapping showed that the 2.2 Mb deletion encompassed only three genes, *TBX5*, *TBX3*, and *RBM19*. Clinical re-examination revealed mild limb features of ulnar-mammary syndrome, i.e. clinodactyly of the fifth digits and hypoplastic hypothenar eminences in the affected mother and both affected daughters. The radial involvement was also rather mild, with only one daughter showing unilateral radial aplasia and the two other affected family members having small but mostly functional hands. Other features of HOS in this family included cardiac septal and conduction defects, whereas UMS features were mammary hypoplasia, axillary gland and hair hypoplasia, and subglottic stenosis. This is the first report of a contiguous deletion of both *TBX5* and *TBX3*, which results in a combined phenotype of Holt-Oram and Ulnar-Mammary syndrome with rather mild radial and ulnar malformations.

Characterisation of genomic instability in Dukes B2 colorectal cancer. *D. Marchetti[1], M.R. Iascone[1], G.D. Beretta[2], A.R. Lincesso[1], S. Mosconi[2], R. Labianca[2]* 1) Laboratorio di Genetica Molecolare; 2) USC Oncologia Medica; Ospedali Riuniti, Bergamo, Italy.

Colorectal cancer (CRC) is one of the most common malignancies in developed countries. The TNM system represents the main tool for identifying prognostic differences among patients. Although the Dukes B2 CRC is an homogeneous histopathological class, 30% of patients has recurrences during lifetime and the role of adjuvant therapy is still unclear in this class. Genomic instability is a driving force in the initiation of CRC development. The molecular genetics of human cancers can be used to categorise CRC in two major types: chromosomal instability and microsatellite instability (MSI). The aim of our study is the evaluation of genomic instability in Dukes B2 CRC patients. After histopathological classification, we studied specimens from 73 Dukes B2 CRC patients. Genomic DNA was extracted from normal and neoplastic microdissected paraffin-embedded tissues. Ten microsatellite markers were used to detect MSI and LOH at 18q and 17q, the chromosomes most frequently affected by LOH in CRC. The product of PCR was sized by WAVE System DNASep Cartridge (Transgenomics) eluted through a linear gradient of TEAA at 50 degree. Four samples were not analysed for scarcity of tumour tissue, 9 (13%) showed MSI, whereas LOH was observed in 25 (36%) at 18q, 5 (7%)at 17q and 15 (22%) at both loci. Fifteen of 69 Dukes B2 CRC (22%) don't show neither MSI neither LOH (noMSI-noLOH). This finding suggests that alternative pathways may be involved in CRC development. To evaluate this hypothesis, 2 noMSI-noLOH cases were tested by 32K array-CGH (TechnoGenetics). The analysis confirmed the absence of LOH at 18q and 17q and showed a common amplification at 13q12-q14 and several different chromosomal gains/losses. This preliminary finding could indicate that 13q12-q14 region may be critical for the progression of CRC but other rearrangements could characterise each tumour progression. Further studies are necessary to verify this hypothesis and to evaluate its clinical utility for the risk stratification of Dukes B2 patients.

MYO7A mutation screening in Usher syndrome type I patients from diverse origins. *T. Jaijo[1], E. Aller[1,2], M. Beneyto[1], C. Najera[2], C. Graziano[3], M. Seri[3], F. Moreno[4], C. Ayuso[5], J.M. Millan[1]* 1) Unit of Genetics, Hospital La Fe, Valencia, Valencia, Spain; 2) Department of Genetics. University of Valencia. Valencia. Spain; 3) U.O. e Catedra di Genetica Medica. Policlínico S. Orsola-Malpighi. Bologna. Italy; 4) Department of Molecular Genetics. Hospital Ramon y Cajal. Madrid. Spain; 5) Department of Genetics. Fundacion Jimenez Diaz. Madrid. Spain.

INTRODUCTION Usher syndrome type I (USH1) is an autosomal recessive disorder which displays severe to profound sensorineural hearing loss, prepuberal onset or RP and vestibular areflexia. Mutations in the MYO7A gene cause USH1B and has been reported as the main responsible of USH1. PURPOSE Screening of the MYO7A gene in probands with USH1 from diverse origins in order to identify the disease-causing mutations. PATIENTS AND METHODS Forty USH1 patients from Spain, Italy, Morocco, Turkey and Czech Republic were screened for mutations in the MYO7A gene. Genomic DNA was extracted from peripheral blood and PCR was used to amplify individual exons. SSCP analysis was performed and fragments displaying different migration pattern were directly sequenced to identify the sequence variant. RESULTS In this study, nineteen different mutations have been found. All mutations were private, with the exception of p.R241G which was found in three Italian families from the same region. Intragenic haplotypes were constructed and the same haplotype linked to this mutation was found. CONCLUSIONS The MYO7A gene is implicated in about 45% of USH1 patients. Most of mutations are private and no hotspots have been found. A common origin has been determined for the mutation p.R241G.

Two novel NOTCH3 mutations not involving cysteine residues in CADASIL patients. *R. Mazzei, C. Ungaro, M. Liguori, F.L. Conforti, A. Gambardella, T. Sprovieri, A. Patitucci, A. Magariello, A.L. Gabriele, A. Qualtieri, M. Muglia* ISN-CNR, C.da Burga, Mangone, Cosenza, Italy.

CADASIL is a cerebrovascular disease caused by mutations in the Notch3 gene. All described mutations resulted in an odd number of cysteine residues. Recently, we reported the first evidence of a small deletion, which did not involve a cysteine residue in an Italian family. The aim of the present study was to confirm that cysteine-sparing Notch3 mutations could cause CADASIL and that these mutations could determine a different phenotype. Two patients suspected of having CADASIL were referred at our Institute. The molecular analysis of Notch3 gene was performed by DHPLC and direct sequencing. A skin biopsy on both patients was also performed. The MRI scans showed a diffuse subcortical leukoencephalopathy suggestive of CADASIL in both patients. The molecular examination showed an amino acid variation in both patients: the R107W and the Q151E mutations. No additional mutation was found. The relatives of the first patient were not available. Three sisters of the second patient underwent to MRI and molecular analysis. The Q151E mutation segregated with the MRI phenotype. Both the two mutations have not been found in 500 control chromosomes. Skin biopsies from the two probands did not show deposits of GOM.Very recently, a novel cysteine-sparing Notch3 mutation causing CADASIL was described in four patients from Korea. T2-weighted hyperintensities of anterior temporal lobe, considered a characteristic MRI feature in CADASIL, were less often detected in patients with cysteine-sparing mutations. Furthermore, electronic microscopy examination of the skin biopsy showed GOMs only in 2 out of 5 patients reported until now with mutations not involving a cisteine residue, and in none of the 2 patients examined in the current study. The description of several patients with clinical features of CADASIL associated with mutations that do not involve cysteine residue, could change the axiom that all patients carry cysteine-related mutations suggesting that they may have some peculiar clinical and pathologic features different from those commonly observed in the classical phenotype.

Quantifying the effects of allele frequency differences and allelic phase on LD captured by tag SNPs derived from incompletely ascertained data: Theoretical basis, models and impact on LD mapping. *R. Lazarus, B. Raby, W. Qiu, E.K. Silverman, S.T. Weiss* Channing Laboratory, Brigham and Womens Hospital and Harvard Medical School, Boston, MA.

Population genetic effects on linkage disequilibrium (LD) are well understood, but the importance of minor allele frequency (MAF) differences and allelic phase are less widely appreciated. For 2 SNPs with MAF of 0.2 and 0.4, the maximum possible pairwise LD (as r^2) is 0.37, falling to 0.17 when the minor alleles are in repulsion. These effects will impact mapping with LD tag SNPs if there are SNPs not known (hidden or HSNPs) at the time of tagging, since HSNP likely have a different MAF spectrum. We tested data from the ten HapMap ENCODE regions using tags from a commercial whole-genome LD tagging SNP panel, designed using HapMap phase I data (Illumina HumanHap300). We quantified the effects of non-tag SNP MAF, the absolute difference in MAF between the best tag and the non-tag SNP, and repulsion between the tag and the non-tag SNP, as independent variables in a regression model for the best LD values captured by the tags. On average, captured r^2 decreased by 0.12 for each 0.1 increase in MAF difference, by 0.04 for each 0.1 decrease in non-tag SNP MAF and by 0.1 for non-tag SNPs in repulsion with the best available LD tag SNP. Similar patters were found in genotypes obtained from dbSNP, for 9590 SNPs with a MAF >= 0.1, discovered by resequencing 240 genes in the 23 CEPH Program in Genomic Applications (PGA) samples. The MAF distribution in the 240 resequenced genes differed substantially from the ENCODE regions (KS D=0.07, p=6.52e-12). The regression model for this data was similar, but captured LD was much lower. A similar pattern was observed among genotypes for 24 African American PGA samples in the same 240 genes, but with even lower levels of captured LD. We conclude that MAF differences and allelic phase differences cause a substantial and predictable loss in LD tagging efficiency when LD tags derived from pilot data with incomplete SNP ascertainment are used, or when tags derived from one population are applied to another population with a substantially different population history.

SIX3, ZIC2 and SHH mutations in a series of holoprosencephaly patients. *J. Herbergs, S. Spierts, D. Tserpelis, H. Smeets* Dept Clinical Genetics, Academic Hosp Maastricht, Maastricht, Netherlands.

Holoprosencephaly (HPE) is a common severe malformation of the brain that involves abnormal formation and septation of the developing central nervous system. The prevalence is 1:250 during early embryogenesis, but the live born prevalence is only 1:16000. The etiology of HPE is extremely heterogeneous and can include both a teratogenic and/or genetic basis. We studied four genes known to be involved in HPE, namely SHH, ZIC2, SIX3 and TGIF by sequence analysis. A series of in total 48 sporadic and familial HPE cases with a variable clinical spectrum has been analysed. We detected 12 pathogenic mutations (25%), 4 out of 39 sporadic cases (10%) and 7 out of 9 familial cases (78%). One of the familial cases was caused by a mutation in parental germ cells. Seven mutations were detected in the SIX3 gene, three mutations in the ZIC2 gene and two mutations in the SHH gene. The familial mutations displayed great phenotypic heterogeneity of the disease, which makes it difficult to establish genotype-phenotype correlations. This phenotypic variability may be due both to environmental factors and to potential modifier genes. HPE development is probably a multihit process , which implicates more genes; illustrating the importance of further identification of new genes.

Distinguishing apparently sporadic CADASIL from other leucoencephalopathies: the role of MRI in the NOTCH3 gene screening. *M. Liguori, C. Ungaro, F.L. Conforti, A. Patitucci, A. Magariello, T. Sprovieri, A.L. Gabriele, M. Muglia, R. Mazzei* ISN, CNR, Mangone, Cosenza, Italy.

CADASIL is a genetically linked cerebrovascular disease resulting from mutations in the Notch3 gene. The aim of the present study is to verify whether MRI criteria might help in the Notch3 gene screening of subjects with sporadic subcortical leucoencephalopathy. We presented data derived from a retrospective analysis (from 2003 to date) on patients referred to the ISN (CS) for conventional brain MRI, whose scans showed subcortical infarcts and leucoencephalopathy. Detailed clinical interviews resulted negative for family history of cerebrovascular diseases. MRI lesions were graded 0 to 4 according to the following criteria: 0= small and scattered brain lesions; 1= periventricular confluence; 2= periventricular confluence + presence of at least one of the following three localizations: external capsule (EC) or anterior temporal pole (ATP) or talamic microbleeds (TM); 3= periventricular confluence + presence of at least two of the following three localizations:EC, or ATP or TM ; 4= periventricular confluence + presence of all the above-mentioned brain localizations. In patients who scored 3/4, a mutational screening of the entire Notch3 gene was performed by DHPLC and direct sequencing. Three-hundred-sixty-one patients with sporadic subcortical leucoencephalopathy were selected on the basis of MRI findings and scored as follows: 66 with score 0; 224 with score 1; 53 with score 2; 17 with score 3 and 1 patient with score 4. The 18 patients with score 3/4 underwent molecular analysis of the entire Notch3 gene; in all but two cases (11.1%), 2 new mutations involving a cysteine residue - in exon 10 (C553S) and in exon 11 (Y574C) of Notch3 gene, respectively - were identified. The present evaluation suggests that patients with a clinical picture suggestive of CADASIL who fulfil the MRI criteria 3/4 should be submitted to the mutation screening of the entire Notch3 gene, even in absence of a positive family history.

Genotype -Phenotype Correlation in Myotonic Dystrophy And CTG Repeat Polymorphism at the Myotonic Dystrophy Locus in Healthy Iranian Population. *K. Kahrizi[1], N. Moradin[1], B. Shojasaffar`[1], A.M. Cobo[2], S. Nafiisi[3], M. Hasanzad[1], A. Soltanzadeh[3], J. Lotfi[3], K. Gharegozli[3], H. Najmabadi[1]* 1) Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran - Iran; 2) Unidad de Genetica, 3a planta,Edificio Aranzazu, Hospital Donostia, Paseo Dr Begiristain s/n ,San Sebastian 20014 Spain; 3) Neurology Department of Shariati Hospital, Tehran - Iran.

Myotonic Dystrophy (DM) type I- the most common form of muscular dystrophy in adults , affecting 1/8000 individuals - is a dominantly inherited disorder with a multisystemic pattern .The normal copy number of 5 - 37 CTG repeat is exceeded in DM patients .There is a correlation between the prevalence of DM1 and the frequency of large size normal alleles in a population. The aim of this study was to determine clinical and genetic characteristics of DM1 and also, to determine the distribution of alleles in healthy Iranian population. Fifty nine patients (31 families) registered with DM were studied .Polymerase chain reaction and Southern Blot analysis were conducted to determine the size of the expansion alleles in DM1 patients and PCR performed for two hundred healthy individuals from different ethnic groups . We studied twenty two DM - families (a total of forty eight patients) out of thirty one families with single band of who thirty five patients were diagnosed with a trinucleotide repeat expansion. We could find correlation between the number of expansion and Muscular Disability Rating Scales (MDRS), a Sum of Symptoms Score (SSS) and age of onset. Also , our data reveals that in 200 healthy individuals , 23.7% of alleles had 5 repeats, 23.3 % had 6-8 repeats, 45.8% had 9-17 repeats and 7.3 % of alleles had CTG repeats of more than 18. Our results on DM1 patients proved correlation of the expansion size and muscular disability. There is no definite correlation of cataract and endocrine dysfunction and the expansion size in DM1 patients. What we have concluded from our data in normal population shows that the frequency of alleles with CTG>18 is comparative to Western Europe and Japan.

Identification of susceptibility genes for Behçets disease using the genomic convergence approach. *T. Krug[1], B.V. Fonseca[1], G. Jesus[2], M.F. Moraes-Fontes[1], A. Bernardino[2], M. Coutinho[2], C. Neves[2], J. Vedes[3], M.J. Serra[4], J. Vaz Patto[5], C. Resende[6], B. Martins da Silva[7], J. Correia[8], C. Vasconcelos[8], J. Demengeot[1], J. Crespo[2], S.A. Oliveira[1], Portuguese Group for the Study of Behçets Disease* 1) Instituto Gulbenkian de Ciência, Portugal; 2) Hospital Infante D. Pedro, Portugal; 3) Hospital de Sousa Martins, Portugal; 4) Hospital dos Capuchos, Portugal; 5) Instituto Português de Reumatologia, Portugal; 6) Hospital de Santa Maria, Portugal; 7) Instituto de Ciências Biomédicas Abel Salazar, Portugal; 8) Hospital de Santo António, Portugal.

Behçet's disease (BD) is a systemic immuno-inflammatory disorder affecting multiple organs with generalized vasculitis of veins and arteries, particularly at mucocutaneous territories (oral and genital ulcers) and eye. The disease etiology remains unknown, but the most widely held hypothesis of disease pathogenesis is that of a profound inflammatory response triggered by an infectious agent in a genetically susceptible host. Although there is evidence for environmental risk factors, epidemiological and family studies strongly support the existence of genetic risk factors. The only established genetic predisposition is the HLA-B*51 gene (chr. 6p21) and its contribution to the overall genetic susceptibility to BD has been estimated to be only 19%. Furthermore, a recent linkage study on Turkish families found strong evidence for linkage with BD at 6p22-24 and 12p12-13. Case-control association studies on biological candidate genes have so far mostly been inconclusive. To identify new susceptibility genes for BD, we are conducting a novel and multidimensional genomic convergence approach, which combines data from whole genome linkage screens with expression studies to determine which genes will be tested in association studies. We will present the results of our linkage screen on several Portuguese multiplex BD families, of our RNA expression studies on blood mononuclear cells from BD patients and controls, and of the association studies on genes differentially expressed in cases and controls which map to linkage peaks.

DNA secondary structure induces genomic instability leading to the recurrent 11;22 chromosomal translocation.

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Human mutations like chromosomal translocations are thought to be random events. However the constitutional t(11;22)(q23;q11) in humans occurs recurrently at restricted breakpoint regions. We identified palindromic AT-rich repeats (PATRRs) at the t(11;22) breakpoint cluster regions both on chromosomes 11 and 22, and suggested that the PATRR induces genomic instability by forming a DNA secondary structure. Recent findings of others regarding potential H-DNA at the t(14;18) breakpoints in follicular lymphomas support our hypothesis. We previously demonstrated polymorphisms of the PATRR that affect the frequency of *de novo* occurrence of the translocation. In this study, we have hypothesized that polymorphism of the PATRR affects its secondary structure-forming propensity, and performed the following experiments; (1) *in silico* calorimetric analysis using the DNA mfold server calculating the free energy for various PATRRs, (2) an *in vitro* cruciform extrusion assay to examine mobility shift in agarose gel electrophoresis using plasmids bearing various PATRR11s, and (3) *in vivo* deletion frequency of PATRR11 plasmids in wild-type *E. coli*. Using these methods, we validated the secondary structure-forming propensity of various PATRR11s, and compared them with the frequency of *de novo* occurrence of the t(11;22) translocation. The results demonstrate that secondary structure-forming propensity correlated well with translocation frequency, Our results provide indirect but strong support to the hypothesis that the PATRR adopts a cruciform conformation in living cells that induces genomic instability leading to the translocation.

Poly-Alanine tract expansion in Aristaless-related homeobox (ARX) causes cognitive impairment in MRX87 family. *M.G. Miano[1], C. Laperuta[1], P. D'Adamo[2], A. Maiorino[3], P. Chiurazzi[4], V. Ventruto[1], C. Carbone[1], M. DUrso[1], M.V. Ursini[1]* 1) Institute of Genetics and Biophysics A.B.Traverso, CNR, Naples, Italy; 2) Tigem, Telethon Institute, Naples, Italy; 3) C.A.R.S.I.C. Institute, Venafro, Italy; 4) Catholic University of Rome, Rome, Italy.

Genetic cognitive impairments are heterogeneous conditions with 10% of forms linked to the X human chromosome and mostly caused by defects of genes involved in brain development and plasticity. One of these is Aristaless (ARX) encoding a homeobox transcription factor of the Q50 Paired-like (Prd-like) class whose murine ortholog regulates the brain organogenesis and neurogenesis. Mutations in ARX comprise a continuous series of developmental brain disorders including macro-alteration of brain such as lissencephaly, and ends with a series of overlapping syndromes with cognition impairment and apparently normal brain structure. Here we report on the clinical and genetic analysis of an Italian family with a syndromic mental impairment segregating with X-linked inheritance (XLMR). Linkage evaluation delineates a new locus, MRX87, between DXS1740 and DXS1214, placing it in Xp22-p21. This is a hot spot region for mental handicap, which comprises many XLMR genes even included ARX. By direct sequencing, we identified in association with the disease a new ARX duplication causing a poly-alanine tract espansion. This mutation was observed to segregate with an intra-familial clinical heterogeneity and penetrance variability as showed by the affected males presenting a mixture of mental handicap and subtle congenital abnormalities. Its pathogenic mechanism is still unknown but similarly to other Ala expansions causing congenital defects, it could disturb the transcriptional properties of Aristaless. Our study of MRX87 locus is consistent with the notions that poly-Ala tract expansions are the main mutations of ARX, a gene hot spot for cognition disorders linked to Xp22-p21 region. Furthermore, our findings enrich the pictures of the correlated phenotypes raising Aristaless as a pivotal transcriptional factor essential to brain/head development and offering the opportunity of unmasking new pleiotropic actions in humans.

Gender-specific linkage of quantitative traits for obesity in Hong Kong Chinese. *M.C.Y. Ng, C.H.T. Tam, V.K.L. Lam, J.C.N. Chan* Department of Medicine & Therapeutics, The Chinese University of Hong Kong, Hong Kong.

Mapping complex traits is difficult due to the complex interaction between genetic and environmental factors. Gender is a key modulator affecting distribution of traits such as anthropometric measures. We hypothesized the presence of gender-specific effect on the quantitative trait loci for obesity measures.

We conducted autosomal genome scans with 355 microsatellite markers in 179 families (897 members, 46% males) ascertained through a proband with Type 2 diabetes from the Hong Kong Family Diabetes Study. We estimated heritability and performed variance component-based linkage analyses on three obesity-related traits: body mass index (BMI), waist circumference (WC) and body fat percentage as measured by bioimpedance (FAT) in all subjects as well as in males and females separately.

Males had significantly higher BMI (25.44.1 vs. 24.54.7 kg/m^2), WC (8610 vs. 7811 cm) but lower FAT (246 vs. 346 %) than females (P 0.05). Heritability estimates suggested stronger genetic loadings of these traits in females than in males: BMI (81% vs. 57% in females and males, respectively), WC (84% vs. 67%) and FAT (63% vs. 50%). Variance component linkage analyses revealed significant linkage at chromosome 1q24-25 for BMI (LOD = 4.4), WC (LOD = 3.7) and FAT (LOD = 4.4) in all samples. Stratification by gender showed similar linkage for BMI (LOD = 3.0 for males and 2.7 for females) and WC (LOD = 2.5 for males and 2.4 for females) in each gender. However, the linkage for FAT at the same region was largely attributed by females (LOD = 6.3) rather than by males (LOD = 0.1).

In summary, we found significant linkage of obesity measures at chromosome 1q24-25. The linkages for BMI and WC were contributed by both gender while the linkage for FAT was only contributed by females. The results suggest the presence of two or more independent loci in this region that contribute to obesity measures, with or without interaction with gender.

An age related homeostasis mechanism is responsible for spontaneous amelioration of hemophilia B Leyden. *K. Kurachi[1], J.S. Huo[2], A. Ameri[2], Z. Zhang[2], E. Kasama[1], T. Tanaka[1], J. Hoff[1], S. Kurachi[1]* 1) Age Dimension Research Ctr, AIST, Tsukuba, Japan; 2) Department of Human Genetics, University of Michigan.

Little has been known about genetic mechanisms regulating diseases with specific patterns of progression over the course of a lifetime. One example of such diseases is hemophilia B Leyden, a unique subset of hemophilia B that shows an unusual pattern of puberty-onset spontaneous amelioration. The mechanisms underlying hemophilia B Leyden have remained a mystery. We previously showed that two genetic elements, ASE and AIE, of the ASE/AIE-mediated genetic mechanism of age-related regulation of gene expression regulate age-related changes in gene expression, specifically from puberty into old age. Through construction of transgenic mouse models reproducing the Leyden phenotype, here we demonstrate that the ASE of this mechanism plays an essential role for puberty-onset expression of human FIX and amelioration of hemophilia B in a sex-independent manner. Binding of a specific liver nuclear protein to ASE parallels with the puberty-onset gradual increase in expression of human factor IX in these animals, and the binding protein was identified as a proto-oncogenic Ets family protein. Human factor IX expression in these transgenic animals was nullified by hypophysectomy, but fully restored by growth hormone, thus agreeing with the sex-independent amelioration of hemophilia B Leyden. This work identifies for the first time the molecular mechanism regulating puberty-onset changes in expression seen in hemophilia B Leyden, and demonstrates the clinical relevance of the ASE/AIE-mediated regulatory mechanism of age-associated changes in gene expression.

mtDNA point mutations are present at various levels of heteroplasmy in human oocytes. *L.J.A.M. Jacobs[1,2], M. Gerards[1,2], P.F. Chinnery[3], J.C.M. Dumoulin[2, 4], J.P.M. Geraedts[1,2], H.J.M. Smeets[1, 2]* 1) Genetics and Cell Biology, University of Maastricht, Maastricht, Netherlands; 2) Research institute GROW, University of Maastricht, Maastricht, The Netherlands; 3) Mitochondrial Research Group, University of Newcastle upon Tyne, Newcastle upon Tyne, UK; 4) Department of IVF, Academic Hospital Maastricht, Maastricht, The Netherlands.

Little is known about the load of mutations and polymorphisms in the mtDNA of human oocytes and the possible effect these mutations may have during life. To investigate this, we optimised at the single cell level the recently developed method to screen the entire mtDNA for mainly heteroplasmic mutations by denaturing high performance liquid chromatography (DHPLC) analysis. This method is sensitive (~1% heteroplasmy detectable), specific and rapid. The entire mtDNA of 26 oocytes of 13 women was screened by this method. Ten different heteroplasmic mutations, of which only one was located in the D-loop and of which two were observed twice, were detected in seven oocytes with mutation loads ranging from less than 5% to 50%. From 8 women more than one oocyte was received and in four of them heteroplasmic differences between oocytes of the same woman were observed. In one of these four also two homoplasmic D-loop variants were detected. Additionally, four oocytes of a single woman were sequenced using the MitoChip (which lacks the D-loop region), but all sequences were identical. It is concluded that heteroplasmic mtDNA mutations are common in oocytes and that depending on the position and mutation load they might give rise to OXPHOS disease early or later in life.

Proposition for a multi-step mutation detection in Hemophilia A. *N. Lannoy[1], I. Abinet[1], Ch. Verellen[1], Ch. Vermylen[2], C. Hermans[3], K. Dahan[1]* 1) Center of Human Genetics, UCL, Saint-Luc Hosp, Brussels, Belgium; 2) Departement of Pediatric, UCL,Saint-Luc hosp, Brussels, Belgium; 3) Departement of Haematology, UCL,Saint-Luc hosp, Brussels, Belgium.

With an incidence of one in 10000 males born worldwide, haemophilia A is the most common hereditary hemorrhagic disorders. Its is caused by a deficiency or dysfunction of blood coagulation Factor VIII (F8). Phenotypically, three classes of hemophilia A are distinguished according F8 residual clotting activity (severe, moderate and mild). Identification of causal mutation is important for proper management of affected boys and detection of female carriers. The size of the F8 gene (26 exons) as well as the large mutational spectrum including intronic inversions and various private small mutations represent a challenge for routine mutation screening. In this context, we developed a rapid multi-step approach for detection of F8 mutations in a large cohort of 74 unrelated hemophilia A patients (46 severe, 5 moderate and 23 mild forms) regulary followed at the Hemophilia Center of the Cliniques universitaires Saint-Luc, Brussels. The first step which includes the intron 22 inversion resulted in the detection of 23 mutations (23/46, 50%) among patients with severe disease. The second step based on the relative existence of hot spot exons (europium.csc.mrc.ac.uk) allowed by direct sequencing of exons 7, 8, 11, 14, 18, 23, 24 and 26 identification of 12 change mutations (4 deletion, 1 insertion, 4 missense, 2 non sense and 1 splice defect) in severe patients (12/46, 26%) and 14 missense mutations in 2 moderate (2 /5, 40 %)and 12 mild (12 /23, 52%) patients. In the third step, the remaining exons were analysed leading to the identification of 19 additional sequence changes in 5 severe, 3 moderate and 11 mild patients. No case of intron 1 inversion was detected. This multi-step approach which can be easily implemented in diagnosis laboratory allowed a rapid identification of 92% mutations (68/74) in a cohort of phenotypic heterogeneous families. Interestingly, 13 mutations identified in this study have, to our knowledge, not been previously reported.

Comparative analysis of the SCA10 ATTCT pentanucleotide repeat indicates the repeat sequence originates from retrotransposons. *T. Kurosaki[1], T. Matsuura[2], K. Ohno[2], S. Ueda[1]* 1) Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo, Japan; 2) Division of Neurogenetics and Bioinfomatics, Center for Neurological Diseases and Cancer, Nagoya University Graduate School of Medicine, Nagoya, Japan.

Spinocerebellar ataxia type 10 (SCA10) is a dominantly inherited neurodegenerative disorder caused by unstable expansion of the ATTCT pentanucleotide repeat in the intron 9 of ATXN10 gene. There is a large gap between the documented normal (10-29) and the mutated repeat length (280-4500) associated with SCA10. The SCA10 expansion has also unique characteristics of instability in both somatic and germ line tissues, which is distinct from other repeat expansions. However, little is known about the underlying molecular basis, since the repeat size of the expansion is too large for its characterization.
For better understanding of the ATTCT repeat instability, we compared the genome sequences in mammals. Interestingly, the repeat was found only in primates. To gain a further insight into the origin and evolutional history of the ATTCT repeat, we performed long PCR and direct sequencing of apes, Old World monkeys and New World monkeys. We found the ATTCT repeat was not preserved throughout the primate lineage. In Old World monkeys, ATTCT was replaced by CTTGT. Moreover, the repeat was exactly placed on a boundary of LINE and Alu, and the 3.2-kb region surrounding the repeat was entirely deleted in New World monkeys. Taken together, it suggests that the repeat-containing fragment was retrotransposed into the intron 9 of ATXN10 in the common ancestor of catarrhines.

Development of a new array-MAPH methodology for detection of copy-number changes and screening of patients with X-linked mental retardation. *A. Kurg[1], L. Kousoulidou[2], K. Männik[1], S. Parkel[1], O. Zilina[1], N. Tõnisson[1], C. Sismani[2], P. Palta[1], H. Puusepp[1], M. Remm[1], P.C. Patsalis[2]* 1) Dept. of Biotechnology, Institute of Molecular & Cell Biology,Univ. of Tartu, Tartu, Estonia; 2) Dept. of Cytogenetics, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus.

Accurate and sensitive genome-wide screening to detect small genomic imbalances has been a technical challenge for a long time. The focus of this study was to introduce a novel methodology, based on a new type of microarray, using the basic principle of Multiplex Amplifiable Probe Hybridization (MAPH) for fast, accurate and reliable determination of copy-number changes of virtually any loci in complex genomes. We have developed a new methodology and software for designing PCR-amplifiable hybridization probes (200-600bp) that can be used for both array-MAPH and array-CGH and also a web interface called MAPHDesigner (http://bioinfo.ebc.ee/MAPH) for the developed programs. We have designed, amplified, cloned and spotted onto arrays 560 target sequences for human chromosome X to cover it uniformly with median spacing of 238kb, while in certain regions of interest with resolution up to 3kb. Another 107 probes from autosomal chromosomes were selected and used as normalization controls. For validation of the new methodology, several normal and patient DNA samples with known and unknown chromosome X aberrations were analyzed. Array-MAPH detected deletions and duplications, which were confirmed by PCR and/or FISH analyses, demonstrating the accuracy and sensitivity of the new approach. The new array-MAPH method was further applied for screening of 20 male patients from families with X-linked mental retardation (kindly provided by EURO-XLMR consortium). One deletion of 500kb and two duplications of 1.6Mb and 0.9Mb were detected and their segregation in the corresponding families was investigated. The new microarray methodology provides an alternative to array-CGH as well as several advantages for high-throughput diagnostic screening by enabling high flexibility to study virtually any region in the human genome.

The genetic variation and population history in the Baltic Sea region. *P. Lahermo[1], V. Laitinen[2], S. Koivumäki[3], P. Sistonen[4], P. Anderson[5], M-L. Savontaus[2,3], K. Huoponen[2], T. Lappalainen[1]* 1) Finnish Genome Center, University of Helsinki, Finland; 2) Department of Medical Genetics, University of Turku, Finland; 3) Department of Biology, Laboratory of Genetics, University of Turku, Finland; 4) Finnish Red Cross Blood Transfusion Center, Helsinki, Finland; 5) Department of Neurology, Umeå University Hospital, Sweden.

Sharp genetic borders within a geographically restricted region are known to exist among the populations around the northern Baltic Sea on the northern edge of Europe. We studied the population history of this area in greater detail from paternal and maternal perspectives with Y chromosomal and mitochondrial DNA markers. Over 1700 DNA samples from Finland, Karelia, Estonia, Latvia, Lithuania and Sweden were genotyped for 18 Y-chromosomal biallelic polymorphisms and 8 microsatellite loci, together with 18 polymorphisms from the coding area of mtDNA and sequencing of the HVR1. Y chromosomal haplogroups from the biallelic data indicate both various phases of gene flow and existence of genetic barriers within the Baltic region. Haplogroup N3, being abundant on the eastern side of the Baltic, differentiates between eastern and western sides of the Baltic Sea, just like R1b that has a reverse frequency pattern to N3. The typically Scandinavian haplogroup Ia1 has a high frequency of up to 40%, separating not only Sweden but also Western Finland from the other populations. The frequency of haplogroup R1a1, most characteristic to Slavic peoples, varied substantially across the populations. In addition to biallelic markers, Y-chromosomal microsatellite loci were analyzed for a more detailed approach to the history of the paternal lineages in the region. We also analyzed mtDNA markers with special interest for sub-haplogroups of H and U, that among other haplogroups, show substantial variation between the populations (e.g. haplogroups H1, H2, T and J1). In conclusion, our current Y-chromosomal and mtDNA data suggest various incidents of gene flow from different sources, each reaching partly different areas of the Baltic region, which can be thus seen as a meeting point of a not only culturally but also genetically diverse set of populations.

COL11A1-gene is Both Linked to and Associated with Human Stature. *J. Kettunen[1], E. Costiander[1], S. Sammalisto[1], L. Peltonen[3,4], M. Perola[1,2]* 1) Department of Molecular Medicine, Finland's National Public Health Institute, Helsinki, Finland; 2) Department of Medical Genetics, University of Helsinki, Finland; 3) Academy Professor, University of Helsinki and National Public Health Institute, Finland; 4) Visiting Professor, the Broad Institute of MIT and Harvard, Boston, USA.

Stature (i.e. adult height) is a quantitative trait with high heritability. Various interesting regions in the human genome have been linked to adult stature but only few have been confirmed by later studies. Recently, we localized a quantitative trait locus locus for stature in chromosome 1p21 (multipoint LOD score 4.25 in sex-statified males-only sample). The most promising candidate gene in this region of interest was COL11A1. We genotyped 24 single nucleotide polymorphisms (SNPs) in 92 families totalling 921 genotyped individuals. In this study we show consistent evidence of linkage in a subset of previously linked families (n = 54, p = 0.05), which is further increased when we add additional family members (p = 0.002) and is replicated in a set of independent families (n = 38, p = 0.04) that have not been analyzed previously for linkage to stature. Joint analysis of all available families yielded yet greater evidence for linkage (p = 0.0004). Two markers in the COL11A1-gene show association to stature in the families with additional family members (n = 54): an intronic SNP with no known function (intron 58, p = 0.008) and a non-synonymous exonic SNP that changes an amino acid 1535 from proline to serine in the proteins major triple helix (p = 0.013). Thus, we have here both replicated the original linkage result in an independent sample and shown association to a genetic variant for human adult stature, implying that genetic variation in the COL11A1-gene contributes to the variation observed in human stature.

Preferential X chromosome loss but random inactivation characterize women with primary biliary cirrhosis. *M. Miozzo[1], C. Selmi[2], P. Invernizzi[3], S.M. Sirchia[1], S. Maitz[3], B. Gentilin[1], M. Zuin[3], M.E. Gershwin[4], M. Podda[3]* 1) Medical Genetics, San Paolo School of Medicine, University of Milan, Italy; 2) Internal Medicine, Department of Clinical Sciences Luigi Sacco, University of Milan, Italy; 3) Internal Medicine, San Paolo School of Medicine, University of Milan, Italy; 4) Division of Rheumatology, University of California School of Medicine, Davis, CA.

Primary biliary cirrhosis (PBC) is a chronic cholestatic autoimmune disease of unknown etiology characterized by 9:1 female predominance.The importance of the genetic background in PBC was demonstrated by the concordance rates among monozygotic twins and by the enhanced frequency of X monosomy in peripheral blood T and B cells in PBC women. We suggest that the genetic mechanism linking X monosomy to PBC relies on haploinsufficiency of genes escaping X inactivation(XCI), and that a non random X loss in monosomic PBC cells might unmask X-linked haplotypes related with autoimmunity. We determined the X dosage and XCI pattern in women with PBC and controls to establish whether: X monosomy is caused by the preferential loss of an X; PBC women display a skewed XCI; X monosomy rates correlate with specific XCI patterns;X defects are restricted to PBC peripheral blood mononuclear cells (PBMC). We performed X dosage in PBMC and buccal cells (BS) from 166 women with PBC and 177 age-matched healthy women by genotyping a panel of 4 X linked polymorphisms using QF-PCR.XCI was carried out by HUMARA test. The frequency of women with skewed XCI (75 or 90) in PBMC and in BS was similar in PBC and controls.The study of X-linked loci demonstrated a preferential X chromosome loss in 47% of PBCs and 18% of controls ($P<0.0001$),independent of the XCI pattern. No differences in X defects were observed between BC from women with PBC and controls. These data highlight that: X loss does not occur randomly in PBC; preferential X monosomy is specific for PBMC; Skewed XCI is similar in PBC to controls.We suppose that hemizygosity of genes escaping XCI, caused by preferential X loss unmask peculiar haplotypes conferring susceptibility to autoimmunity.

A cohort of patients with generalized Fibromuscular Dysplasia and features of Ehlers-Danlos Syndrome: A new phenotype. *N.B. McDonnell[1], J. Yang[1], W. Chen[1], B. Griswold[1], C.A. Francomano[1,2]* 1) National Institute on Aging, NIH, Baltimore, MD; 2) Harvey Inst Human Gen, Greater Baltimore Medical Center, Baltimore, MD.

The Ehlers-Danlos syndromes (EDS) are a heterogeneous group of hereditary disorders of connective tissue. Vascular dissections and aneurysms are a cardinal feature of the vascular form of EDS (VEDS) caused by mutations in COL3A1. Loeys-Dietz syndrome, a closely related phenotype, is caused by mutations in TGFBR1 or TGFBR2. We have identified a group of patients without mutations in COL3A1, TGFBR1 or TGFBR2 who presented with arterial dissections and aneurysms as well as stenotic lesions with a diagnosis of fibromuscular dysplasia (FMD) by pathology or radiology. 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. Several of the patients had a family history of premature death from vascular events, as well as a family history of joint and skin abnormalities compatible with an autosomal dominant inheritance pattern. There were no reports of uterine or bowel rupture, or pregnancy related death in personal or family histories. The facial features were not characteristic of VEDS or Loeys-Dietz syndrome. The first patient identified was a 44 year old woman who had a history of carotid dissection, ruptured cerebral aneurysms, FMD of renal arteries and iliac vessels, multiple atrophic scars, frequent joint dislocations, and stretchy and doughy skin. A cohort of thirty patients with this syndrome has been identified and detailed phenotype and family history information has been assembled. The etiology of fibromuscular dysplasia is thought to be heterogeneous, with genetic and environmental factors proposed as possible contributors. Our findings suggest that there is a previously unrecognized variant of EDS, distinct from the VEDS and Loeys-Dietz syndrome, with FMD as a major clinical feature in addition to the skin and joint abnormalities.

MYH7 and MYBPC3 gene mutations in pediatric hypertrophic cardiomyopathy. *M.R. Iascone[1], D. Marchetti[1], A. Iacovoni[2], A.R. Lincesso[1], C. Mammana[2], S. Pentiricci[2], M. Triggiani[2], A. Gavazzi[2], P. Ferrazzi[2]* 1) Molecular Genetics Lab; 2) Cardiovascular Dept, Ospedali Riuniti, Bergamo, Italy.

Sarcomeric gene mutations are a well known cause of hypertrophic cardiomyopathy (HCM) in adults. Although these alterations may present before adult life, they were not extensively investigated in children. The present study is a description of clinical, pathological and genetic findings in a series of children (pts) with HCM referred to our Center. From 2001 to 2005, 40 HCM unrelated pts were investigated for MYH7 and MYBPC3 genes mutations. The disorder was diagnosed by echocardiography in 37 pts, while at autopsy in the other 3. At diagnosis, the age was <1yr in 8 pts, 1-6yrs in 8, 7-12yrs in 7 and 13-17yrs in 17. Among these 40 pts (27 males), 17 were previously transplanted, 14 were referred for transplant evaluation (3 died in waiting list), 6 were hospitalized for surgical myectomy, while 3 were diagnosed at autopsy because of sudden cardiac death. Myocardial tissue was available for histological analysis in 29 pts. The concordance between clinical and pathological diagnosis of HCM was good in almost all cases, except for 2 pts that had a different diagnosis after pathological examination. Re-examination of clinical records of these 2 pts revealed a latent neuromuscolar disorder and were excluded from the study. After informed consent, genomic DNA was extracted from blood and all exons and flanking intronic regions of MYH7 and MYBC3 genes were analyzed by DHPLC and direct sequencing. A gene mutation was present in 19/38 cases (50%). Six are de novo and 13 had a positive family history for HCM, confirmed by genetic analysis. Twelve mutations (57%) were not previously described in literature. Two pts showed a double mutation, 9 had mutations in MYH7 gene and 8 in MYBPC3 gene. There was no correlation between type of mutation or mutant gene and severity of clinical manifestations, although the double mutant patients were younger (age <1yr) and had a very severe phenotype. Our data show that sarcomeric gene mutations could play a major role in the pathogenesis of pediatric HCM. Further studies will improve our understanding of genetic causes in this cohort of patients.

ESRETNET: A Spanish network for the clinical and genetic study of inherited retinal disorders. *J.M. Millán[1], D. Valverde[2], M. Baiget[3], G. Antiñolo[4], M. Carballo[5], C. Ayuso[6]* 1) Unit of Genetics, Hospital La Fe, Valencia, Spain; 2) Department of Biochemistry, Genetics and Immunology. University of Vigo, Vigo, Spain; 3) Department of Genetics. Hospital Sant Pau, Barcelona, Spain; 4) Department of Genetics, Hospital Virgen del Rocio, Sevilla, Spain; 5) Laboratory, Hospital de Terrassa, Barcelona, Spain; 6) Department of Genetics, Fundacion Jimenez Diaz, Madrid. Spain.

EsRetNet is a Spanish network composed of six groups that arose in 1991 in order to achieve the following objectives: § Development of a National Retinal Degeneration (RD) Register § Create a Case Report Format § Review and Update of clinical and molecular procedures § Implementation of molecular technology (direct and indirect screening) § Mutational Screening of Known Genes § Identification of new loci/genes § Clinical and Genetic characterisation of syndromic forms of RD § Genotype-Phenotype correlation § Generation of homogeneous patient groups for future clinical trials § Spreading of results among patient organizations, researchers ophthalmologist, ENTs. Up to date, EsRetNet has collected DNA samples of over 3000 RD patients from over 2000 different families. Among them, 70% are patients suffering from isolated Retinitis Pigmentosa (RP), 18% syndromic retinitis pigmentosa (most of them Usher syndrome but also Bardet-Biedl syndrome and others) and 12% other RD (Autosomal dominant macular degeneration, Norrie disease, choroideremia, X-linked retinoschisis, Stargardt disease and Congenital Leber amaurosis). Further details about the genetic findings for every disorder will be shown.

Genetic Analysis of PHOX2B Gene in Congenital Central Hypoventilation Syndrome (CCHS). *C.C. Hung[1], Y.N. Su[2], W.L. Lin[1]* 1) Institute of Biomedical Engineering, National Taiwan University, Taipei, Taiwan; 2) Department of Medical Genetics, National Taiwan University Hospital, Taipei, Taiwan.

BACKGROUND Congenital central hypoventilation syndrome (CCHS) is a rare neurological disorder which is characterized by abnormal autonomic central nervous system control of breathing during sleep. The mutation in PHOX2B gene, including point mutations, frameshift and a polyalanine expansion, is relevant to the pathogenesis of this disease.

METHODS We analyzed PHOX2B mutations in 7 CCHS patients, the family members and 1520 healthy individuals from general populations. Our approach is based on polymerase chain reaction (PCR), capillary electrophoresis (CE), denaturing high-performance liquid chromatography (DHPLC) and direct sequencing with high sensitivity and resolution.

RESULTS By applying this technique, we identified 7 mutations in PHOX2B gene, including 2 frameshift mutations and 5 polyalanine expansions in the 20-residue polyalanine tract. Additionally, allele and genotype distributions showed significant differences noteworthy. $(GCN)_{20}$ had the highest allele frequency of 94.84% in populations, and $(GCN)_{15}$ was the second common, whereas its frequency was 4.51%. The remaining allele were $(GCN)_{13}$ and $(GCN)_{7}$, with frequency 0.59% and 0.06% respectively.

CONCLUSIONS The homeobox gene PHOX2B mutations in CCHS patients present variable phenotypes suggest the size of expansion allele was associated with the risk of CCHS. We demonstrate that capillary electrophoresis can be used to improve detection of polyalanine expansions in the PHOX2B gene. It is a fast, simple, reliable and non-fluoresce tool for identification of PHOX2B gene mutations. We believe the described method will be suitable for routine clinical application and screening in trinucleotide repeat tracts.

The TRIM50 and TRIM37 complex: a link between Williams Beuren Syndrome and Mulibrey Nanism. *G. Merla[1], L. Micale[1], B. Augello[1], C. Fusco[1], C. Ucla[2], G. Meroni[3], L. Napolitano[3], A. Reymond[4]* 1) Medical Genetics Unit, IRCCS Casa Sollievo Della Sofferenza Hospital, S.G. Rotondo, Italy; 2) Department of Genetic Medicine and Development, University of Geneva Medical School, Geneva, Switzerland; 3) TIGEM, Telethon Institute of Genetics and Medicine, Naples, Italy; 4) Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland.

Williams-Beuren Syndrome (WBS, OMIM#194050) is a neurodevelopmental disorder with multisystemic features caused by a microdeletion at 7q11.23. Using comparative genomics approaches we identified a TRIpartite Motif-containing gene (TRIM) in each of the three LCRs that flank the WBS deletion, TRIM50, 73 and 74. TRIM50, the only one hemizygous in WBS patients encodes a putative protein that contains a RING, a B-box type 2, a coiled-coil and an RFP-like domain, while TRIM73 and TRIM74 miss the last domain. Some TRIM proteins were recently shown to play a role in ubiquitylation. Consistently we show that the TRIM50 protein has ubiquitin-ligase activity and interacts with E2 ubiquitin-conjugating enzymes. Interestingly it colocalizes with TRIM37 in discrete cytoplasmic structures. This association was further confirmed by coimmunoprecipitation of the two proteins. TRIM37 encodes an additional E3-ubiquitin ligase, that when mutated is responsible for Mulibrey Nanism (MUL, OMIM # 253250). MUL is a recessive disorder that includes severe growth failure, dysmorphic features, pericardial constriction. The tissue expression of Trim50 shows significant positive correlation with expression of Trim37, being both genes highly expressed in testis, pancreas and brain. In conclusion we found an unexpected relationship between two apparently distinct disorders suggesting a possible common role for the TRIM50:TRIM37 complex in both Syndromes probably involved in the Proteasome-mediated degradation pathway.

Association of IL4R gene polymorphisms with asthma in Chinese population. *X. Kong[1,7], H. Zhang[1], Q. Zhang[1], L. Wang[2], H. Chen[3], Y. Li[4], T. Cui[5], W. Huang[6], L. Zhang[1], F. Yan[1], L. Wang[1], Y. Xu[2], L. Hu[1]* 1) Health Sci Ctr, Sibs, Shanghai, China; 2) Department of Respiratory Disease of Renji Hospital, Shanghai Second Medical University, 200025, Shanghai, China; 3) Department of Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing 100005, China; 4) Department of Pediatrics, the First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China; 5) Union Hospital, Tongji Medical School, Huazhong University of Science and Technology, Wuhan 430022, China; 6) Chinese National Human Genome Center at Shanghai, Shanghai 201203, China; 7) State Key Laboratory of Medical Genomics, Ruijin Hospital, Shanghai 200025, China.

Cytokines, having central functions in immunological and inflammatory process, are expected to play important roles in the pathogenesis of various diseases, such as asthma. Genetic polymorphisms of those cytokine and cytokine receptor genes are the focus of genetic association studies. In an effort to identify gene(s) whose variant(s) are associated with the asthma, we screened all exons and their flanking regions, as well as the promoter region (1.5kb) of eight genes, including IL4, IL13, IL12B, IL5, IL3, IL9, CD14 and IL4R. We identified 42 single nucleotide polymorphisms, 15 of which were novel. Then, we examined the genetic effects of 30 single nucleotide polymorphisms in eight cytokine and cytokine receptor genes on asthma in a Chinese asthma cohort (n=537). Genetic association analysis of polymorphisms revealed that six polymorphisms (c.899-2C>A, c.1199A>C, c.1242G>T, c.1291T>C, c.1299T>C, c.1507T>C) in IL4R gene, three of which resulted in an amino-acid change, showed significant association with the risk of asthma (P=0.00023). Further analysis indicates that these six polymorphisms segregated in strong linkage disequilibrium. The genetic association of IL4R with asthma might provide valuable insights into the pathogenesis of asthma.

A *de novo* mutation in *endoglin* gene in a Japanese family with hereditary hemorrhagic telangiectasia. *N. Matsui*[1], *Y. Izumi*[1], *H. Azuma*[2], *R. Kaji*[1] 1) Neurology,The University of Tokushima, Tokushima, Tokushima City, Japan; 2) Medicine and Bioregulatory Sciences, Institute of Health Biosciences, The University of Tokushima, Tokushima, Japan.

Hereditary hemorrhagic telangiectasia (HHT), also known as Rendu-Osler-Weber syndrome, is an autosomal dominant disorder that affects vascular malformations and hemorrhage. The *endoglin* (*ENG*), which is located on chromosome 9q33-34, is responsible gene for HHT1. HHT1 is associated with a higher incidence of pulmonary arteriovenous malformations (PAVMs). We had an 18-year-old Japanese woman, who suffered from a repeating epistaxis and an embolic stroke associated with PAVMs. Because her sister and mother also showed epistaxis, we examined the clinical course and gene related to the proband and her family history. We report here that a *de novo* mutation in the endoglin gene segregated perfectly with HHT1 in this Japanese family. More than 130 *endoglin* mutations have been reported all over the world so far. This mutation (IVS3+1GA), which is the second report in Japan, has been reported four times in three ethnic groups, Canadian, German and Japanese. Taking advantage of the past reports, we suggests that this splice site mutation resulted in skipping of exon3.

A Non-coding SNP Is Associated With Lethal Congenital Contracture Syndrome. *H. Nousiainen[1], N. Pakkasjärvi[1], R. Herva[2], M. Kestilä[1], L. Peltonen[1,3,4]* 1) Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland; 2) Department of Pathology, University of Oulu, Finland; 3) Department of Medical Genetics, University of Helsinki, Finland; 4) The Broad Institute, Boston, MA, USA.

Lethal Congenital Contracture Syndrome (LCCS) is a severe developmental disorder that results in the death of the fetus before 32nd gestational week. Affected fetuses display the FADS (Fetal Akinesia Deformation Sequence) phenotype, which includes joint contractures, small jaw, lowset ears, fetal hydrops, and pulmonary hypoplasia. Histopathological analysis reveals degeneration of muscles and the anterior horn of the spinal cord. LAAHD is another form of lethal arthrogryposis, in which the neuropathological findings are similar to LCCS, but the fetuses may survive to full term and the degeneration of muscles and the spinal cord is slightly less severe. Both syndromes are inherited in the autosomes recessively. The LCCS locus has been mapped to chromosome 9q34. By monitoring for shared haplotypes in affected individuals, we have restricted the critical chromosomal region to 1 Mb between markers D9S1827 and D9S972. We sequenced 27 regional genes and identified only one nucleotide variant differentiating patients and controls: a previously uncharacterized single nucleotide polymorphism (SNP) in the 3UTR region of the SLC27A4 gene that shows a strong association with LCCS and immediately provided us with a powerful diagnostic tool to help the families affected with the disease. 100% of the fetuses with an assertive LCCS diagnosis are homozygous for this previously uncharacterized nucleotide variant. By sequencing control samples we have estimated the carrier frequency of the variant allele to be 2% in the Finnish population. No homozygotes for the variant allele were found among the healthy family members of the affected fetuses or in the control samples; however, heterozygotes were found in families affected with LAAHD. Ongoing sequencing efforts of the LCCS and LAAHD alleles will reveal whether these two syndromes are truly allelic. Functional studies concerning the pathogenity of the 3UTR SNP are being carried out.

Prevalent filaggrin mutations are a major genetic factor for atopy, atopic dermatitis and eczema-associated asthma. *W.H.I. McLean[1], A. Sandilands[1], A. Terron-Kwiatkowski[1], S.P. Lee[2], Y. Zhao[1], H. Liao[1], G. O'Regan[3], A.D. Irvine[3], C.N.A. Palmer[2], F.J.D. Smith[1]* 1) Human Genetics Unit, University of Dundee, Dundee, Scotland, UK; 2) Biomedical Research Centre, University of Dundee, Scotland, UK; 3) Our Lady's Children's Hospital, Dublin, Ireland.

The FLG gene on chromosome 1q21 encodes 10-12 repeats of the 37 kDa filaggrin peptide essential for skin barrier formation. We recently reported the first FLG mutations, R501X and 2282del4, leading to complete loss of filaggrin expression. These mutations, carried by up to 10% of European-origin populations, cause mild or severe ichthyosis vulgaris (IV) and importantly, are a genetic predisposing factor for AD and related atopic phenotypes. Here we ascertained a cohort of 150 Irish children with AD and identified further loss-of-function mutations, 3702delG in filaggrin repeat 3, R2447X in repeat 7 and S3247X in repeat 9, all of which are carried by <1% of the population. These variants allow synthesis of 3-9 repeats but biochemical analysis showed that these truncated profilaggrin molecules cannot be processed into mature filaggrin and so act as null-alleles. Consistent with this, homozygosity for these mutations, or compound heterozygosity with R501X or 2282del4, causes severe IV accompanied by AD. In this cohort with moderate-severe AD, ~48%; carried one or more of these 5 FLG variants. Comparing our cohort to the Irish population gave a Chi-square p value of $<10^{-50}$ and Fishers exact test odds ratio of 7.13 with 95%CI 4.9-10.6. We have also replicated the association of FLG null-alleles with AD and other atopic phenotypes, including AD-associated asthma, in a total of 10 independent studies within 6 different European populations. In addition to association studies, we used genetic linkage analysis, transmission disequilibrium testing and a longitudinal association study, all of which gave highly statistically significant correlations. These data conclusively demonstrate that in European populations at least, FLG is a major predisposing gene for atopy.

Is alpha-1-antitrypsin a modifier gene for Cystic Fibrosis? *R. Mirfakhraie[1,2], M. Gorgipoor[3], F. Mirzajani[1]* 1) Medical Genetics, NIGEB, Tehran, tehran, Iran; 2) Science and Research Campus, Islamic Azad University of Tehran; 3) Khatam University.

Cystic Fibrosis (CF) transmembrane conductance regulator (CFTR)genotype does not explain the heterogeneity observed in CF pulmonary disease severity. Modifier genes are implicated for this heterogeneity. Alpha-1-antitrypsin (Alpha-1-AT) is one of the few antiprotease capable of inactivating neutrophil elastase. We investigated whether Alpha-1- AT alleles (Z, S deficient alleles and the 3 G1237 A mutation) are associated with increased disease severity and Alpha-1-AT acute phase response during pulmonary exacerbations. 70 CF patients were genotyped for the S and Z, mutations and G1237 A polymorphism using PCR-RFLP Method. 200 control individuals were also screened for GA polymorphsm in order to find the frequency of this polymorphism in Iranian population. Our result suggest no correlation between allelic alterations in Alpha-1- AT gene and CF.

Development of a densely genotyped Population Reference Sample (POPRES): a resource for population, disease, and pharmacological genetics research. *M.R. Nelson[1], M. Klotsman[1], A.M. McNeill[2], Y. Maruyama[1], C.E. Bowman[1], D. Morris[2], M.E. Ehm[1], E.H. Lai[1]* 1) Genetics Research, GlaxoSmithKline, Research Triangle Park, NC; 2) Medicine Development Centers, GlaxoSmithKline, Research Triangle Park, NC.

Recent technologic and scientific advances, stemming in large part from the Human Genome and HapMap projects, have made large-scale, genome-wide investigations feasible and cost-effective. These advances have the potential to dramatically impact drug discovery and development by identifying genetic factors that contribute to variation in disease risk as well as drug pharmacokinetics, treatment efficacy, and adverse drug reactions. In spite of the technological advancements, successful application in biomedical research would be limited without access to suitable sample collections. To facilitate exploratory genetic research, we are developing an extensive DNA resource collected from a large number of participants, sampled throughout the world. This nascent sample resource is being initially genotyped using a commercially available genome-wide 500,000 SNP panel. This project currently includes over 2500 subjects of Mexican, Chinese, Japanese, European, and Indian Asian origin. Approximately 2,500 additional samples that are broadly representative of the global population will be ascertained and genotyped. As the genotypic data from these samples are assembled, they will be made publicly available for use by the wider genetics community. To further develop such a public resource, we welcome the opportunity to work with academic, non-profit, and industry partners to populate this resource with representative and genetically diverse collections, while maintaining the highest ethical standards. Here we present the goals and design of this collection, a summary of the samples and data currently available, as well as initial exemplary applications in the study of the genetic basis of adverse drug reactions.

Generation of *Trim37* knock-out mice. *R.H. Hämäläinen, A.L. Träskelin, A.E. Lehesjoki* Folkhälsan Institute of Genetics and Neuroscience Center, University of Helsinki, Helsinki, Finland.

TRIM37 is a peroxisomal ubiquitin E3 ligase that belongs to the TRIM-family of tripartite motif proteins. In humans, mutations in the *TRIM37* gene underlie a developmental disorder, mulibrey nanism. Mulibrey nanism patients manifest with prenatal onset growth retardation without later catch-up growth, typical craniofacial features, progressive cardiopathy, hepatomegaly, failure of sexual maturation, an increased risk for developing tumors and type 2 diabetes. The mouse *Trim37* gene is highly homologous to the human *TRIM37*, with 88 % identity between the cDNA sequences and 92 % identity on protein level, suggesting that mouse would be a suitable organism to model the human disease. Further, the *Trim37*$^{-/-}$ mice may provide tools to study growth and development in general as well as serve as a model for type 2 diabetes and metabolic syndrome. Towards understanding the disease mechanisms underlying mulibrey nanism, we are currently generating *Trim37*$^{-/-}$ mice. A search for *Trim37*-deficient mouse strains or ES cells in the IMSR (International Mouse Strain resource) resulted in identification of a BayGenomics genetrap ES cell line (RRO307) that was ordered from UC Davis, California. In these ES cells, we found that the -geo insertion vector is localized in intron 2 of *Trim37* and we developed a PCR based analysis for genotyping the mice. The ES cells were microinjected into C57Bl wild-type blastocysts and altogether 10 chimeric male mice were born. Thus far three of the chimeric mice have shown germ line transmission and produced heterozygous offspring, which we are currently breeding. In homozygous offspring we first aim to study the developmental effects of nonfunctional Trim37 in embryos. If viable offspring is produced, our primary aim is to characterize the mice to evaluate their potential to mimic the human disease.

Mapping trait loci using inferred Ancestral Recombination Graphs. *M.J. Minichiello, R. Durbin* Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

Large-scale association studies are being undertaken with the hope of uncovering the genetic determinants of complex disease. We will describe a computationally efficient method for inferring genealogies from population genotype data, and will show how these can be used to fine map disease loci and dissect association signals.

These genealogies take the form of the Ancestral Recombination Graph (ARG). The ARG defines a genealogical tree for each locus, and as one moves along the chromosome, the topologies of consecutive trees shift according to the impact of historical recombination events. There are two stages to our analysis. First, we infer plausible ARGs using a heuristic algorithm, which can handle unphased and missing data, and is fast enough to be applied to large-scale studies involving thousands of individuals. Second, we test the genealogical tree at each locus for a clustering of the disease cases beneath a branch, thus determining whether a causative mutation occurred on that branch. Since the true ARG is unknown, we average this analysis over an ensemble of inferred ARGs.

We have characterised the performance of our method across a wide range of simulated disease models. Compared to single marker and haplotype based tests, our method gives increased power and accuracy in positioning untyped causative loci. It can also be used to estimate the frequencies of untyped causative alleles and the haplotypic background on which causative mutations occurred. We have applied our method to Ueda et al.'s association study of CTLA4 and Graves' disease, showing how it can be used to dissect the association signal, giving interesting results suggesting allelic heterogeneity and epistasis.

Similar approaches analysing an ensemble of ARGs inferred using our method may be applicable to many other problems of inference from population genotype data, such as detecting population substructure and selection.

New evidence on the molecular evolution of the ADH gene cluster in primates. *H. OOTA[1,3], R. KAUL[2], K. HUI[1], W.C. SPEED[1], A.J. PAKSTIS[1], J.R. KIDD[1], M. OLSON[2], K.K. KIDD[1]* 1) Department of Genetics, Yale University School of Medicine, 333 Cedar St., New Haven, CT; 2) Department of Medicine, University of Washington Genome Center, 225 Fluke Hall, Seattle, WA; 3) Department of Integrated Biosciences, University of Tokyo, Chiba 277-8562, Japan.

We are sequencing the alcohol dehydrogenase (ADH) gene cluster (370kb in humans) in 10 primates (3 great apes, 3 old world monkeys (OWMs), 2 new world monkeys (NWMs), 2 prosimians) and one bat to explore the evolutionary mechanisms for the duplicated genes in the primate lineage. A preliminary comparison shows that both the OWMs and prosimians have 7 ADH genes including 3 Class I-like genes. Mice and rats have only one Class I gene. Thus, the Class I duplication event(s) seem to have occurred after primates diverged from rodents but before the primate radiation. Other mammals (sheep, horse, rabbit) studied have two Class I genes making the timing of the duplication event(s) unclear since their homologies to the 3 primate genes are unclear. The three Class I genes in OWMs and great apes show very high nucleotide similarity both in exons (90%) and introns (70%) implying recent duplication events and/or possible gene conversion(s). Our analyses show no indication of any gene conversions, suggesting the Class I genes have evolved independently in higher primates.

The 7 ADH genes, located on human chromosome 4 in tandem, are classified into five classes: the Class I (*ADH1A*, *ADH1B*, *ADH1C*) and the Class II (*ADH4*) genes are expressed primarily in liver, the Class IV (*ADH7*) gene is in stomach, and the Class III (*ADH5*) gene is in all tissues. The expression pattern of the Class V (*ADH6*) gene remains unclear. Mice have six *ADH* genes located on mouse chromosome 3 in the same order as humans. In addition to the Class I triplication in primates, differences between primates and rodents include two genes and one pseudogene of Class V in mice. We shall present analyses of our new sequence data for the whole *ADH* cluster on various primate species as well as comparisons with published rodent (mouse, rat) sequence. Supported in part by US NIH AA09379.

Sleep Disturbance in Ehlers-Danlos Syndromes: Related to Chronic Pain or an Independent Entity? *K.W. Mandel[1], B. Griswold[1], C.A. Francomano[1,2], N.B. McDonnell[1]* 1) National Institute on Aging, Baltimore, MD; 2) Harvey Inst Hum Genetics, GBMC, Baltimore, MD.

Patients with Ehlers-Danlos Syndomes (EDS) often complain of disturbed and non-refreshing sleep. There is a paucity of data available on the etiology of this complaint in EDS. Since many patients also suffer from chronic musculoskeletal pain, we hypothesized that the reported sleep abnormalities are related to the chronic pain syndrome. Multiple sleep and pain assessment tools were administered to sixty five consecutive patients with a diagnosis of EDS enrolled in the National Institutes of Aging protocol 2003-086 on the natural history of hereditary disorders of connective tissue. Data was collected utilizing the Epsworth Sleepiness Scale (ESS), Pittsburgh Sleep Inventory (PSI) as well as a sleep questionnaire designed to elucidate the specific problem areas such as difficulty falling or remaining asleep, snoring, early morning awakening and non-refreshing sleep. Pain was assessed through the Brief Pain Inventory (BPI) and review of systems data focusing on musculoskeletal and generalized pain. Results indicate that 33/65 (50%) of patients had an ESS scale > 9, and 49/65 (75%) a PSI Quotient > 5, suggesting the presence of a sleep disorder. Almost all patients (62/65; 95%) had pain in at least one location, and 29/65 (44 %) reported chronic generalized pain. Of the patients with ESS > 9, a complaint of generalized pain was present in 15/33 (45 %). Of the 49 patients with PSIQ > 5, generalized pain was present in 24 (48 %). The discordance of sleep disturbance with chronic generalized pain in EDS suggests that the pathogenesis of sleep complaints in this disorder may be unrelated to the pain syndrome, at least in part. Further investigation regarding the etiology of this phenomenon in EDS, using formal sleep studies, is warranted.

Investigation of Leu/Lys tRNA gene mutation in Iranian Ataxia Telangiectasia patients. *S. Kasraie[1,2], S. EtemadAhari[1,2], M. Houshmand[2], M. Moin[3], M. Bahar[1]* 1) Azad uiversity of Science and research,Tehran,Iran; 2) Molecular Medical Genetic,National Institute for Genetic Engineering and Biotechnology (NIGEB),Tehran,Iran; 3) Immunology, Asthma & Allergy Research Institute, Tehran, Iran.

Ataxia-Telangiectasia (AT) is a rare human neurodegenerative autosomal recessive multisystem disease that is characterized by a wide range of features including, progressive cerebellar ataxia with onset during infancy, occulocutaneous telangiectasia, susceptibility to neoplasia, occulomotor disturbances, variable immunodeficiency with susceptibility to sinopulmonary infections, impaired organ maturation ,x-ray hypersensitivity ,chromosomal instability and growth and developmental abnormalities. About 20% of those with A-T develop cancer, most frequently acute lymphocytic leukemia or lymphoma. AT is the result of mutations in the AT-mutated (ATM) gene. ATM protein is required for radiation-induced apoptosis and acts before mitochondrial collapse. The tRNA gene mutations are the one of the hot spots cuase mitochondrial disorders, AT can be due to mutation in mitochondrial genes. We performed mutations screening of leu/lys tRNA genes and also ATPase6 genes in 20 patients who referred as Ataxia Telangiectasia. Mitochondrial leu/lys tRNA genes and ATPase 6 genes were studied by PCR method and automated DNA sequence to evaluate any possible mtDNA damage. We have found a mutation in patients that could be related to cause a disease. Now, were confirming the sequence results and finding mutations. Keywords: Mitochondrial tRNA gene, ATPase 6,Point mutation, Ataxia Telangiectasia.

Genetic association between interferon regulatory factor 5 (IRF5) and systemic lupus erythematosus in three ethnic groups. *J.A. Kelly[1], K.M. Kaufman[1,2], A.J. Adler[1], B.J. Herring[1], S.G. Frank[1], J. Kilpatrick[1], J.T. Merrill[1], J.A. James[1,3], G.R. Bruner[1], J.B. Harley[1,2,3]* 1) OMRF, OKC, OK; 2) US Dept of Veterans Affairs Medical Center, OKC, OK; 3) Oklahoma Univ Health Sci Ctr, OKC, OK.

Systemic lupus erythematosus (SLE) is a systemic autoimmune disorder with complex genetics. Two recent reports have demonstrated genetic association with the interferon regulatory factor 5 (IRF5) gene and SLE. This study evaluates IRF5 in our collection of European-American (EA), Hispanic (HI), and African-American (AA) SLE patients. A total of 3027 samples (1420 cases and 1607 controls) were genotyped at polymorphisms within the IRF5 gene. Population-based case-control association designs were employed. We observed significant association in our EA SLE samples with rs729302 (AIC: 8.82, ^{2}=10.83, p=0.001, OR=1.63 (95% CI: 1.22-2.19)), rs2004640 (AIC: 21.68, ^{2}=24.6, p=7x10^{-7}, OR=2.12 (95% CI: 1.57-2.86)), rs752637 (AIC: 13.83, ^{2}=15.98, p=0.00006, OR=1.81 (95% CI: 1.35-2.42)) and rs3807306 (AIC: 16.77, ^{2}=19.70, p=0.000009, OR=2.01 (95% CI: 1.47-2.75)), in our HI SLE samples with rs2004640 (AIC: 3.898, ^{2}=5.94, p=0.01, OR=1.89 (95% CI: 1.13-3.16)), rs752637 (AIC: 4.62, ^{2}=6.69, p=0.009, OR=1.95 (95% CI: 1.17-3.26)) and rs3807306 (AIC: 16.82, ^{2}=17.74, p=0.00003, OR=3.50 (95% CI: 1.47-2.75)) and in our AA SLE samples with rs729302 (AIC: 4.65, ^{2}=6.06, p=0.01, OR=1.72 (95% CI: 1.11-2.64)) and rs3807306 (AIC: 9.66, ^{2}=11.28, p=0.0008, OR=1.99 (95% CI: 1.33-3.0)), thereby confirming previously observed associations with IRF5 and EA and HI SLE and establishing association in AA SLE and with rs3807306. The greatest significance was observed in all three ethnic groups when using a recessive model. In addition, we confirm the previously identified rs729302-rs2004640-rs752637 haplotype, which was observed in all three ethnic groups (EA: 61% cases, 49% controls, ^{2}=22.91, p=0.00002; HI: 58% cases, 43% controls, ^{2}=12.81, p=0.0003; AA: 45% cases, 38% controls, ^{2}=4.64, p=0.03). In summary, we confirm association with IRF5 and SLE in European-Americans and Hispanics, establish association in African-Americans and confirm association with a three-marker haplotype that is enriched in the SLE cases.

Imprinted genes related with MECP2 in 7q21 are not major causative genes for autism. *N. Nakashima, T. Yamagata, M. Imai, M. Mori, M.Y. Momoi* Dept Pediatrics, Jichi Medical Sch, Shimotsuke, Tochigi, Japan.

MECP2 is a gene for Rett syndrome (RTT) that shows autistic phenotype. Therefore, downstream genes of MECP2 are candidate genes for autism. DLX5 is regulated by MECP2 and is maternally expressed. In MECP2 knockout mice, expression level of DLX5 in the brain is twice of wild type mice. And both of maternal and paternal alleles were expressed in lymphoblastoid cells from RTT patients. It is interesting to know whether the expression and imprinting status of DLX5 is altered in autistic patients to detect the presence of common mechanism between autism and RTT. DLX5 is located in the imprinted region of 7q21, and two MECP2 binding sites are reported to exist in this region, one is near DLX5 and DLX6 and the other is near PEG1. Therefore, we analyzed the expression level of the genes near MECP2 binding sites in lymphoblastoid cells to detect the alternation of imprinting status. In addition, we analyzed each gene for mutation among autistic patients using their DNA from lymphoblastoid cells for possible link for the cause of autism as the downstream molecular process of MECP2.Patients diagnosed as pervasive developmental disorder or autism according to the criteria of DSM-IV were enrolled in this study after the informed consents by their parents. The genes studied included DLX5, DLX6 and PEG10. Expression level of each gene in five autistic patients was compared with five control individuals, respectively. For mutation analysis, up to one hundred autistic patients were enrolled. All exons and promoter regions of these genes were amplified by PCR and the mutation was screened by DHPLC, and finally confirmed by direct sequencing. The expression level of each gene in lymphoblastoid cells were not different between patients and controls. No causative mutation was detected in all genes analyzed. It is considered that autism and RTT did not share common pathway after MECP2. Addition to the previous reports that no causative mutation of DLX5 and DLX6 was detected on autistic patients, both genes can be excluded from the candidate gene for autism. It is required to analyze another genes located in this region further to clarify the contribution to autism.

Whole Genome Association Scan for Alzheimer Disease Using Multiple SNP Panels. *L. Li[1], P.L. StJean[1], M.R. Barnes[1], S.L. Chissoe[1], S. Subramanian[1], N.P. Goodgame[1], R.A. Gibson[1], D.D. Kelly[1], K.S. King[1], E.H. Lai[1], J. Marchini[2], K.L. Nangle[1], M.R. Nelson[1], M. Owen[3], J.C. Richardson[1], A.D. Roses[1], P. Skelding[1], S. Stinnett[1], J. Williams[3], M.G. Ehm[1], GenADA Investigators (Investigator sites across Canada)* 1) GlaxoSmithKline; 2) University of Oxford, Oxford, UK; 3) Cardiff University, Cardiff, UK.

Alzheimer disease (AD) is a progressive neurological condition characterized by a gradual but persistent decline in memory and other cognitive, social and ultimately physical functions. Identifying variants associated with AD provides important information during for drug development. Variants that are associated with disease susceptibility and progression can be used to: validate target genes, identify biomarkers, and optimize disease indications for safe therapeutics. A genome-wide association study was carried out on 2584 Caucasian subjects using 500K common SNPs, 20K cSNPs, and 3000 cSNPs in druggable genes. This comprehensive scan tags greater than 70% of common variants and additional functional variants. This sample includes 850 Canadian and 450 British case-control pairs. To identify single variants associated with AD, we performed single point association analysis in the combined sample by controlling for the group effect. For SNPs showing the strongest signals, we examined the genetic effects in each subset under a variety of disease models. Analyses were also conducted by conditioning on ApoE carrier status. To detect SNPs exhibiting epistatic effects, a comprehensive interaction analysis was carried out for all pairs of SNPs using a full logistic model, followed by a detailed diagnosis of the interaction effects observed for promising pairs. We summarize the properties and information capture of these genome scans. Graphical summaries including the genomic context of these regions and interaction results are used to illustrate results. We will report the challenges and opportunities faced in conducting such comprehensive scans and implications for future research. Findings from this study provide evidence that comprehensive genome scans can provide important genetic findings.

Pilot Study of Patients Affected With Leber Congenital Amaurosis (LCA) Clinically Selected With Regard to the Previously Established Genotype-Phenotype Correlations. *S. Hanein[1], I. Perrault[1], N. Delphin[1], S. Gerber[1], J.L. Dufier[2], A. Munnich[1], J.M. Rozet[1], J. Kaplan[1]* 1) INSERM U781, Hopital des Enfants Malades, Paris, Cedex 15, France; 2) Ophthalmology, Hopital des Enfants Malades, Paris, Cedex 15, France.

The purpose of this study was to evaluate the improvement of the genotyping of new patients affected with LCA after pre-selection of genes to screen in priority with regard to the genotype-phenotype correlations previously established for the nine hitherto identified genes. This pilot study focused on the two major LCA genes, GUCY2D and CRB1, which account for about 1/3 of all patients. Thirty-nine unrelated patients were selected on their clinical history and ophthalmologic findings: i) 18/39 suggested the involvement of GUCY2D, the major LCA gene responsible for the most severe form (cone-rod type) of the disease and ii) 21/39 suggested the implication of CRB1, the major gene responsible for less severe forms (rod-cone type). In each group of patients, the selected gene only was screened for mutations using DHPLC and direct sequencing. Ten out of the 18 patients compatible with the involvement of GUCY2D were found to carry mutations in this gene i.e. 55.5% vs 22.1% in a non-clinically pre-selected population of LCA patients. Along the same lines, 11/21 patients of the CRB1 subgroup harboured mutations in this gene i.e. 52.4% vs 10.1% in a non-clinically pre-selected population of patients. In conclusion, this study emphasizes the absolute necessity to obtain full and detailed clinical findings for all patients affected with LCA. These data must include: i) the natural history of the disease since birth, ii) the early light behaviour of the child, iii) the exact measurement of the refraction, iv) a precise description of the fundus aspect including the existence or the absence of early macular rearrangements, and finally v) the visual acuity and the visual field recordings when possible.

Capillary array SSCP analysis of pooled DNA for association testings. *K. Hayashi, K. Masumoto, Y. Okazaki, A. Yoshinaga, K. Higasa, Y. Kukita, T. Tahira* Division of Genome Analysis, Res Ctr Genetic Info, Med Inst Bioreg, Kyushu Univ, Fukuoka, Japan.

Allele frequency estimation of SNPs using pooled DNA is a practical primary screening strategy in the association study, where hundreds to thousands of samples must be examined. However, the effectiveness of this approach depends on how accurately the frequency can be estimated. We evaluated the pooled DNA analysis using an SSCP-based method, PLACE-SSCP, and found that the estimation by this method is reproducible and accurate. In PLACE-SSCP, the PCR products are post-labeled with fluorescent dyes, subjected to automated capillary electrophoresis under nondenaturing (SSCP) conditions, and separated allele peaks are identified and quantified. To facilitate the analysis, we developed a new software, called *QSNPlite*, for a systematic and quantitative analysis of the SSCP data, and used in the evaluation. This software is designed, so that the allele frequencies are calculated from the peak height ratios of alleles in the pooled samples after correcting the different representation of each allele using the data of heterozygotes included in each PCR plate. Partially overlapped peaks can be deconvoluted and quantified using this software. We compared the results of this SSCP-based frequency estimation with the true frequencies that were obtained by counting the alleles after genotyping all individuals in the pool. The estimates and the counts agreed closely (R = 0.997), and the averaged absolute values of the differences between the estimates and true values (i.e., the mean absolute error) were 1.4%. Thus, we conclude that the present method is far more accurate than other methods, such as mass spectrometry or electrophoresis after primer extension. We applied this system in the association studies of systemic lupus erythematosus and breast cancer, and found that the PLACE-SSCP analysis of pooled DNA is useful, in the search for the disease responsible genes by the candidate gene approach.

Analysis of eQTL gene expression data for the identification of complex genetic interactions. *K.A. Kim[1], J. Huang[1], T.E. Scheetz[1], R. Swiderski[1], A.R. Philp[1], E.M. Stone[1,2], V.C. Sheffield[1,2]* 1) Univ of Iowa, Iowa City, IA; 2) Howard Hughes Medical Institute.

Identification of genes involved in complex and genetically heterogeneous disorders presents challenges and requires the use of diverse and integrated approaches. Recent advances in microarray technology have made it possible to perform experiments that examine the expression of thousands of genes in related individuals and to use these data to identify regions of the genome that regulate the expression of multiple genes. Such studies are referred to as expression quantitative trait locus (eQTL) mapping. In a novel application of eQTL data we have performed pair wise correlation of gene expression to identify sets of genes that appear to be co-regulated. The use of eQTL data has the potential to aid in the identification of interacting genetic loci involved in complex genetic disorders. However, it is essential that proper statistical methods be utilized for such applications of eQTL data. In a typical genome scan, linkage is evaluated at each marker locus or in between loci, assuming one eQTL (or QTL). The assumption of one eQTL is potentially inaccurate in cases of complex inheritance resulting from the presence of interacting genes. To enhance the statistical modeling of complex inheritance, in particular to better detect interactions, we propose two statistical models: Tukeys 1-df model for interaction (TM1) and a modified version of Tukeys model (TM2). The two models add only one additional parameter to estimate and test for interaction between two loci. TM2 has a novel interpretation of the interaction term when compared to TM1. We applied the two models to eQTL data to detect interaction effects of genetic loci on the expression of genes causing Bardet Biedl Syndrome (BBS; obesity, retinopathy, polydactyly, mental retardations and urogenital anomalies), as well as eye disease genes. The study involves profiles of 31,000 probes represented on an Affymetrix expression microarray from 120 F_2 rat eyes including 399 genetic markers. We detected a statistically significant interaction effect under TM2 for the expression of *Opn1sw*, a gene that encodes for blue cone opsin.

Identification of a new locus of an X-linked dominant male-lethal isolated microphthalmia/anophthalmia/coloboma (MAC). *N. Meola[1], D. De Brasi[2], M. Morleo[1], V. Ginocchio[2], T. Caramico[1], P. D'Adamo[1], O. Zuffardi[3], G. Sebastio[2], S. Banfi[1]* 1) Telethon Institute of Genetics and Medicine, Naples, Italy; 2) Department of Pediatrics, Federico II University, Naples, Italy; 3) Department of Medical Genetics, University of Pavia, Italy.

Microphthalmia, anophthalmia and coloboma (MAC) are major structural eye malformations. Anophthalmia is characterized by the complete absence of the eye, microphthalmia is characterized by a small eye while the coloboma results from a failure in the closure of the optic fissure. These disorders have a combined birth incidence of approximately 1 in 5000. Here we report the analysis of an Italian family characterized by the presence of isolated MAC with a highly variable expression. The five affected females show a variety of eye malformations either unilateral or bilateral, ranging from microcornea to complete anophthalmia . Moreover, no affected males are present in the family while there is a high frequency of spontaneous abortions some of which were documented to affect male pregnancies. These observations strongly suggested that the MAC phenotype in this family is transmitted with an X-linked dominant male-lethal pattern. Detailed cytogenetic analysis, including also high-resolution comparative genomic hybridization (CGH), failed to reveal the presence of any chromosomal rearrangement. Linkage analysis carried out with a panel of X-chromosome polymorphic markers using a model of X-linked dominant transmission allowed us to exclude two already known loci for X-linked dominant syndromic microphthalmia/anophthalmia, namely microphtalmia with linear skin defects (MLS) and oculocardiofaciodental syndrome (OFCD) and suggested a linkage of the disease to a region of approximately 8 cM on the short arm of the X-chromosome (Xp22.1). We are currently carrying out systematic mutation analysis on the genes present in the critical region and determining the X-inactivation status of the affected individuals to verify the presence of a skewed X-inactivation pattern. The identification of this novel MAC gene is expected to shed further light on the pathogenesis of this complex group of eye developmental disorders.

Pharmacokinetics and tolerability of miglustat in juvenile GM2 gangliosidosis. *G. Maegawa[1], B. Banwell[2], J. Hayes[3], S. Blaser[4], C. Hawkins[5], P. van Giersbergen[6], J. Clarke[1]* 1) Div. of Clinical and Metabolic Genetics Hospital for Sick Children; 2) Div. of Neurology Hospital for Sick Children; 3) Div of Anaesthesia Hospital for Sick Children; 4) Dept. fo Diagnostic Imaging Hospital for Sick Children; 5) Dept. of Paediatric Laboratory Medicine Hospital for Sick Children; 6) Dept. of Clinical Pharmacology Actelion Pharmaceuticals Ltd.

GM2 gangliosidosis (GM2) is an inherited neurodegenerative disorder caused by lysosomal -hexosaminidase A deficiency. Substrate reduction therapy is currently one of the therapeutic options for GM2. Objective: To establish the pharmacokinetics (PK) of miglustat (Zavesca) given as single and multiple doses in juvenile GM2, and evaluate its safety and tolerability in a 12-month follow-up period. Methods: Five patients (mean age 14.6$\pm$4.5 yrs) receiving oral miglustat at the dose of 100-200 mg t.i.d. based on body surface area. Patients underwent two PK assessments at day 1 and month 3. Blood samples were drawn immediately before and at intervals within 24 hours after drug administration. Results: The mean plasma Cmax after multiple dose regimen was statistically higher than the one performed after the single dose regimen. The accumulation index was 1.80. No differences were found between t_{max}, Cmax, and $t_{1/2}$ for single and multiple dose administration. The major side effects observed among the 5 studied patients were diarrhea, vomiting and weight loss. Diarrhea responded well to diet modification, lactaze and loperamide. Four patients had weight loss, and only one recovered previous weight. Two patients who showed frequent and severe diarrhea showed the highest values mean Cmax in multiple dose profile. Laboratory tests showed mild hypernatremia, mild elevation of AST, ALT and low WBC with low neutrophils. One patient developed peripheral neuropathy after the 12 month-studied period thought possibly due to disease progression. Conclusion: The PK of miglustat was similar after single- and multiple-dose administration. The exposure to and plasma concentration of miglustat appeared to correlate with the severity of adverse events. Basic biochemical and haematology test should be done routinely for patients on miglustat.

Clinical and genetic correlations in subjects with the *puratrophin-1* (-16C>T) genetic change. *K. Ishikawa, T. Amino, N. Sato, T. Ishiguro, T. Tsunemi, S. Toru, H. Mizusawa* Dept Neurology, Tokyo Medical & Dental Univ, Tokyo, Japan.

Autosomal dominant cerebellar ataxia (ADCA) is a group of heterogeneous neurodegenerative conditions showing late onset progressive cerebellar ataxia as a cardinal neurologic manifestation. We have identified that a single-nucleotide (C-to-T) change in the 5-untranslated region (-16C>T) in the gene, *puratrophin-1*, is strongly associated with the ADCA linked to chromosome 16q22.1 region (Ishikawa K and Toru S et al. Am J Hum Genet 2005) (OMIM #117210). This chromosomal region is also known as the spinocerebellar ataxia type 4 (SCA4) locus (OMIM %600223), although this genetic change has not been observed in SCA4 subjects. The gene product puratrophin-1, a novel protein with spectrin repeat and Rho GEF domains, is predicted to have a role in maintaining the cytoskeletal structure particularly related to the Golgi apparatus. Although the -16C>T genetic change completely segregated with the 16q22.1-linked ADCA in more than 100 families, one affected individual without this genetic change has recently been reported. This finding may suggest that the *puratrophin-1* (-16C>T) genetic change is not sufficient for the diagnosis of 16q22.1-linked ADCA. We examined correlations between the clinical finding on 60 ADCA families linked to 16q22.1 and the presence of *puratrophin-1* genetic change. Among our cohort of the 60 families with the *puratrophin-1* (-16C>T) genetic change, we found that only one family showed questionable correlation. In this family, two individuals with the -16C>T genetic change showed obvious ataxia, whereas one individual (P87-II-03) without the genetic change also showed down-beat nystagmus, suggestive of presence of cerebellar dysfunction. Haplotype analysis demonstrated that this individual P87-II-03 had different haplotype for the region where all 16q22.1-linked ADCA patients show a founder haplotype. The present observation indicates that both detailed clinical investigation and haplotype analysis are important for the diagnosis of 16q22.1-linked ADCA.

Novel autosomal dominant syndrome in a three-generation family with broad first digits, oral anomalies, and speech delay. *V. Mardo[1], E. Lisi[1], D. Riegert-Johnson[2], S.A. Boyadjiev[1,3]* 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Division of Gastroenterology, Mayo Clinic, Rochester, MN; 3) Department of Pediatrics, MIND Institute, University of California-Davis School of Medicine, Davis, CA.

A characteristic combination of digital, facial, and oral anomalies was observed in a three-generation Caucasian family, with a segregation pattern suggestive of an autosomal dominant inheritance. The facial dysmorphisms included prominent forehead, almond shaped eyes with upslanting palpebral fissures and medial epicanthal folds, broad nasal bridge, short philtrum with thin upper lip, and posteriorly rotated ears. Observed oral anomalies included high arched palate and central groove of the lower lip. All affected family members had broad thumbs and great toes, speech delay, and mild mental retardation. Moreover, macrocephaly, somatic overgrowth, congenital heart defects (VSD with coarctation of the aorta), and cryptorchidism were also observed among various members of this family. Microsatellite analysis excluded linkage to CREBBP and EP300, the genes associated with Rubinstein-Taybi syndrome. Additionally, Simpson-Golabi-Behmel syndrome was considered, but X-linked inheritance was excluded by the presence of male-to-male transmissions and two affected females. Cytogenetic studies, including karyotype, subtelomere screen, and FISH for 22q11 deletion syndrome, were normal. Taken together, we believe that this condition represents a novel genetic syndrome. Further molecular analyses are currently in progress to identify the causative gene.

Sample recollection using an improved cytobrush methodology. *M.M. Jenkins[1], K.L. Rose[2], A. Woomert[3], P.A. Romitti[2]* 1) Battelle contractor to CDC, Columbus, OH; 2) University of Iowa, Iowa City; 3) Battelle Memorial Institute, Durham, NC.

A request for buccal cell samples from birth parents and index children was integrated into the National Birth Defects Prevention Study (NBDPS) in 1998. Samples were collected using cytobrushes that were stored and transported in air-tight tubes (wet brushes). These brushes did not dry during transport, and initial extractions showed considerable variation in DNA yield. In 2003, NBDPS sites modified sample collection to include cytobrushes that were stored and transported in open-to-air containers (dry brushes) allowing them to dry during transport. Use of dry brushes produced higher and more consistent DNA yield than wet brushes. To improve the quality of samples in the NBDPS tissue bank, two sites, Georgia (GA) and Iowa (IA), evaluated the feasibility of recollecting dry brush samples from families who initially provided wet brush samples. Eligible families were those of case children (n=68) diagnosed with spina bifida (SB) or longitudinal limb defects (LD) and those of unaffected control children (n=66) born between 1997 and 2003. Additional eligibility criteria were the index child was living and the family previously provided wet brush samples. Contact has been attempted with 49 IA case (SB=36; LD=13) and 47 IA control families and with 19 GA case (SB=11; LD=8) and 19 GA controls. Of the 96 IA families, 72 (75%) provided samples, 17 (18%) refused, and 7 (7%) were not located. Of the 38 GA families, 18 (47%) provided samples, 3 (8%) refused, 5 (13%) are pending, and 12 (32%) are being traced for a current address. Mean DNA yield for 11 GA families using wet brushes and dry brushes were 0.7g (0-2.6g) and 1.5g (0.1-4.3g), respectively. Among recollected samples, 99% had DNA yields >0.1g and successful microsatellite sequence amplification. Preliminary results showed that half or more families are willing to recollect buccal samples, providing support for extending recollection to additional case groups and NBDPS sites. Other genetic epidemiology studies might benefit from modifications to buccal cell collection methods the NBDPS has implemented to optimize DNA yield.

A new Bayesian method to detect complex multifactorial interactions in complex diseases using the variance-component-threshold model. *A. Narita, A. Tajima, I. Inoue* Dept. Molecular Life Science, Tokai University School of Medicine, Isehara, Kanagawa, Japan.

It is now well known that gene-gene and/or gene-environment interactions play an important role in etiologies of most common human diseases. The objective of this study is to introduce a new Bayesian method to investigate multifactorial interactions responsible for complex diseases in the variance-component-threshold model. In the variance component model, each genotype is regarded as a random effect and thus, instead of dealing with all the effects as independent parameters, we only have to estimate the mean and the variance of the most likely distribution of the genotypic effects. This reduces substantially the degrees of freedom that are required to investigate a high-dimensional interaction. In combination with the threshold model, where it is assumed that an apparently discrete trait is actually controlled by continuously-distributed liability, our method can be applied to binary, or even polychotomous, complex human diseases, as well as quantitative traits. Additionally, since the Markov chain Monte Carlo algorithm is adopted in our method, the most likely model is heuristically searched out of all the possible combinations of varying numbers of SNPs and thus its computational time and loads can be considerably reduced. The method also calculates weighting coefficients for respective loci that indicate the degrees of individual contribution of each locus. The genotypic relationship matrices that consist of the coefficients can also provide the estimated degrees of correlation between multilocus genotypes. In the present study, we demonstrate the effectiveness of the proposed method using both simulated populations and a real dataset. The versatility of the method will enable a later extension to analyze a variety of types of datasets, using not only genetic but also epidemiological and/or clinical information, for the diagnosis of diseases and the prediction of onsets.

Genetics of gene expression for Amyotrophic Lateral Sclerosis (ALS). *R.A. Ophoff[1,2,3,4], C.-Y. Lee[1], C.G.J. Saris[3], P. van Vught[3], S. Horvath[1], L.H. van den Berg[3]* 1) Human Genetics, Univ California Los Angeles, Los Angeles, CA; 2) UCLA Cntr for Neurobehavioral Genetics, Los Angeles, CA; 3) Rudolf Magnus Institute, University Medical Center, Utrecht, The Netherlands; 4) Dept of Medical Genetics, University Medical Genetics, Utrecht, The Netherlands.

ALS is the most common form of motor neuron disease in humans, characterized by progressive degeneration of motor neurons in brain and spinal cord leading to muscle weakness and death within 3 years for 50% of patients. The predominant presentation of ALS is sporadic with a familiar variant affecting <10% of the patients. Studies of ALS suggest a large degree of genetic heterogeneity but with extensive clinical homogeneity. This suggests that at the molecular level only a limited number of pathways are immediately involved in disease etiology. For this reason we set out to study pathways by means of gene expression profiling in peripheral blood of ALS patients. We collected genome-wide gene expression data from 30 ALS cases and 30 controls. Using the most informative 8,000 genes, an absolute T-statistic was calculated for each gene and a power adjacency function was used to calculated connectivity for the data set. The 3,600 most connected genes were included for subsequent module detection. Using average linkage hierarchical clustering, we used a dynamic cut tree algorithm to select branches (modules). Nine modules were obtained and each gene was clustered. The mean gene significance was calculated for each module, which represents the differential gene expression of genes in these modules between ALS patients and controls. Four modules were identified with differential expression. In two of these modules gene significance was strongly correlated with gene connectivity, suggesting that hub genes play an important role in these modules. We further applied functional enrichment analysis to identify gene categories within these modules based on their gene ontology. Our data clearly identified differential expression of genes in ALS patients within distinct functional modules. This approach allows for an unbiased approach of pathway reconstruction and may lead to new insight into the etiology of ALS.

Deletion 10q26 in a patient with inv(10)(p11.23q21.2): clinical report and characterization by comparative genomic hybridization (CGH) microarray analysis and FISH with locus specific probes. *S.C. Newman[1], P.I. Bader[1], F.J. Bader[1], S. Blend[2], D.E. Mensing[2], J. Li[2], S.W. Cheung[2]* 1) Northeast Indiana Genetic Counseling Center, Parkview Hospital, Fort Wayne, IN; 2) Department of Human and Molecular Genetics, Baylor College of Medicine, Houston, TX.

Clinical findings in patients with 10q deletion include mental retardation (MR), growth retardation (GR), congenital heart disease (CHD), genitourinary (GU) anomalies, microcephaly, and facial features such as broad nasal bridge with beaked or prominent nose, strabismus, and low-set malformed ears. Behavioral abnormalities are paramount in the disorder and may not correlate with the degree of MR. Attention Deficit Hyperactivity Disorder(ADHD), Autism Spectrum Disorder(ASD), and Bipolar Disorder have all been diagnosed in patients with 10q deletion.

We report a girl with inv(10)(p11.23q21.2), ADHD, MR, and ASD who had strabismus surgically corrected. Her height was at -3SD, weight at 10 %ile, OFC at -4SD. Facial features included a metopic ridge, hypotelorism, small palpebral fissures (-3SD), prominent nose and nasal bridge, thin upper lip, flat philtrum, chin cleft, heart murmur, clinodactyly, Sydney lines, poor balance. Echocardiogram revealed atypical PDA or an aortopulmonary window. Ultrasound showed a hypoechoic area in the superior right kidney. Her behavior was characterized by hyperactivity, repetitiveness, and mouthing/licking objects.

Chromosomal microarray analysis designed to interrogate clinically relevant genomic regions revealed haploinsuffiency at 10q26.2 with the following clones: starting from RP11-422P15 to RP11-140A10 encompassing approximately 7 Mb of subtelomeric region. Interestingly, high-resolution chromosome analysis revealed the deletion present in the inverted chromosome 10 at bands 10q26.2-q26.3. Candidate genes for behavioral anomalies/MR include dihydropyrimidinase-like 4(DPYSL4) involved in neuronal differentiation and dopamine receptor D1 interacting protein (DRD1IP) that encodes a protein interacting D1 domamine receptor.

Intravenous MCT Oil for Nutritional Support in a Preterm Infant with LCHAD Deficiency. *T. Markello* Dept of Genetics & Metabolism, Childrens Natl Med Ctr, Washington, DC.

Long Chain 3-Hydroxy Acyl CoA Dehydrogenase(LCHAD) deficiency, and trifunctional protein deficiency, both result in the partial inability to metabolize fat acyl chains. They both increase the risk for preterm delivery due to their association with the HELLP syndrome (Hypertension, Elevated Liver enzymes and Low Platelets during pregnancy). Oral MCT oil has been used to optimize the diet for older children with LCHADD. Its use has improved neurological development and growth. It may also aid in reducing the cardiac myopathy seen in LCHADD. Extremely preterm infants (birth weight <1500gm, gestational age <28weeks) frequently need extended time without oral intake, and are at risk for heart failure from a variety of mechanisms. Using MCT containing intravenous lipids in LCHADD preterm infants would extend the optimal LCHADD diet to this group of patients. It would also achieve increased caloric intake with less fluid administration than nutrition based on glucose solutions alone. The case of a 26 week infant with LCHADD is described. The course was complicated by critical fluid management and cardiac dysfunction. A single use FDA IND was obtained to administer intravenous MCT oil as part of total parenteral nutrition. The initial objective of decreasing the total daily fluid load was achieved while maintaining sufficient calories for growth. No adverse acute toxicity was detected. LCHADD diagnoses are expected to increase, due to implementation of extended newborn screening together with the increased awareness of the association between HELLP syndrome and LCHADD. A significant fraction of these infants will be preterm. The use of intravenous MCT oil in preterm LCHADD infants extends the range of therapeutic options for this condition as well as for trifunctional protein deficiency. Currently no USA/FDA approved form of intravenous MCT oil is commercially available. Advocacy for a commercially produced and FDA approved form of intravenous MCT oil, to be used in the nutritional support of LCHADD and other appropriate metabolic disorders, is warranted.

***SLC26A3* and *CFTR* variants in men with primary failure of spermatogenesis.** *S. Hihnala[1,2], R. Eklund[1], O. Hovatta[3], C. Holmberg[2], J. Kere[1,4], P. Höglund[1]* 1) Department of Medical Genetics, University of Helsinki, Finland; 2) Hospital for Children and Adolescents, University of Helsinki, Finland; 3) Department of Clinical Science, Karolinska Institute, Stockholm, Sweden; 4) Department of Biosciences at Novum, Karolinska Institute, Stockholm, Sweden.

A rare disease with an autosomal recessive inheritance is congenital chloride diarrhea (CLD), which results from over 30 different mutations in the *solute carrier family 26 member 3* (*SLC26A3* alias *DRA*) gene. *SLC26A3* is located on chromosome 7q22-31.1, near the *cystic fibrosis transmembrane conductance regulator* (*CFTR*) gene, and it encodes for an apical epithelial Cl^-/ HCO_3^- exchanger. In duodenum and ductal systems, interaction between the STAS-like domain of SLC26A3 and the R-domain of CFTR promotes secretion of HCO_3^- and fluid from the epithelium.

Finnish CLD-males with the founder mutation *V317del* are subfertile. Reduced fertility involves poor morphology and motility of sperm, and high seminal plasma Cl^- with a low pH. Interestingly, the low seminal plasma pH and poor sperm quality appear in males with cystic fibrosis (CF) as well, although the major cause for infertility among them is congenital bilateral absence of the vas deferens.

SLC26A3 is expressed at multiple sites of the male reproductive tract together with the interacting protein CFTR. In case of *CFTR* mutations, the only manifestation of CF can be male infertility, and even in carrier males, subfertility emerge. The aim of this study is to find whether *SLC26A3* mutations are associated with primary male infertility - alone or together with those of *CFTR*.

So far, direct sequencing of all coding regions and exon-intron boundaries of *SLC26A3* among Finnish men (n=83) with primary failure of spermatogenesis has revealed five new heterozygous variants. Additionally, one of these infertile men was carrier of the Finnish *V317del* genotype of CLD. Among control men (n=33) with known etiology of infertility, however, all these novel variants were non-existent. Future studies include variant analysis among men with proved fertility, in addition to analysis of possible compound heterozygosity for the novel variants and *CFTR* mutations.

SMN1 and SMN2 deletion of exons 7 and 8 concurrent in one family. *T. Majidizadeh, S. SABER, P. JAMALI, M. HOUSHMAND* medical genetic, NIGEB, Tehran, tehran, Iran.

Spinal Muscular Atrophy (SMA) is an autosomal recessive disorder that characterized by loss of - motor neurons due to degeneration in the lower motor neurons . The SMN gene exists in two highly homologous copies, telomeric (SMN1) and centromeric (SMN2). SMA is caused by mutations in SMN1 but not SMN2. In this study one family who were heterozygote for both SMN1 and SMN2 gene deletion screened for common deletion on exon 7 and 8 in both genes. They missed only their child because of SMA type I caused by deletion in exons 7 and 8 in SMN1 gene. Heterozygosity of parents was found when they referred to prenatal diagnosis in next pregnancy .PND showed deletion of exons 7 and 8 concurrent in SMN2 gene. It proved that parents were heterozygote for deletion of exons 7 and 8 in both genes SMN1 and SMN2. Mutations in SMN1 are responsible for SMA and mutations in SMN2 just influence on severity of SMA. Also, so far authors have not been observed any report about deletion of exons 7 and 8 together in SMN2 gene. Genetic counseling could have opinion about SMA because of found intact SMN1 gene in PND, but we can not ignore the possible role of homozygous SMN2 deletion in exons 7 and 8 either in SMA or other disorder such ALS and CMD that SMN2 gene can act as a prognostic factor and may be a phenotypic modifier in sporadic ALS.

The accuracy of family history questionnaires as a genetic counseling tool in a familial cancer setting. *J.M. McCuaig, S. Randall Armel, A. Finch, R. Demsky, B. Rosen* Familial Ovarian Cancer Clinic, Princess Margaret Hospital, Toronto, Ontario, Canada.

Over time, the number of people receiving genetic counseling at the Familial Breast and Ovarian Cancer Clinic (FBOCC) at Princess Margaret Hospital has steadily risen. To help reduce patient waiting times and shorten lengthy cancer counseling sessions, the use of family history questionnaires has been implemented. Currently across the province of Ontario, 22 familial cancer clinics exist: of these, 13 mail questionnaires to obtain family histories, 6 attain them by phone, and 3 acquire them during the counseling session itself. While many familial cancer clinics use questionnaires to obtain family histories, no literature exists on the validity of this approach. To explore the accuracy of the questionnaires used by the FBOCC, we compared the pedigrees generated from the questionnaires to those updated during the genetic counseling session for all new families seen between May 1st, 2005 and May 31st, 2006. All differences between the two pedigrees were scored based on the nature of the information changed and whether the changes altered the patients risk assessment and/or eligibility for BRCA1/2 genetic testing as set forth by the Ministry of Health. Of 80 families recruited, 70 (87%) had no change to their risk assessment and/or eligibility for genetic testing. To summarize the nature of the inaccuracies noted between the two pedigrees: 19 (24%) incorrectly provided information regarding relationship lines, 7 (1%) regarding ancestry or consanguinity, 24 (30%) regarding which family members were diagnosed with cancer, 17 (21%) regarding the type of cancer diagnosed, and 19 (24%) regarding biopsies/abnormal test results, or cancer reducing medications/surgeries. We therefore conclude that through carefully worded questionnaires, genetic counsellors can obtain accurate pedigrees without the need to devote valuable time to obtaining these pedigrees by phone or in person. Further, by using these questionnaires the average genetic counseling session can be reduced by approximately 20 minutes.

Dystrophin gene's hotspots in Iranian patients suspected to DMD or BMD. *M. Dehghan manshadi, T. majidizadeh, M. shafa, S.M. seyed hassani, M. houshmand* medical genetics, NIGEB, tehran, tehran, Iran.

The dystrophinopathiesDuchenne muscular dystrophy (DMD)and Becker muscular dystrophy (BMD)are the most common inherited disorders of muscle. Although reliable prevalence data are lacking, the prevalence of DMD is generally estimated at 1:3,500 live male births . Both DMD and BMD are due to mutations in the dystrophin gene , located at Xp21,which comprises 79 exons and 8 tissue-specificpromoters distributed across ~2.2 Mb of genomic sequencemaking dystrophin the largest gene yet described. Dystrophin gene deletions are found in ~55%of patients with BMD and 65% of patients DMD; point mutations account for ~30% of mutations, and duplications account for the remainder. Genetic testing for deletions relies on a multiplex PCR technique, with amplification of fragments containing 20 of the genes 79 exons and with deletions detected as absent or size-shifted bands on poly Acryl amide gel analysis. Because deletions tend to occur in hotspots within the dystrophin gene, analysis of this limited number of exons can detect 98% of dystrophin deletions. Hot spots are exons3-19 and 42-60.We studied all of these exons for 23 Iranian families. In our study most common of deletion were in exon6, exon44, exon50, exon4 respectively.

Association of the *GAD1* candidate gene with autism. *M. Martins[1,2], C. Correia[1,2], A. Currais[2], A.M. Coutinho[2], C. Marques[3], A. Ataíde[3], T.S. Miguel[3], J. Almeida[3], C. Bento[3], T. Morgadinho[4], G. Oliveira[3], A.M. Vicente[1,2]* 1) Neurogenetics & Mental Health Unit, Instituto Nacional de Saúde Dr Ricardo Jorge, Lisbon, Portugal; 2) Genetic Epidemiology Group, Instituto Gulbenkian de Ciência, Oeiras, Portugal; 3) Hospital Pediátrico de Coimbra, Coimbra, Portugal; 4) Faculdade de Medicina da Universidade de Coimbra, Coimbra, Portugal.

Autism, a childhood-onset neurodevelopmental disorder, is characterized by marked deficits in communication and social interaction skills, language impairment, and by repetitive and stereotyped behaviors. Although there is considerable evidence for a strong genetic component to idiopathic autism, the underlying genetic causes have yet to be identified. Several genome-wide scans for susceptibility genes have been carried out and, among others, a putative susceptibility locus on chromosome 2q31-33 has been identified. In the present study we tested the candidate gene glutamate decarboxylase 1 (*GAD1*), mapping to this linkage region, for genetic association with autism. The *GAD1* gene encodes GAD67, a protein responsible for the conversion of glutamic acid into GABA which has been found reduced in autistic patients. We have examined the association of autism with five SNPs within the *GAD1* gene, two located in the 5UTR region, and three in intronic regions, in a sample of 174 nuclear families and 132 controls. The Extended Transmission Disequilibrium Test (ETDT) showed significant association with autism for the marker rs3749034 (P=0.0190), in the 5UTR region. The Transmit test showed a significant transmission for six haplotypes ($0.0061<P<0.02$), suggesting association with autism. Moreover, given the known interactions between the glutamate, GABA and serotonin neurotransmission systems, we tested the hypothesis of an involvement of *GAD1* in the occurrence of hyperserotonemia in autism. Quantitative transmission disequilibrium test (QTDT) results showed no evidence of association between serotonin distribution and *GAD1* variation. The positive results obtained for ETDT of the 5UTR marker and the significant transmission for six haplotypes are suggestive of a contribution of the *GAD1* gene to autism pathogenesis.

Assessing the roles of GABABR2, DDC, and CHRNA4 in clefting of the lip in a large sample of Norwegian case and control triads. *A. Jugessur[1], R.T. Lie[1], A.J. Wilcox[2], H.K. Gjessing[3], R.M. Nilsen[1], J.C. Murray[4]* 1) University of Bergen, Bergen, Norway; 2) National Institute of Environmental Health Sciences, Durham, North Carolina, USA; 3) Norwegian Institute of Public Health, Oslo, Norway; 4) University of Iowa, Iowa City, Iowa, USA.

Genetic variants of DDC, CHRNA4 and GABABR2 have recently been linked to nicotine dependence. These variants might also be associated with clefting, since maternal smoking is a known risk factor. A maternal effect alone could reflect confounding from smoking, whereas an effect in the child could point to a direct effect of these genes, contributing to an apparent effect of maternal smoking. Twelve SNPs (GABABR2: rs10985765, rs1435252, rs3780422, rs2779562, and rs3750344; DDC: rs2060762, rs3757472, rs1451371, rs3735273, and rs921451; CHRNA4: rs4522666 and rs1044393) were genotyped on 377 case and 762 control triads from Norway. The software HAPLIN was used to estimate relative risks for a single or double dose of a haplotype in the mother or the child. Mothers with a single dose of haplotype GGCGC of DDC had a 2.5-fold risk of having a child with cleft lip (95%CI:1.4-4.3, $p<0.01$). For a double dose, the risk was 5.8-fold (95%CI:1.1-31.0, $p=0.04$). This haplotype was not overrepresented among the control mothers. Maternal haplotypes of CHRNA4 were not associated with cleft lip. For GABABR2, mothers with the haplotype CGTG in single dose had a reduced risk (RR=0.4, 95%CI:0.2-0.9, $p=0.03$). None of these haplotypes were associated with risk when present in the child. Children with a GC haplotype of CHRNA4 had a 2-fold risk (95%CI:1.3-3.2, $p<0.01$). In double dose, the risk was 2.1-fold (95%CI:1.1-4.0, $p=0.02$). Other associations were observed, but no clear pattern emerged. We also found a few irregularities in the haplotype distribution among control triads, but no deviations from Hardy-Weinberg equilibrium. To conclude, maternal haplotypes of DDC and GABABR2 were associated with cleft lip, consistent with an increased propensity to smoking in mothers with certain genetic variants of these genes. A haplotype of CHRNA4 in the child raised the risk of cleft lip, but not enough to explain the association with maternal smoking.

The mouse ortholog of KIAA2022, a candidate gene for mental retardation is highly expressed in the postnatal ventral premammillary nucleus. *A. Lossi[1], V. Cantagrel[1], L. Van Maldergem[2], L. Villard[1]* 1) INSERM U491, Faculte de Medecine La Timone, Marseille, France; 2) Institut de Pathologie et de Genetique, Loverval, Belgique.

We have previously reported a family in which a pericentric inversion of the X chromosome was segregating and in which two male individuals were affected by severe mental retardation. In this family, we demonstrated the complete absence of expression of the KIAA2022 gene in the two affected patients following its disruption by the intrachromosomal rearrangement. However, despite the subsequent screening of many additional patients affected by mental retardation, we failed to detect additional mutations in this gene, thus questioning its contribution to the observed neurological phenotype. In order to determine if KIAA2022 could be involved in the development or normal function of the central nervous system, we have cloned its murine ortholog, Kiaa2022, and we have studied its expression during the development. Using quantitative RT-PCR and in situ hybridization, we show that Kiaa2022 is preferentially expressed in the nervous system although its transcript can also be detected in other tissues. The expression pattern of this gene is temporally and spatially regulated. Kiaa2022 transcripts are detected at E11 in postmitotic cells of the central nervous system and Kiaa2022 expression increases rapidly during the development to reach a maximum a few days after birth at P3. The postnatal peak of expression is detected in hippocampus, in enthorinal cortex and in a discrete area of the hypothalamus, the ventral premammillary nucleus where Kiaa2022 is highly expressed. This nucleus was shown to be an important target of melatonin and also involved in conspecific agression and sexual behavior in rats. After P3, the expression of Kiaa2022 subsequently decreases and it is maintained at low levels during adulthood. Taken together, our results suggest that Kiaa2022 could play a role in the developing brain perhaps through a specific action in ventral hypothalamic nuclei.

Molecular pathogenic studies of Hutchinson-Gilford progeria syndrome. *Y.H. Li[1], W. Ju[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, Staten Island, NY.

Hutchinson-Gilford progeria syndrome (HGPS, OMIM176670) is an early-onset premature aging disorder. It is caused by a heterozygous silent mutation at cordon 608 (G608G: GGC>GGT) of LMNA gene that encodes nuclear lamin A/C. This mutation creates a cryptic splice donor site in exon 11 of LMNA gene and consequently produces a truncated progerin with a deletion of 50 amino acid (50-aa) residues near the C-terminus of lamin A. Presently, the pathogenic mechanisms underlying HGPS are yet unknown. Earlier, we have proposed that progerin may have a dominant-negative effect on lamin A/C. This effect may interfere with the normal functions of lamin A/C or generate novel associations with non-lamina proteins. We identified four novel progerin-interactive partner proteins (PIPs) and showed that the PIPs did not differential interact with lamin A/C, compared to progerin. Further studies (Ju et al., in this meeting) demonstrated that these PIPs associate with lamina proteins in both normal and HGPS cells without significant difference. Considering that progerin lacks the 50-aa of pre-lamin A, we hypothesize that the truncated 50-aa may have a critical function in the development of HGPS. We propose that there might exist unidentified proteins that normally can be interacted by lamin A while not with progerin in the HGPS condition due to the loss of function that progerin lacks the 50-aa residues. Therefore, we undertook an investigation to search for the unidentified protein(s) that may be uniquely interacted by the 50-aa resides with a yeast two-hybrid system. Ten candidate proteins interacted by the bait peptide of the missing 50-aa were identified, including fibulin C and filamin B. The fibulins are a family of proteins that are associated with basement membranes and elastic extracellular matrix fibres. We therefore investigated if there is any differential association between the lamin A/C and the progerin. Our data did not provide any difference between normal and HGPS cells. Further studies on the other candidates such as filamin B are being undertaken.

Clinical and molecular characterization of patients with microdeletions of 6p21. *R. Mendoza-Londono[1], S.A. Yatsenko[2], D. Napierala[2], L. Medne[3], E.H. Zackai[3], K. Armfield Uhas[4], F. Kendall[5], S. Unger[6], A. Hunter[7], P. Stankiewicz[2], B. Lee[2,8]* 1) Div Clin & Metab Genetics, Hosp Sick Children, Toronto, Canada; 2) Dept of Mol & Hum Genetics, Baylor College of Medicine, Houston, TX; 3) Div of Hum Gen & Mol Biol, The Childrens Hospital of Philadelphia; 4) Childrens Healthcare of Atlanta, Atlanta, GA; 5) Horizon Molecular Medicine, Atlanta, GA; 6) Institute for Human Genetics, Freiburg, Germany; 7) Childrens Hospital of Eastern Ontario, Sudbury, Canada; 8) Howard Hughes Medical Institute, BCM,Houston, TX.

Chromosomal microdeletions have become an important diagnostic consideration in patients with developmental delay and multiple congenital anomalies. The advent of systematic testing for these defects, available in chromosomal microarray platforms, has increased the identification of these common disorders, but the delineation of some of them is limited. We present the clinical and molecular characterization of patients with microdeletions of chromosome 6p21. We have identified 4 patients with microdeletions that range between 3 and 5 Mb in size in the 6p21 region. The region of the microdeletion harbors over 30 known and hypothetical genes including *VEGF* and *RUNX2*. Three of the patients presented with features of cleidocranial dysplasia (CCD) and developmental delay, and their samples were referred to our laboratory for mutation analysis of *RUNX2*. The fourth patient was identified through diagnostic microarray analysis. Patients with microdeletion 6p21 have characteristic facial features expressed as wide open fontanels in infancy, metopic groove, and frontal bossing. The patients have short stature and moderate developmental delay. Additional skeletal findings explained by haploinsuficiency for RUNX2 include supernumerary teeth, clavicular hypoplasia, and delayed ossification of the pubic bone. Other characteristics seen only in some of the patients include, congenital heart disease, hypospadias, growth hormone deficiency and megalocornea with signs of glaucoma in-utero. Further evaluation of patients with 6p21 microdeletions will allow us to refine the characterization of this under recognized syndrome.

Interstitial deletion of the long arm of chromosome 2 in a child with severe limb abnormalities and craniosynostosis : implication of the HOXD cluster? *N. Marle[1], L. Faivre[2], P. Callier[1], A.L. Mosca[1], S. Beer[3], C. Thauvin[2], F. Mugneret[1]* 1) Laboratoire de Cytogénétique, CHU Le Bocage, Dijon, France; 2) Centre de Génétique Clinique, Hôpital d'Enfants, Dijon, France; 3) Service de Néonatologie, Hôpital d'Enfants, Dijon, France.

Interstitial 2q deletion including the band 2q31 are associated with a large spectrum of limb malformations. This band contains the HOXD gene cluster, whose five most 5 members (HOXD9 through HOXD13) are known to play major roles in the growth and patterning of the developing limb. Here we report on the second child of healthy parents born from intracytoplasmic sperm injection. A cystic hygroma was evidenced at 12 weeks of gestation, investigated with normal cytogenetic studies. Examination at birth revealed a birth length of 46 cm with normal OFC at 36 WG. Facial dysmorphism included a high forehead, hypertelorism with broad nasal bridge, down-slanted palpebral fissures, small and low-set ears, a short neck and retrognathism. Only four fingers on the right hand with the presence of a thumb, an hypoplastic forearm and a luxation of the right elbow were noted, as well as a syndactyly between the 2nd-3rd and 4th-5th toes bilaterally and hypoplastic 5th toe. Examination of the left arm and mammary development were normal. X-ray studies revealed hypoplastic and curved left radius and ulna with four metacarpals. Other investigations showed an atrial septal defect with persistant ductus arteriosus. Follow-up at 4 months of age revealed cranial asymmetry and 3D CT scan confirmed a craniosynostosis of the metopic suture. Postnatal cytogenetic analysis with high resolution banding revealed a male karyotype with a deletion of the band 2q31 : 46,XY,del(2)(q31.1-q31.3. The deletion was confirmed by FISH study with BAC probe RP11-387A1 spanning the 2q31.1 band and including the HOXD gene cluster. Both parents had a normal karyotype. Further molecular studies using BAC probes are in progress in order to evaluate the size of the deleted segment. We compare clinical features and cytogenetic findings in our case with the previously reported observation and discuss the potential implication of HOXD in the phenotype.

Suggestive evidence for linkage to chromosome 4qter for autosomal dominant distal motor neuronopathy. *M. Muglia[1], A. Magariello[1], L. Citrigno[1], L. Passamonti[1], A. Patitucci[1], F.L. Conforti[1], A.L. Gabriele[1], R. Mazzei[1], T. Sprovieri[1], C. Ungaro[1], M. Bellesi[2], A. Quattrone[1,3]* 1) ISN-CNR, Mangone Cosenza, Italy; 2) Institute of Neurological Sciences, Ospedale Regionale "Torrette", University Ancona, Italy; 3) Institute of Neurology, University Magna Graecia, Catanzaro, Italy.

Distal hereditary motor neuronopathy (dHMN)is a rare genetically and clinically heterogeneous early-onset disorder, characterized by weakness and wasting of distal limb muscles with possible pyramidal dysfunction, in absence of overt sensory abnormalities. To date nine clinically different forms have been recognized, and seven loci have been identified for autosomal dominant distal HMN on chromosomes 2p13, 2q14, 7p14, 7q11, 9q34, 11q12, 12q24. A large four generation family with autosomal dominant dHMN was studied. All known loci responsible for dHMN were previously investigated. A genome wide search was performed by using 206 microsatellite markers from the ABI PRISM Linkage Mapping Set LD 20. Additional markers for fine mapping were obtained from Marshifield Genetic map. The two point lod scores obtained for all the analysed markers were negative. Suggestive positive Lod scores at =0 were obtained in region 4qter with three consecutive markers D4S424 (1.15), D4S415 (0.48) and D4S426 (1.07). Ten additional markers were tested in the family, and a maximum LOD score of 2.71 at D4S1607 in the absence of recombinants was obtained. Multipoint linkage analysis with markers D4S1607-D4S3041-D4S3051 resulted in a maximum LOD score of 3.78 at D4S1607 allowing assignment of a novel dHMN locus to this region. Two recombination events occurred in the family: in an affected individual with the proximal marker D4S2991 and in an unaffected subject with the distal marker D4S426. Haplotype construction and analysis of recombination narrowed this locus to a 20cM region between markers D4S2991 and D4S2924. Many genes are known to be located in this region; we screened the SNX25 gene, but no mutations have been identified. Further studies are needed to demonstrate the specific gene alteration.(Supported by a Telethon-Italia grant NGUP04009).

Development of a genotyping microarray for Usher syndrome. *H. Kremer[1], W.J. Kimberling[2], M. Külm[3], H. te Brinke[1], C.W.R.J. Cremers[1], L.H. Hoefsloot[4], F.P.M. Cremers[4]* 1) Dept Otorhinolaryngology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; 2) Center for the study and treatment of Usher syndrome, Boystown National Research Hospital, Omaha, NE; 3) Asper Biotech, Tartu, Estonia; 4) Dept Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.

Usher syndrome, a combination of retinitis pigmentosa (RP) and sensorineural hearing loss with or without vestibular dysfunction, displays a high degree of clinical and genetic heterogeneity. Three clinical subtypes can be distinguished, based on the age of onset and severity of the hearing impairment and the presence or absence of vestibular abnormalities. In addition, there are patients with atypical Usher syndrome. Thus far, 8 genes have been implicated in the syndrome, which together comprise 347 protein coding exons. Therefore, sequence analysis and the most routinely used mutation scanning techniques are not cost-effective for molecular diagnostics of the syndrome. To improve DNA-diagnostics for patients with Usher syndrome, we developed a genotyping microarray based on the arrayed primer extension (APEX) method. Allele-specific oligonucleotides corresponding to virtually all 298 Usher syndrome-associated sequence variants known to date were arrayed. Approximately half of these variants were validated using original patient DNAs which yielded an accuracy of >98%. The efficiency of the Usher genotyping microarray was tested using DNAs from 370 unrelated European and American patients with the syndrome. Sequence variants were identified in 64/140 (46%) patients with Usher syndrome type I, 45/189 (24%) patients with Usher syndrome type II, 6/21 (29%) patients with Usher syndrome type III, and 6/20 (30%) patients with atypical Usher syndrome. The chip also identified two novel sequence variants, c.400C>T (p.R134X) in PCDH15 and c.1606T>C (p.C536S) in USH2A. The Usher genotyping microarray represents a versatile and affordable screening tool for Usher syndrome. Its efficiency will improve with the addition of novel sequence variants with minimal extra costs, making it a very useful first-pass screening tool.

A bivariate whole-genome linkage analysis identified several shared genomic regions contributing to both total body lean mass and femoral neck bone geometry in a Caucasian sample. *S.F. Lei[1], F.Y. Deng[1], P. Xiao[2], D.H. Xiong[2], L.J. Zhao[2,3], H.W. Deng[1,2,3]* 1) College of Life Sciences, Hunan Normal University, China; 2) Osteoporosis Research Center, Creighton University, USA; 3) School of Medicine, University of Missouri-Kansas City, USA.

Our previous study found that the two highly heritable traits of total body lean mass (TBLM) and femoral neck cross-sectional (FNCS) geometry had highly genetic correlation. To identify the specific genomic regions shared by them, a bivariate whole-genome linkage analysis (WGLA) was performed in a large Caucasian sample with 4,498 individuals from 587 pedigrees. Bone mineral density (BMD) and bone size at the femoral neck, and TBLM were measured by Hologic DXA scanners. FNCS geometric parameters, including sub-periosteal diameter (W), cross-section area (CSA), cortical thickness (CT), section modulus (Z), and buckling ratio (BR), were calculated from BMD and bone size of femoral neck. Peak bivariate multipoint LOD scores were 1.74 (17p13), 3.32 (20q13), 3.88 (20q13), 2.39 (20q13), 1.89 (14q32) between TBLM with Z, BR, CT, CSA, and W, respectively. Other genomic regions with bivariate multipoint LOD score over 2.0 were 3p13 (LOD score = 2.18) and 6q27 (2.24) between TBLM and CT; 20p11 (2.30) between TBLM and CSA; and 3q12 (2.49) between TBLM and BR. Interesting, peak LOD scores in 20q13 are consistent between the three pairs of TBLM with CT, CSA and BR. Subgroup analysis also demonstrated the presence of sex-specific genomic regions shared by TBLM and FNCS geometry such as 7q11 and 10q24. We also performed bivariate WGLA between TBLM and two principal components derived from the above five parameters, and found the results supported the above findings. The results suggested that some functional loci in the shared genomic regions may exert pleiotropic effects on both TBLM and bone geometry, or that the loci respectively regulating TBLM and bone geometry are in linkage disequilibrium. In addition, due to the high phenotypic correlation between TBLM and other important bone strength phenotypes such as BMD, bivariate WGLA between them is also ongoing, with some preliminary interesting results found.

Expression of Proteins in Cortical Neurons Following DNA Damage. *M. Hosseini[1], K. Parivar[2], M.H. Ghahremani[3], N. Ostad[3]* 1) Science, Islamshahr Branch-Islamic Azad University, Tehran, Asia, Iran; 2) Dept. Animal Biology, Science & Research Campus, Islamic Azad University, Tehran,Asia, Iran; 3) Dept.Toxicology-Pharmacology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran,Asia, Iran.

DNA damage, as an important initiator of neuronal cell death, has been implicated in numerous neurodegenerative conditions. In this experimental model, primary embryonic cortical neurons were prepared from E14-16 rat embryos.A density of 1.2×105 cells/well plated on the wells, pre-coated with poly-D-lysine. Camptothecin (10-5 M)was added to neuronal culture after 24 hours. After 4,6,24 and 48 hours , expression of the P38 and ATF-2 was studied using primary antibody in the Immunocytochemistry technique,and number of healthy and death nuclei were counted by cell lysis buffer.Then percentage of the healthy , death and expression of the cells was analyzed by one way ANOVA followed by Tukeys post test . Percentage of the expression of P38 was 4%, 20%, 40% and 55%, and percentage of the survival was 95%, 85%, 64% and 50% for 4, 6, 24 and 48 hours, respectively.The expression of ATF-2 was also 3%, 20%, 30% , 45% and percentage of the survival was 97%, 85%, 64% and 50% , respectively. Percentage of the expression and survival of the P38 neurons for 24 hours were 40 and 64 percent and it was for ATF-2 , 30 and 64 percent respectively which these results compared to control were increased significantly ($p<0.05$). Expression of the proteins at 4 hours was not changed significantly.This study revealed that expression of the P38 and ATF-2 was increased simultaneously.Thus, in this model, Camptothecin induces neuronal death by stimulation P38-ATF-2 pathway.

Identification of RNAs interacting with N-Terminal Domain of FMRP (NDF). *B. He[1,2], W. Ju[1,2], W.T. Brown[1], N. Zhong[1,2]* 1) Human Genetics, New York State Institute for BAsic Research, Staten Island, NY; 2) Peking University Center of Medical Genetics.

Fragile X syndrome, as one of the most common inherited mental retardations, is caused by methylation-induced transcriptional silencing of fragile X mental retardation 1 gene (FMR1), which leads to absence of its translational product FMRP, the FMR1 encoded protein. FMRP was found to interact with protein and RNA molecules both in vitro and in vivo, suggesting that it may be involved in a complicated network of protein-protein and protein-RNA interactions. FMRP contains two known types of RNA-binding motifs: an RGG box and two heterogeneous nuclear RNP K homology (KH) domains. Previous studies have identified RNA partners of these two motifs. Recently, an N-terminal domain of FMRP (NDF) was determined to be a new RNA-binding domain in FMRP by using RNA homopolymers as partners. However, little study was performed to characterize the RNA-binding feature of NDF. Here, we undertook a study to determine the RNA-binding function of NDF by screening a yeast three-hybrid cDNA library we constructed earlier. Three RNA candidates, tubulin, alpha 3 (TUBA3); v-rel reticuloendotheliosis viral oncogene homolog B, nuclear factor of kappa light polypeptide gene enhancer in B-cells 3 (avian) (RELB); latent transforming growth factor beta binding protein 4 (LTBP4) have been identified to bind to NDF. Among them, TUBA is able to interact with metabotropic glutamate receptor 7, which mediates a variety of responses to glutamate in the central nervous system. Further characterization of these candidates will be presented.

A missense mutation in the canine ortholog of *ATF2* in standard poodles with neonatal seizures and encephalopathy. *G.S. Johnson[1], X. Chen[1], R.D. Schnabel[2], J.F. Taylor[2], H.G. Parker[3], E.E. Patterson[4], T. Awano[1], S. Khan[1], D.P. O'Brien[5]* 1) Veterinary Pathobiology, University Missouri, Columbia, MO; 2) Animal Sciences, University of Missouri, Columbia, MO; 3) Cancer Genetics Branch, NHGRI, NIH Bethesda, MD; 4) Veterinary Clinical Sciences, University of Minnesota, St Paul, MN; 5) Veterinary Medicine and Surgery, University of Missouri, Columbia, MO.

A previously undescribed autosomal recessive neonatal seizures and encephalopathy syndrome of standard poodles is characterized by decreased cerebellar size, cerebellar dysplasia, and hypomyelination in the cerebrum. Affected pups nursed poorly and were smaller than their littermates. Puppies surviving to 3 weeks of age were weak, ataxic, and mentally dull. By 4 to 6 weeks, they developed severe generalized clonic-tonic seizures. Weakness progressed to lateral recumbency with extensor rigidity, opisthotonus, and death. A panel of microsatellite markers was used to genotype DNA from a 73-member Standard Poodle family containing 20 affected puppies. Linkage analysis localized the disease locus to an 8.7 Mb segment of CFA36 between markers REN179H15 and REN252E18. The maximum two-point LOD score was 3.31. Fine mapping with 12 additional markers restricted the target region to a 3.76 Mb chromosomal segment containing 29 orthologs of genes from HSA2 including LOC478806, the canine ortholog of *ATF2* which encodes activating transcription factor 2. A LOC478806 missense mutation changes the ancestral methionine-51 to an arginine at a position immediately adjacent to a key alpha helix within the transactivation domain in the gene product. The mutant allele co-segregated with the disease in our standard poodle family: all 20 affected poodles were homozygous for the mutant allele; whereas, the normal relatives were either heterozygous (n = 44) or homozygous for the normal allele (n = 9). One hundred and eighteen representatives of other breeds were all homozygous for the ancestral allele. A pyrosequencing-based DNA test that identifies heterozygous carriers of the disease is available to standard poodle breeders.

Rapid implementation of MLPA using synthetic probes increases the mutation detection rate in DNA diagnostics.
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For the identification of genomic mutations during the forthcoming years sequence analysis will stay the golden standard. The major drawback of this technique is that heterozygous (multiple) exon deletions and duplications will not be detected. Deletion/duplication analysis for genes such as BRCA1, BRCA2 and MECP2 has demonstrated that such changes may be important sources of pathogenic mutations. Multiplex ligation-dependent probe amplification (MLPA) is a recent addition to routine DNA diagnostics allowing detection of copy gains or losses in an efficient, low-cost, robust way, with a high sensitivity and specificity. MLPA-kits have become available commercially for several genes with known deletions, including those mentioned above We have developed a system in which we can rapidly implement MLPA-analysis for any desired gene by the use of fully synthetic probes, as we expected that exon deletions and duplications would be an important source of pathogenic changes in other genes as well. Briefly, 5 control probes with different lengths have been designed to be included in all kits. Additional gene-specific synthetic probes can be added to up to 15 fragments in total, each fragment 4 nucleotides longer than the previous one. To date, we have been successful for the autosomal genes CHD7 , EYA1, LMX1B, and SPG4, and X-linked CHM, NDP and EHMT1. Preliminary data indicate that indeed 1-5% of mutations in the autosomal genes is an exonic deletion or insertion. For the X-linked disorders, it allowed us the easy diagnosis of female carriers with a deletion.

Mapping quantitative personality trait loci in opioid receptor genes: utility of stepwise MANCOVA and Roy Bargmann Stepdown ANCOVA. *X. Luo[1,2], H.R. Kranzler[3], L. Zuo[1,2], H. Zhang[1,2], S. Wang[4], J. Gelernter[1,2]* 1) Dept Psychiatry, Yale Univ Sch Medicine, West Haven, CT; 2) VA CT Healthcare System, West Haven, CT; 3) Dept Psychiatry, Univ CT Sch Med, Farmington, CT; 4) Dept Biostatistics, Columbia Univ Mailman Sch Pub Health, New York, NY, USA.

Human personality traits are among the most complicated quantitative traits. Certain personality traits are associated with substance dependence (SD); genetic factors may influence both. Associations between opioid receptor (OPR) genes and SD have been reported. We aimed to investigate the relationship between *OPR* genes and personality traits in the present study. We assessed dimensions of the five-factor model of personality in 250 subjects with SD [181 European-Americans (EAs) and 69 African-Americans (AAs)] and 306 healthy subjects (266 EAs and 40 AAs). We genotyped twenty *OPRM1* markers, eight *OPRD1* markers, and seven *OPRK1* markers, and thirty-eight unlinked ancestry-informative markers in these subjects. The relationships between *OPR* genes and personality traits were comprehensively examined using Multivariate ANalysis of COVAriance (MANCOVA), controlling for gene-gene interaction effects and potential confounders. Then, associations were decomposed by Roy Bargmann Stepdown Univariate ANalysis of COVAriance (ANCOVA). Generally, SD patients, older individuals, males, and African-Americans scored higher on Neuroticism and lower on other personality factors (i.e., Extraversion, Agreeableness, Conscientiousness, and Openness to Experience). There were main or interactive effects of haplotypes, diplotypes, alleles or genotypes at all three *OPR* genes. We demonstrate that *OPRM1*, *OPRD1*, and *OPRK1* may contribute to variation in personality traits, both directly and through gene-gene interactions.

Negative association between polymorphism rs225014 of DIO2 gene and bipolar disorder. *T. Zhang[1], B. He[1,2], N. Zhong[1,2]* 1) Peking University Center of Medical Genetics, Beijing, China; 2) New York State Institute for Basic Research.

Bipolar disorder (BPD) is a form of mood disorders that involves one or more episodes of serious mania and depression in affected individuals. The etiology of BPD may include genetic, developmental, social, cultural and environmental factors. Multiple genealogical£¨twin and adoption research showed that genetic background played an important role in the pathogenesis of BPD. Molecular genetic studies have identified several genes that may be associated with BPD, including 5-HTT, HTR, MAOA, COMT, IMPA1, DAOA, BDNF, etc. In previous studies, associations between depression and hypothyroidism and between mania and hyperthyroidism were described. A recent study demonstrated that patients hospitalized with hypothyroidism have a greater risk of readmission with BPD. Taking into consideration these data, together with other evidence such as association between the level of thyroid hormones (T3, T4) and mood instability, we are interested in exploring the relationship between T3, T4 and BPD. The deiodinase, which is encoded by DIO gene, is a key enzyme in the process of conversion between T3 and T4 and regulates the absolute and relative amount of T3 and T4. The DIO2 gene, a tissue-specific regulator of intracellular T3 concentrations in the brown fat, brain and pituitary, is particularly important for the conversion from T4 to T3 in the brain. In this study, we took DIO2 as our target to investigate the possible association with BPD. Eighty-two clinically diagnosed BPD patients were recruited as experimental subjects, while ninety-two normal individuals were included as controls. In both groups the DIO2 gene polymorphism rs225014 was in Hardy-Weinberg equilibrium. Our results reveal that (1) there is no significant difference between rs225014 allele frequencies of BPD patients and normal controls and (2) no significant difference was found among rs225014 allele frequencies of BPD patients with different features (gender, family history and suicide tendency). Our preliminary study indicates that there is no association between polymorphism rs225014 of DIO2 and BPD. Further investigations, such as more markers and haplotype assay will be pursued.

Fanconi Anemia: Congenital abnormalities in patients homozygous for the *FANCG637-643* deletion mutation. *T. Haw, L. Wainwright, J. Poole, A. Krause* Division of Human Genetics, National Health Laboratory Service and University of the Witwatersrand, Johannesburg, South Africa.

Fanconi anemia (FA) is a chromosome breakage disorder caused by mutations in one of at least twelve different genes. Individuals with FA have diverse clinical features but the disease is characterized by aplastic anemia (AA). There has been evidence to show genotype phenotype correlations. A deletion in the *FANCG* gene (*FANCG637-643*) accounts for 78% of all FA mutations in the black South African (SA) population. We aimed to delineate the congenital abnormalities associated with this mutation and to determine whether hematological outcome could be predicted from the number of congenital abnormalities a patient had.

The frequency of congenital abnormalities in 25 black SA patients homozygous for *FANCG637-643* was compared to FA patients described by two other research groups, one from Europe and one from the US. The US group was composed of patients with mutations in unknown FA genes (mixed group) while the patients in the European group had mutations only in *FANCG*.

No significant differences in the frequency of congenital abnormalities were found between the SA group and the *FANCG* cohort. However, significantly more patients from the SA group had head abnormalities, including dysmorphic features and microcephaly, and significantly fewer had developmental delay and hearing impairment compared to the mixed group. Therefore mutations in the different genes may be associated with specific abnormalities. We have identified that thumb abnormalities, head abnormalities and growth retardation are the congenital abnormalities most frequently associated with *FANCG637-643*.

It was reported previously that the greater the number of congenital abnormalities, including developmental delay, heart, kidney, hearing and head abnormalities, in a FA patient, the larger their risk for developing AA. We found that *FANCG637-643* is associated with a high risk of early development of AA even though individuals have a low number of these congenital abnormalities.

Identification of a novel risk gene for Progressive Supranuclear Palsy by a genome-wide scan of 500,288 SNPs. *S. Melquist[1], M.J. Huentelman[2], D.W. Craig[2], M. Baker[1], R. Crook[1], J.V. Pearson[2], V.L. Zismann[2], J. Gass[1], J. Adamson[1], S. Szelinger[2], J. Corneveaux[2], A. Cannon[1], K.D. Coon[2], D.W. Dickson[1,3], D.A. Stephan[2], M. Hutton[1]* 1) Neuroscience, Mayo Clinic College of Medicine, Jacksonville, FL; 2) Neurogenomics, Translational Genetics Research Institute, Phoenix, AZ; 3) Pathology, Mayo Clinic College of Medicine, Jacksonville, FL.

We hypothesized that additional genetic loci may be involved in conferring risk for Progressive Supranuclear Palsy, which could be identified through genome-wide association methods. Affymetrix 500K SNP chips were therefore used in a pooled genome-wide association analysis of a large, pathologically-confirmed PSP case and control set. The *MAPT* H1 haplotype, a previously identified risk locus, was strongly detected by this methodology, providing proof of concept. In addition, a second major locus on chromosome 11p11.2 and a minor locus on chromosome 4 showed evidence for association at a genotypic (p=0.0002, p=0.004, respectively) and haplotypic level. The chromosome 11p11.2 locus could be narrowed to a small region containing just a few candidate genes, with the well-described DNA repair gene *DDB2* showing the strongest evidence for association. The identification of *DDB2* as a gene likely harboring risk for PSP suggests that alteration in DNA repair capacity could play a vital role in the neurodegenerative process in PSP and perhaps other related disorders.

Candidate gene analyses for nonsyndromic cleft lip (CL) with or without cleft palate (CP) in families from six countries. *M.L. Marazita[1], B.S. Maher[1], J.C. Murray[2], A. Lidral[2], L.L. Field[3], T. Goldstein McHenry[1], M.E. Cooper[1], S. Daak-Hirsch[2], A. Jugessur[2], B. Riley[2], L. Moreno[2], P. Chines[4]* 1) Ctr Craniofacial and Dental Genet, U of Pittsburgh, PA; 2) U of Iowa; 3) U of British Columbia, Canada; 4) Genome Technology, NHGRI.

Our research group identified several chromosome regions that reached genome-wide significance for containing susceptibility loci for CL/CP, including 1q, 2p, 3q, 9q, 14q, 16p (Marazita et al 2005). To follow-up those results 1,476 SNPs from candidate genes and regions were genotyped using the Illumina platform by the Center for Inherited Disease Research (CIDR). The study population consisted of 5,232 individuals (2,850 genotyped of whom 1,008 were affected) from 499 families ascertained from six countries (Philippines, China, India, Turkey, U.S.A., Spain). Reported here are the family-based-association-test (FBAT) results. To control for multiple testing and to include information from our previous genome scans we performed a weighted approach to false discovery rate (FDR; Roeder et al 2006). In this approach, per marker FBAT p-values were adjusted using a weighting scheme devised from our published genome scan meta-analysis (Marazita et al, 2004) allowing for a maximum 20% FDR. Analyses were done for the TOTAL dataset, CL, CLP and CLCLP subsets (CL=families with CL-alone affecteds, CLP=all affecteds have CL plus CP, CLCLP=some affecteds with CL and some with CLP). SNPs in IRF6 are the only ones reaching formal FDR-adjusted significance in the TOTAL ($p<10^{-7}$) and CLCLP ($p=7x10^{-5}$) datasets. Although not reaching formal significance, notable results include SNPs on 9q in TOTAL ($p<0.001$) and CLCLP ($p=0.001$), and on 6q in CLP (gene DDO, $p<0.001$). The 6q region has a 90% probability of containing a gene for CL/CP (Govil et al, this meeting). Further genotyping suggests that FOXE1 is likely to be the associated gene on 9q (Mansilla et al, this meeting). In summary, IRF6 is confirmed as a susceptibility locus for CL/P, and genes on 9q and 6q require additional confirmatory studies. NIH grants R01-DE09886, R01-DE012472, R37-DE08559, R01-DE016148, P50-DE016215, M01-RR00084; CIDR NIH contract N01-HG-65403.

Genotyping of Common Variants Near the HNF4A Locus in a Norwegian Population-Based Sample of 1850 Diabetes Cases and 1879 Controls. *S. Johansson[1,2], H. Raeder[1], S.A. Eide[1], K. Midthjell[3], O. Søvik[1], A. Molven[4], P.R. Njolstad[1,5]* 1) Department of Clinical Medicine, University of Bergen; 2) Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital; 3) Department of Public Health and General Practise, Norwegian University of Science and Technology; 4) The Gade Institute, University of Bergen; 5) Department of Pediatrics, Haukeland University Hospital, Norway.

Introduction: Several recent publications from different populations have shown an association between SNPs near the P2 promotor of the HNF4A gene and Type 2 diabetes, whereas no effect has been seen in other populations. Hence, it is still not clear whether genetic variations in this region might predispose to diabetes. We therefore genotyped four common SNPs near the HNF4A gene in a Norwegian population. Material: 1850 patients with diabetes and 1879 controls were recruited from a large population based study (HUNT) in a county (Nord-Trøndelag) in Norway. Genotyping was performed using the Sequenom single base extension technology. Results: When comparing all diabetes patients with controls a non-significant trend towards increased OR was seen for the minor alleles at the two SNPs flanking the P2 promotor rs1884613 (OR=1.09, 95% CI: (0.97-1.23)) and rs2144908 (OR=1.11, (0.98-1.25)) and for the major allele at rs3818247 (OR=1.09 (0.99-1.20)) located downstream of the gene (three-marker- haplotype p=0.07). The putative effect remained similar in size for all three SNPs after exclusion of subjects with possible autoimmune diabetes as measured by GAD autoantibodies or C-peptide <150 pmol/L: OR=1.08-1.10 (95% CI range 0.95-1.25). Conclusion: The results from this Norwegian population, although not significant, are consistent with the associations found both in the Swedish and Danish population. These populations are considered genetically similar and we hypothesize that the discrepant results between different populations may reflect differences in LD-pattern with unidentified disease-associated SNPs in the region.

Is the $GABA_A$ receptor a possible target for drug therapy in fragile X syndrome? *F. Kooy[1], C. D'Hulst[1], P.P. De Deyn[2]* 1) Dept Medical Genetics, Univ Antwerp, Antwerp, Belgium; 2) Department of Neurochemistry and Behavior, Institute Born-Bunge, Univ Antwerp, Antwerp, Belgium.

After our initial discovery of under expression of the $GABA_A$ receptor subunit in a genome wide expression profiling study in fragile X mice, a mouse model for the most frequently inherited form of mental retardation, we analyzed expression of the 17 remaining subunits of the $GABA_A$ receptor by real-time PCR. We found significant under expression of the $_{1, 2 \text{ and } 3}$, $_{1 \text{ and } 2}$, and $_{1 \text{ and } 2}$ in the cortex of fragile X mice. $GABA_A$ receptors are the main inhibitory receptors in brain, implicated in anxiety, epilepsy, sleep-wake cycles and learning and memory, processes also disturbed in fragile X patients. Many factors lead us to believe that the $GABA_A$ receptor is a novel target for drug therapy in fragile X syndrome. Patients with 1p36 deletions, lacking the $GABA_A$ receptor subunit among other genes, show neurological and neuropsychiatric anomalies as well as epilepsy, and it was suggested that the subunit contributes to this phenotype. In addition, evidence for involvement of the $GABA_A$ system in fragile X syndrome is strengthened by recent observations that the ratio between inhibitory (taurine+GABA) and excitatory (aspartate+glutamate) amino acids in fragile X mice brain is decreased. Other groups analyzed differential expression of the -subunits at protein level and found, in line with our results at RNA level, 2-fold under expression of these subunits in cortex. Powerful new therapeutic opportunities may arise from the $GABA_A$ receptor pharmacology. Numerous drugs of clinical importance bind to various types of the $GABA_A$ receptor, including benzodiazepines like diazepam (Valium), barbiturates, such as phenobarbital and neuroactive steroids, like ganaxolone. Currently we are investigating whether fragile X mice have an altered susceptibility to these drugs by testing their effect in a battery of behavioural tests. This may elucidate the physiological consequences of under expression of the GABA-ergic system in fragile X mice.

***FGFR1* overexpression and 5' CpG Island hypomethylation in primary rhabdomyosarcoma tumors.** *A. Orr-Urtreger[1,2], M. Goldstein[1,2]* 1) Genetics Inst, Tel Aviv Sourasky Medical Ctr, Tel Aviv, Israel; 2) Sackler Faculty of Medicine, Tel Aviv University, Tel-Aviv, Israel.

Rhabdomyosarcomas (RMS) are highly aggressive tumors likely to result from deregulation of proliferation and differentiation during skeletal myogenesis program. The three histopathological RMS subtypes are: the prevalent embryonal (ERMS), the more aggressive alveolar (ARMS) and the rare adult variant pleomorphic RMS (PRMS). The association between genetic alterations in the *PAX3*, *PAX7* and *FOXO1A* genes and RMS tumorigenesis was well established, however, the entire spectrum of genetic changes underlying the development and progression of this neoplasm remains to be studied. Recently, we reported multiple novel genetic aberrations in a series of primary RMS tumors, including 2 ERMS, 7 ARMS and one PRMS (Goldstein, et al., Neoplasia 2006). Notably, the amplification of the *Fibroblast Growth Factor Receptor 1* (*FGFR1*) gene was detected in the PRMS tumor. We further investigated *FGFR1* involvement in RMS tumorigenesis by quantitative mRNA expression and methylation analyses, as well as mutation screening in the 10 primary RMS tumors and in 4 RMS cell-lines. *FGFR1* overexpression was detected in all primary RMS tumors and cell-lines tested. Using DHPLC and sequencing, activating mutations were ruled-out in the entire *FGFR1* coding sequence and exon-intron boundaries. However, hypomethylation of a CpG island upstream to *FGFR1* exon 1 was identified in the primary RMS tumors by the sodium bisulfite modification method, suggesting a molecular mechanism for *FGFR1* overexpression. Expression analysis of three additional genes, the cell survival regulator *AKT1*, *NOG* (*Noggin* homolog) and its antagonist *BMP4* (*bone morphogenetic protein 4*), which interact downstream to FGFR1, demonstrated significant expression differences between the primary RMS tumors and normal muscle. Thus, our data suggest that aberrant sustained or reactivated FGFR1 transduction signals in RMS cells may lead to deregulation of the downstream network and perturbation of the delicate equilibrium between proliferation and differentiation in skeletal myoblasts.

Prenatal detection of pure complete trisomy 1q: A case report. *N. Le Meur[1], S. Patrier[2], V. Ickowicz[3], A. Laquerriere[2], M. Trehin[1], E. Verspyck[4], P. Leboullenger[5], T. Frebourg[6], A. Rossi[1]* 1) Laboratory of Cytogenetics, EFS-Normandie, Bois-Guillaume, France; 2) Department of Pathology, University Hospital, Rouen, France; 3) Department of Radiopediatry, University Hospital, Rouen, France; 4) Department of Obstetrics, University Hospital, Rouen, France; 5) Department of Obstetrics, Mathilde Hospital, Rouen, France; 6) Department of Genetics, University Hospital, Rouen, France.

We report on cytogenetic, ultrasonographic, foetopathological and neuropathological description of a fetus with pure complete trisomy 1q. Prenatal chromosome analysis was performed because of intra-uterine growth restriction and showed, in a mosaic state, a translocation of the entire long arm of chromosome 1 on chromosome 22: 46,XX,der(22)t(1;22)(q12;q11)[5]/46,XX[32]. Subsequent ultrasound examination revealed craniofacial dysmorphism with low set malformed ears, hypertelorism and micrognathia and multiple congenital malformations including cerebral ventriculomegaly, vermis hypoplasia, and camptodactily. In agreement with the french law, the parents decided to terminate the pregnancy at 27 weeks of gestation. Autopsy confirmed sonographic findings and showed bilateral abnormal lung lobulation, an accessory spleen and asymmetric kidneys. Neuropathological examination displayed hypertrophy of ventricular septum, corpus callosum, olfactif bulbs and internal capsule. Cytogenetic analysis performed in lung, thymus, skin, liver, muscle and placenta confirmed the mosaicism. Only 9 cases of trisomy of the long arm of chromosome 1 have previously been reported. The fetus that we described shared therefore physical features with other reported cases but some phenotypic features had never been described (hypertrophy of ventricular septum, corpus callosum, olfactif bulbs and internal capsule). Trisomy 1q is obviously a very rare condition but the frequency of trisomy 1q mosaic may be underestimated. This case report highlights the importance to perform extensive cytogenetic analysis on different tissues from fetus or newborns with multiple congenital malformations when trisomy 1q phenotype is suspected.

Association of Eukaryotic Translation Initiation Factor 2-alpha Kinase 3(*EIF2AK3*/*PERK*) with Bone Mineral Density in the Old Order Amish. *N. Hoppman[1], E.A. Streeten[1], A.R. Shuldiner[1,2], J.R. O'Connell[1], B.D. Mitchell[1], D.J. McBride[1]* 1) University of Maryland School of Medicine, Baltimore, MD; 2) GRECC, Veterans Administration Medical Center, Baltimore, MD.

The Amish Family Osteoporosis Study (AFOS) was designed to identify genes influencing bone mineral density (BMD) in the Old Order Amish. Based on observations in humans and mice, we identified *EIF2AK3*, also known as *PERK*, as a candidate gene for osteoporosis. *PERK* spans approximately 71 kb of chromosome 2p12 and consists of 16 exons with 6 known splice patterns. The function of PERK is to phosphorylate the alpha subunit of eukaryotic translation-initiation factor 2, resulting in its inactivation and thus a rapid reduction of translation initiation and repression of global protein synthesis. Mutations in *PERK* have been shown to cause Wolcott-Rallison syndrome, a rare, autosomal recessive disorder characterized by permanent neonatal or early infancy insulin-dependent diabetes. These patients develop epiphyseal dysplasia, osteoporosis, and growth retardation at later ages. Additionally, *Perk* -/- mice display pancreatic and skeletal defects including deficient mineralization, osteoporosis, and abnormal compact bone development.

Sequence analysis of a subset of AFOS subjects (n=40) was used to identify *PERK* SNPs in this population. All 16 exons, including UTR and splice junctions, and approximately 5kb upstream of exon 1 were sequenced. Eleven SNPs were identified: 4 in the promoter, 3 nonsynonymous coding SNPs, 1 synonymous coding SNP, 1 intronic SNP and 2 just 3 to the gene. We genotyped all 4 promoter variants and 2 nonsynonymous coding variants in AFOS participants (n=900) and compared mean BMD by genotype to test for associations. All 6 polymorphisms were significantly associated with BMD of the forearm ($P < 0.001$), spine ($P < 0.01$), and/or hip ($P < 0.05$). In stratified analyses, some SNPs were significantly associated with BMD in both sexes separately (one promoter and both nonsynonymous SNPs) while the others were associated in only one sex. These findings suggest that sequence variation in *PERK* may influence BMD.

Balanced interchromosomal insertional translocation resulting in partial trisomy 3p in two brothers with mental retardation. *A.L. Mosca[1], N. Marle[1], C. Thauvin[2], P. Callier[1], L. Faivre[2], F. Mugneret[1]* 1) Laboratoire de cytogénétique, CHU le Bocage, Dijon, France; 2) Centre de Génétique, Hôpital d'Enfants, Dijon, France.

Interchromosomal insertional translocations are rare chromosomal rearrangements with an incidence of about 1:80,000. Most insertional translocations are familial with a maternal origine and lead to pure trisomy or monosomy. Balance carriers have a high risk of unbalanced offspring, theoretically up to 50%. Here, we report on 2 brothers with moderate mental retardation. The elder brother was 3.5 years old and presented with normal growth, minor dysmorphic features including long eyelashes, thin lips and a pointed chin and severe behavioural abnormalities. The younger brother was 2.5 years old and presented with high forehead, a pulmonary valve stenosis and speech difficulties. Familial history revealed mental retardation in two maternal cousins. High resolution chromosomal analysis performed in the both children showed the presence of extra material on the short arm of chromosome 6. FISH with a whole chromosome- specific paint for the chromosome 6 (Vysis) was normal and FISH analysis using the 3p ter probe showed two signals on both chromosome 3p homologs. CGH studies revealed that the additional material was of chromosome 3p. Parents karyotype showed an insertional translocation of the short arm of the chromosome 3 into the long arm of chromosome 6 in the father. The karyotype was defined as follows : 46,XY,ins(6;3)(p23;p25.3p26.1) while the children karyotype was: 46,XY,der(6)ins(6;3) (p23;p25.3p26.1). Further FISH studies using BACs and PACs of the 3p region are in progress in order to characterise the chromosomal breakpoints and the size of the trisomic region. This familial case is of interest in defining the phenotype of 3p25-26 pure trisomy.

Screening and testing using the same data set: A strategy for case/control genomewide association studies. *J. Lasky-Su[1,3], M. McQueen[2,3], N. Laird[3], C. Lange[3]* 1) Psychiatry, SUNY Upstate Medical University, Syracuse, NY; 2) University of Colorado, Boulder, CO; 3) Harvard School of Public Health, Boston, MA.

We propose a 2-stage testing strategy for case/control studies that applies the screening step and the testing step to the same data set. The screening step is constructed so that it is statistically independent of the association tests that are computed in the testing step. The most "promising" SNPs that are identified by the screening step can therefore be tested for association in the testing step without the need to adjust the significance level for the statistical analysis in the screening step. In simulation studies for 100k scans, we observe substantial power differences by several magnitudes between the proposed testing strategy and standard case/control testing methodology. The practical relevance of the approach is illustrated by an application to a genome-wide association studies in which SNPs are identified and reach genome-wide significance that would not have been detected by standard case/control testing methodology.

Microsatellite Instability in Chinese Epithelial Ovarian Carcinoma. *X.S. Liu[1], Y. Lu[1], N. Zhong[2,3]* 1) Dept. Gynecology, OBGYN Hospital, Shanghai, China; 2) Peking University Center of Medical Genetics; 3) New York State Institute for Basic Research.

Ovarian carcinoma (OC) is one of the most lethal cancers of women in China. About 90% of OC are epithelial ovarian tumors. The association of microsatellite instability (MSI) with several cancers has been shown to play a significant role in the development of cancers. However, The role and incidence of MSI in ovarian cancer are matters of great controversy. In some studies, MSI frequencies have ranged from 0 to 53% in sporadic ovarian cancers. These studies varied greatly in terms of the type and number of loci studied, the criteria used for defining MSI, and the histological subtypes of ovarian cancer analyzed. The role and incidence of MSI in epithelial ovarian tumor remains unknown. Our current study is to evaluate the frequency of MSI in epithelial ovarian tumors and its relationship with clinicopathologic features. Ninety fresh specimens of epithelial ovarian tumors (primary 74, secondary 16) were collected from our gynecology clinic of Medical Center of Fudan University from 2004 to 2005. Microsatellite analysis was carried out using 5 mono- and dinucleotide markers from the National Cancer Institute Consensus Panel by fluorescence-labeled polymerase chain reaction. Of the 90 epithelial ovarian tumors analyzed, 18 demonstrated a high level of MSI (MSI-H), 30 demonstrated a low level of MSI (MSI-L), and the remaining 42 exhibited microsatellite stability (MSS). Frequency of MSI at loci BAT-25 was higher than that at any other loci. No correlation was found between MSI level and patient age, tumor type, tumor differentiation ($P > 0.05$). But the microsatellite instability-high phenotype correlate with clinical stage, it tended to occur more frequently in early-stage tumors ($P = 0.03$). Our results indicate that there are frequent MSI in epithelial ovarian tumors. It is an early event and it is involved in the development of epithelial ovarian tumors.

Detection of large deletions and duplications in genomic DNA using a semi-quantitative multiplex PCR-based assay on capillary electrophoresis systems. *S. Karudapuram[1], S. Jankowski[2], M. Barrois[3], A. Miniere[3], A. Rico[2]* 1) Applied Biosystems, Foster City, CA; 2) Applied Biosystems France, 25 Avenue de la Baltique B.P.96, 91943 Courtaboeuf Cedex, France; 3) Laboratoire de Génétique, Institut Gustave Roussy, Villejuif, France.

Deletions and duplications in genomic DNA have been implicated as pathogenic mutations in many diseases. Traditionally, detection of these types of mutations is done using southern blot hybridization or fluorescence in situ hybridization, techniques which can be laborious, time-consuming and require high quantities of starting material. In this study we present a semi-quantitative multiplex PCR-based method that uses relative quantitation of fluorescently-labeled short fragments. Fragments from BRCA1, BRCA2, 9p21 and MMR (MSH2) regions were amplified using FAM-labeled primers from DNA that had been isolated from blood. Amplified samples were then run on an Applied Biosystems capillary electrophoresis platform and the data was analyzed in GeneMapper software. After normalization to a control amplicon, peak regions that had undergone deletions or duplications were identified using the GeneMapper software v4.0 report manager feature and verified using the dye scale functionality. Our results will highlight an easy to use, optimal system that can be used for both small and large scale studies.

Assessment of specimen storage and DNA integrity for optimal aCGH results. *H.A. Marble, M. Lucas, T. Perez, M. Ziegler, K. Wilber, E. Pestova* Abbott Molecular, Inc., Des Plaines, IL.

Array Comparative Genomic Hybridization (aCGH) is a multiplex molecular technique that detects chromosomal gains and losses, as well as high-resolution genomic disparities such as microdeletions and microduplications, across the entire genome by measuring differences in DNA copy number of a test sample relative to a normal reference standard. In this study, we describe limitations of aCGH imposed by the test specimen quality, demonstrate the effect of DNA integrity on aCGH performance, and show the importance of standardized quantitative software algorithms in evaluating the reliability of aCGH results. We assessed performance of DNA from cell lines, blood, and archived peripheral blood lymphocytes on a BAC array of 333 clones, printed in triplicate, using an image quality assessment software algorithm, Overall Quality Rating (OQR), that rejects suboptimal array results based on an experimentally selected quality measure cutoff. In a set of 200 hybridizations, the distribution of OQR scores varied dramatically depending on the DNA source. Archived blood lymphocyte DNA exhibited the greatest performance variability (10-20% failure rate). In these specimens, we showed the dependency between test DNA size distribution (fragmentation below 9 KB) and poor aCGH results. Furthermore, we proved that the integrity of DNA purified from blood lymphocytes fixed in Carnoys reagent was affected by specimen storage conditions. Fresh whole blood specimens afforded the least variability in aCGH assays (failure rate 6%). However, regardless of the type of collection/storage vessel, 3-week blood storage resulted in deterioration and low DNA yields, leading to suboptimal aCGH performance. The effect of DNA integrity on aCGH was further confirmed by artificial enzymatic degradation of test DNA. In these experiments, we observed a strong linear correlation between the DNA size distribution and OQR scores. Finally, we demonstrated the feasibility of modifying aCGH assay conditions to recover suboptimal-quality specimens for aCGH analysis. Together, these results provide guidelines for specimen storage and DNA sample integrity assessment to achieve reliable aCGH results.

Preconceptional or prenatal Fragile X screening. Experience of 14 years in a Belgian Center for Human Genetics. *P. Hilbert, S. Boulanger, L. Van Maldergem, Y. Gillerot, Ch. Verellen-Dumoulin* Centre de Genetique Humaine, Institut de Pathologie et de Genetique, Loverval, Belgium.

Fragile X syndrome is one of the most frequent cause of inherited mental retardation. The high frequency of this disease seems to justify the prenatal or preconceptional screening of women but the question raised is controversial. We present here the results of 14 years of premutation screening in a Belgian Center for Human Genetics. Prenatal or preconceptional screening was performed on request of clinicians, mainly geneticists and gynaecologists, for women with no known family history of fragile X syndrome. On a total of 8043 women, a full mutation was found in a patient and a premutation in 34 patients (1/237). The cut-off for premutation was set at 59 repeats which seems to be the smallest repeat who ever amplified to a full mutation in one generation With the frequently used premutation threshold of 55 repeats, we obtain a premutation frequency of 1/149. The availability of a prenatal diagnosis was offered to all the premutated women. On a total of 36 foetus from 23 different women, the premutated allele was transmitted 16 times. In 11 cases, mothers had expansion below 70 repeats and the inherited premutation in the foetus ranged from -3 to +12 repeats compared to the mothers premutation. In 5 cases, the mothers expansion was over 70 repeats. In 4 males, the transmission resulted in 3 full mutations and 1 mosaïcism for full mutation and premutation and , in one female, in an unmethylated expansion of about 200 repeats. Pregnancy was terminated in the 4 affected males. This confirms that alleles over 70 repeats are at high risk of expansion to full mutations when transmitted. Such alleles concerned 15 of the 34 premutated women which means 1/536 women of an unselected population. Based on theses results, we suggest that either preconceptional or prenatal screening is to be recommended to women in childbearing age.

Single nucleotide polymorphism-comparative genomic hybridization (SNP-CGH) of PBMC and buccal DNA from B-CLL subjects using the Illumina Human -1 SNP Genotyping BeadChip. *A. Lee[1], N. Chiorazzi[1], K. Rai[2], A. Liew[1], P. Gregersen[1]* 1) Dept Genomics & Human Genetics,The Feinstein Institute for Medical Research, Manhasset, NY; 2) Dept Hematology/Oncology Long Island Jewish Hospital, New Hyde Park, NY.

B-cell chronic lymphocytic leukemia (B-CLL) accounts for 30% of all leukemias in Western countries and is characterized by the clonal proliferation and accumulation of neoplastic B lymphocytes, which can account for more than 90% of total PBMCs in some patients. Chromosomal changes are common and have some prognostic value in disease outcome. Deletion of 13q14 is more often associated with indolent/benign B-CLL while trisomy 12 and deletions of 17p and 11q22-23 are associated with aggressive disease. The aim of this study was to compare and contrast PBMC and buccal DNA from the same subjects in order to distinguish somatic from germline genetic variation. To accomplish this we performed single nucleotide polymorphism-comparative genomic hybridization (SNP-CGH) on PBMC and buccal DNA using the Illumina 109K SNP Human-1 beadchip. This whole genome genotyping assay allows for the high resolution of chromosomal changes in DNA copy number, allelic imbalance/loss of heterozygousity (LOH) and other chromosomal aberrations. The SNP-CGH analysis was concordant with FISH data available for 4 PMBC B-CLL samples. In addition, several other chromosomal aberrations were observed throughout the genome that were not detected by the B-CLL FISH panel. Consistent with the somatic origin of these changes, these chromosomal aberrations were observed in PBMC DNA, but were not present in the buccal samples. Thus, SNP-CGH appears to be a more sensitive method of detecting somatic genetic changes in B-CLL. Furthermore, these results emphasize that linkage or association studies of B-CLL may be confounded by the use of DNA derived from PBMCs, and buccal DNA may be more appropriate for studying the effect of germline polymorphisms in disease susceptibility.

Influence of MTHFR genotype on the teratogenic effects of antiepileptic drugs. *U. Kini[1], R. Lee[3], A. Jones[3], S. Ramsden[3], A. Fryer[4], J. Clayton-Smith[2]* 1) Dept of Clinical Genetics, Churchill Hospital, Oxford, United Kingdom; 2) Regional Genetics Service, St. Mary's Hospital, Manchester; 3) Regional Molecular Genetics Service, St.Mary's Hospital, Manchester; 4) Dept. of Clinical Genetics, Alder Hey Children's Hospital, Liverpool.

Antiepileptic drug(AED) therapy in pregnancy has an additive effect on the physiological reduction in folate levels. This may be further enhanced by the presence of the common C677T polymorphism in the MTHFR (methylenetetrahydrofolate reductase) gene which lowers the activity of the MTHFR enzyme. Folate deficiency has been linked to birth defects eg. neural tube defects,seen frequently with exposure to AEDs such as valproate and carbamazepine. Aim: To examine the influence of the maternal and childs MTHFR genotype on the rate of major malformations (MM) and developmental delay (DD) in children with AED exposure. Methods:Between January 2000-August 2004,in a prospective, population-based study women on AED treatment (cases) and matched control mothers (without epilepsy)were recruited from antenatal clinics. Buccal cells from the mother and child were screened for the C677T variant by PCR. Children were examined for MM, as defined by EUROCAT criteria and DD was assessed using the Griffiths Test. Fishers exact test was used to analyse the statistical significance of the results. Results: We studied a total of 343 mothers -128 cases (102 on monotherapy and 26 on polytherapy) and 215 controls.6.1% (4/66) of case mothers with C677T variant had children with MM compared to 1.9% (2/107) controls(p=0.262).11.8% (2/17) of TT homozygote case mothers had a child with MM (both exposed to valproate) compared to 0% in controls (p=0.56).The rate of MM was found to be similar between the wild type cases(4.4% or 2/45) and controls(4.8% or 4/84)(p=1.000). There was no correlation between the maternal or childs MTHFR genotype with DD at the age of 2 years or between the child's MTHFR genotype and the rate of MM. Conclusion: Although these results do not reach statistical significance due to small sample size, they show a trend for increased risk of MM in children of case mothers who are C677T heterozygotes or TT homozygotes.

Managing gene-specific information at NCBI. *D.R. Maglott* NCBI, National Institutes of Health (NIH;NLM), Bethesda, MD.

The Entrez Gene database at the National Center for Biotechnology Information (NCBI) represents the result of identifying, annotating, and integrating information about genes from an increasing number of key genomes. The data flow includes close coordination with NCBI's Reference Sequence group (RefSeq), model organism databases and nomenclature committees, and multiple annotation projects. The GeneID assigned to each gene, and the sequences used to define that gene, provide a standard for communication about genes among databases within and external to NCBI.

This presentation will summarize key aspects of the dataflow used by Entrez Gene and other databases within NCBI that manage gene-specific information, such as GEO, HomoloGene, Map Viewer, OMIM, and UniGene. Recent efforts to improve the scope of and access to the data will be reviewed. Highlights will include web interfaces for multiple NCBI databases, genomic gene-specific RefSeqs, and reports from Gene and Map Viewer. Methods to review sequence and functional annotation, including phenotype, will be emphasized.

Compound heterozygous mutations in *LMNA* cause MADA phenotype. *F. Lombardi[1], F. Gullotta[1], C. Catalli[1], V. Brugiati[1], S. Considera[1], A.M. Nardone[1], E. Grosso[2], G. Lattanzi[3], N.M. Maraldi[3], M.R. D'Apice[1], G. Novelli[1]* 1) Dept. of Biopatology Diagnostic Imaging, University of Rome "Tor Vergata" Roma, Italy; 2) Laboratory of Medical Genetics, A.O. S.G Battista Torino, Italy; 3) Laboratory of Cell Biology, Istituti Ortopedici Rizzoli, Bologna, Italy.

Mandibuloacral dysplasia type A [MADA; OMIM # 248370] is a phenotypically heterogeneous, rare autosomal recessive disorder. Almost all MADA patients carry the same homozygous missense mutation (R527H) in C-terminal tail domain of *LMNA* gene, encoding lamin A/C, an intermediate filament component of the nuclear envelope. We report a 27-yr-old Italian girl with MADA clinical features. The patient shows hypoplastic mandible (micrognathia), acro-osteolysis of the finger distal phalanges, pointed nose, generalized loss of subcutaneous fat, acrogeric features. She also presents muscular hypotrophy and generalized hypotonia. We examined the *LMNA* gene, identifying a compound heterozygous with the classical R527H mutation and the rare V440M allele in exon 7, previously detected in a patient with a Dunnigan-type partial lipodystrophy (FPLD). In order to investigate the effects of these mutations on the nuclear architecture, usually affected in MADA fibroblasts, we examined the nucleus morphology and the distribution pattern of the nuclear lamina proteins lamin A and lamin C and the unprocessed toxic precursor pre-lamina A protein. Lamins A and C resulted not strongly altered in their expression and localization, but a percentage of nuclei showed accumulation of pre-lamin A in the nuclear envelope, associated with formation of intranuclear pre-lamin A-labeled structures. These results shows a MADA-like cellular phenotype suggesting that the R527H in compound heterozigosity state with V440M behaves as dominant mutation.

Variants in the 3'UTRs of different isoforms of the *NTRK3* candidate gene for panic disorder. *M. Muiños-Gimeno[1], M. Gratacòs[1], R. Martín-Santos[2], ML. Ímaz[2], M. Torrens[2], X. Estivill[1,3], Y. Espinosa-Parrilla[1]* 1) Genes and Disease Program, Center for Genomic Regulation, Barcelona; 2) Psychiatry Department, Hospital Mar & Pharmacology Research Unit, IMIM, Barcelona; 3) Department of Health and Life Sciences, Pompeu Fabra University, Barcelona, Catalunya, Spain.

Neurotrophins and their receptors have been implicated in the common pathophysiology of depression and anxiety and in the therapeutic action of some antidepressant drugs. Due to its involvement in synaptic transmission, survival and differentiation of neurons and in neuronal plasticity, the neurotrophin-3 receptor gene (*NTRK3*) is a good candidate for these psychiatric disorders. The *NTRK3 loci* encode a variety of isoforms, including the full-length (FL) receptor and a truncated kinase-deficient form (TF) with a different and longer 3UTR. Recently, and in contrast to previous hypothesis, it has been shown that these two isoforms are able to activate different pathways. Moreover, levels of mRNA and protein of the different *NTRK3* isoforms are discordant during human lifespan suggesting that factors other than direct transcriptional control may contribute to their regulation. We suggest that 3UTRs of *NTRK3* isoforms may be involved in the genetic susceptibility to psychiatric disease, particularly anxiety and depression. 3UTRs are important for post-transcriptional regulation mechanisms involving polyadenilation or miRNAs silencing. Therefore, according to their conservation in evolution and the presence of putative miRNA target sites, we have re-sequenced the 3UTRs of the TF (3kb) and FL (2kb) of *NTRK3*. Re-sequencing was performed in 62 chromosomes of patients with panic disorder with/without major depression. We have identified 3 previously known SNPs (MAF>0.4 for all of them), and 6 new sequence variants (3 with MAF>0.1) in the TF. A known SNP (MAF= 0.45) and a new sequence variant (MAF=0.02) were identified in the FL. Case-control studies are being performed in a group of 261 patients and 340 controls. Finally, the possibility that these variants disrupt the sites of regulation by miRNAs has been analyzed by the use of *in silico* approaches. Supported by the Spanish Government (SAF2005-01005; FI05/00061; G03/184).

Enhancement of SMN2 gene expression by the DNA demethylating agent 5-aza-2'-deoxycytidine. *E. Hahnen, J. Hauke, B. Wirth* Institute of Human Genetics, Institute of Genetics and Center for Molecular Medicine Cologne (CMMC), University of Cologne, Germany.

Spinal muscular atrophy (SMA) is an autosomal recessively inherited neuromuscular disorder. The disease determining survival motor neuron gene 1 (SMN1) is homozygously deleted or mutated in approximately 97 % of all SMA patients. Within the SMA region (5q13), the SMN genes exist in two almost identical copies, SMN1 and SMN2, which are ubiquitously expressed and encode identical proteins. Since all SMA patients lacking SMN1 carry at least one SMN2 copy, transcriptional activation of the SMN2 gene is likely to be clinically beneficial. We and others have shown that histone deacetylase (HDAC) inhibitors such as valproic acid (Brichta et al., 2003) and the highly potent second generation HDAC inhibitors SAHA and M344 (Hahnen et al., 2006; Riessland et al., 2006) increase SMN2 expression levels in fibroblast cell lines derived from SMA patients as well as in several neuroectodermal tissues, including rat and human hippocampal brain slice cultures and motoneuron-rich cell fractions. Here we report for the first time that the DNA demethylating agent 5-aza-2'-deoxycytidine (5-Aza) increases SMN2 transcript- and protein levels in SMN1-deleted fibroblasts. This finding indicates that both, histone acetylation and DNA methylation, regulate SMN2 gene activity. SMN2 promoter analyses indicated that the human SMN2 gene contains 4 putative CpG islands (CpG1-4). Bisulfite treatment of DNA derived from SMN1-deleted fibroblasts followed by methylation-specific PCR, cloning of PCR products and sequencing revealed methylation of specific cytosine residues within and adjacent to the predicted SMN2 CpG-1, CpG-2 and CpG-4, while CpG-3 appears to be not methylated. The SMN2 methylation patterns were subsequently analyzed using DNA isolated from blood samples of 10 type I SMA patients. In each case we found almost identical SMN2 methylation patterns, while the analyses of other tissues are in progress. These results improve the understanding of how expression of the SMN2 gene is regulated and may contribute to the development of a treatment of SMA.

Simultaneous detection of changes in copy number and CpG methylation of the differentially imprinted chromosome 11p15 BWS locus using Methylation-specific MLPA (MS-MLPA). *A.O.H. Nygren[1,2], B. Baskin[3], R.R.T. Brekelmans[2], D.J.G. Mackay[4], J.P. Schouten[2]* 1) DNA Diagnostics Lab, Free University Medical Center, Amsterdam, Netherlands; 2) MRC-Holland bv, Amsterdam, Netherlands; 3) The Hospital for Sick Children, Toronto, Canada; 4) Wessex Regional Genetics Labs, Salisbury District Hospital, Salisbury United Kingdom.

Beckwith-Wiedemann Syndrome (BWS) is a clinically heterogeneous overgrowth syndrome associated with an increased risk for embryonal tumor development. This condition is either caused by a genetic or an epigenetic alteration within two domains of imprinted growth regulatory genes on human chromosome 11p15 leading to dysregulation of the expression of the imprinted genes within this region. The incidence of BWS is estimated to be approximately 1 in 15000 and around 85% of the cases are sporadic. 60-70% of these have imprinting abnormalities at one of two imprinted domains H19DMR or KvDMR. Other causes of BWS are Uniparental disomy (UPD)(20%), maternally inherited translocations (1%), trisomy with paternal duplication (1%), mutations in the CDKN1C gene (10%) as well as small deletions. We have developed a novel application of Methylation-Specific MLPA (MS-MLPA) capable of detecting most causes of BWS, including UPD, in a single reaction. Besides, this MS-MLPA assay can distinguish H19DMR and KvDMR methylation defects. The MS-MLPA assay was validated on DNA samples from BWS patients and identified methylation defects as well as chromosome 11p15 duplications. Apart from generating next day results of both copy numbers and methylation status using only 20ng of DNA, the MS-MLPA assay for BWS might also be a great aid in the screening of childhood cancers, in particular Wilms tumor. A strong linkage between methylation of the H19DMR locus, but not KvDMR, has been described in these patients resulting in biallelic expression of the IGF2 gene.

Fatal form of KID syndrome. *S. Hadj-Rabia[1], D. Hamel-Teillac[1], C. Parsy[1], C. Thibaud[2], L. Jonard[3], M. Levêque[3], D. Feldmann[3]* 1) Service de dermatologie Hôpital Necker, Paris, France; 2) Service de gynécologie Centre Hospitalier Intercomunnal, Créteil, France; 3) Laboratoire de Biochimie et Biologie Moléculaire, Hôpital A Trousseau, AP-HP Hôpital Trousseau, Paris, France.

Keratitis-ichtyosis-deafness syndrome (KID) is a rare ectodermal dysplasia caracterized by vascularizing keratitis, profound sensorineural hearing loss (SNHL) and progressive erythrokeratoderma. Dominant mutations in the connexin 26 gene GJB2 cause KID syndrome. Most of the patients bared the D50N mutation. Recently, a de novo mutation, G45E, have been described in a fatal form of the disease. The G45E mutation has not been previously reported in Caucasian patients, however it is the third most common GJB2 mutation in Japanese patients with autosomal recessive nonsyndromic hearing loss. Different modes of action of G45E depending on genetic background have been suspected. We described a new family from Africa with twins affected by a fatal form of KID syndrome and a G45E de novo mutation. G,3 month old boy, had pachyderma and erythroderma, palmoplantar keratoderma, complete atrichia, dystrophic nails, congenital absence of foreskin, hyperplasic vocal cord hyperplasia. Ophthalmologic examination and immunity exploration were normal.Several episodes of uncontrolled septicaemia lead to death at 5 months. His brother, with the same phenotype, died at 1 month. Molecular analysis of GJB2 revealed an heterozygous transition 134G-A causing an exange of glycine 45 by glutamic acid in the twins only. In order to search for other genetic variation that could modify the phenotype, analysis of mitochondrial DNA and of the CX 30 gene GJB6 was performed. The mtDNA sequence differed from the revised Cambridge reference sequence by 67 variations including 3308T-C and 5655T-C. The patients mtDNA sequence belongs to African haplogroup L1b. But no deleterious variation could be observed. Sequencing of GJB6 gene in the patients showed an heterozygous transition 15G-A that do not change the aminoacid threonine 5. However 15G-A is a silent variation, it is possible that this rare variation could influence the expression level of CX30 and increase the potentially effect of mutant CX26 on the function of CX30.

Genetic liability to schizophrenia in Oceanic Palau: a search in the affected and maternal generation. *L. Klei[1], B. Devlin[2], J. Tiobech[3], C. Otto[3], M. Myles-Worsley[4], W. Byerley[5], K. Roeder[6]* 1) Computational Genetics Program, Dept of Psychiatry, UPMC, Pittsburgh, PA; 2) Dept. of Psychiatry, Univ. of Pittsburgh SOM, Pittsburgh, PA; 3) Belau National Hospital, Korror, Palau; 4) SUNY Upstate Medical Center, Syracuse, NY; 5) Dept. of Psychiatry, UCSF, San Francisco, CA; 6) Dept. of Statistics, Carnegie Mellon Univ., Pittsburgh, PA.

We postulate a two-hit model for Schizophrenia (Scz), in which initial liability is generated during fetal development. We hypothesize this hit to the developing fetal brain is precipitated by environmental stressors interacting with maternal genetic vulnerability to stress. The second hit occurs perinatally, again due to genetic vulnerability. To evaluate the putative genetic vulnerability, we search in the genome of affected individuals and their mothers for variation that differs from that in the general population. We used a sample from the Palauan population that contained 175 individuals who were diagnosed with Scz, broadly defined. DNA was available from these affected individuals, 87 of their mothers and 45 of their fathers; for analyses of parents, mothers were treated as affected, not offspring, and fathers were treated as controls. Pedigree and diagnostic data were available on 2953 living and deceased subjects, most clustering into one pedigree. DNA from 553 individuals was genotyped for STRs spaced every 10 cM across the genome. We tested for association between affection status and STR alleles, which was reasonable, despite the widely-spaced markers, because the population has far-ranging linkage disequilibrium. Results were consistent with the hypothesis of maternal genetic liability. For a recessive model and a test for excess allele matching across mothers, significant findings occurred for D20S481, D10S1221, D6S1021, D13S317, and D18S976. Other noteworthy results occur for D9S1118, D2S1356, D13S325, and D17S1290. Regions with two adjacent markers with substantial statistics include 2p12-11.2, 2q24.1-32.1, 6q12-14.1, 10q23.2-24.21, 12q23.2-24.21 and 17q23.2-23.3. Results for the truly affected individuals were modest. The strongest association occurs for D2S434, at 2q35.

SNP Regulation of LDLR Splicing Efficiency. *I.F. Ling, H.M. Tucker, S. Estus* Department of Physiology, University of Kentucky, Lexington, KY.

Low density lipoprotein receptor (LDLR) is critical to cholesterol homeostasis. We have identified a single-nucleotide polymorphism (SNP) that decreases the splicing efficiency of exon 12. The exon 12 deleted mRNA is predicted to produce a soluble form of LDLR. However, the mechanism whereby this SNP regulates splicing is unclear. We utilized a NucleofectorTM kit to co-transfect HepG2 cells with an LDLR mini-gene and several SR protein splicing factors. Twenty-four hours later, we purified the resulting mRNA and performed RT-PCR to evaluate the splicing efficiency of exogenous LDLR. We found that overexpression of SRp40 appeared to rescue the decreased splicing efficiency due to the SNP. In contrast, SC35 further exacerbated the splicing inefficiency. We interpret these results as implying that the splicing regulation of LDLR exon 12 is complex and the SNP modulates a functional exonic splicing enhancer (ESE) for SRp40. Therefore, we will mutate several nucleotides in LDLR exon 12 to define the functional ESE and investigate interactions between the ESE and splicing factors.

Integration of imaging and genetics: an analytical strategy to perform genome-wide studies for Imaging Genetic Phenotypes (IGPs). *F. Macciardi[1], J. Turner[2], L. Friedman[2], H. Stern[3], J. Fallon[2], S. Potkin[2]* 1) Dept. of Science and Biomedical Tehcnology, Univ of Milano, Milano, Italy; 2) Brain Imaging Center Dept. of Psychiatry and Human Behavior University of California Irvine; 3) Dept. of Statistics University of California Irvine.

Our goal is to develop novel approaches that address the interplay of imaging and genetics, within the context of a specific disease. Key to our approach is a general linear model (GLM) analysis of a quantitative imaging phenotype. The model can accommodate a range of factors expecting to affect the observed imaging activation (e.g., ethnicity, behavior/performance): in the most basic form, the GLM is: Imaging Phenotype = Overall Mean + Genotype Effect + Diagnosis Effect + Genotype-Diagnosis Interaction Effect To perform a genome-wide analysis with 500,000+ SNPs, we developed a method building on the work of Satagopan et al. (2004) and modifying their approach to use the GLM summary test statistic on imaging phenotypes instead of traditional test statistics comparing allele frequencies across diagnostic groups. Our approach is to assume that a set T of the 500,000+ SNPs are loci with true genotype-diagnosis interaction effects on the imaging phenotype. The goal is to identify a subset t of these T true disease-related loci using the loci corresponding to the highest t test-statistics. Note that the value of t is determined by considerations of power. The power of the statistical strategy is defined as the probability that the t highest test statistics correspond to t out of the T true disease (and imaging) related loci. This power can be calculated by simulation as a function of sample size, T and t, the minor allele frequencies in the patient and control groups and the interaction effect size.

Hutchinson-Gilford Progeria Syndrome (HGPS): Consistency of phenotype in 15 children. *W.J. Introne[1], M.A. Merideth[1], L.B. Gordon[2], M.B. Perry[3], M. Turner[4], S. Clauss[5], V. Sachdev[6], J. Graf[3], A.C.M. Smith[1], L.H. Gerber[7], J.C. Reynolds[3], J.A. Yanovski[8], R. Cannon[6], W.A. Gahl[1]* 1) NHGRI, NIH, Bethesda, MD; 2) Brown University, Providence, RI; 3) Clinical Center, NIH, Bethesda, MD; 4) NCI, NIH, Bethesda, MD; 5) CNMC, Washington, DC; 6) NHLBI, NIH, Bethesda, MD; 7) GMU, Fairfax, VA; 8) NICHD, NIH, Bethesda, MD.

HGPS, an extremely rare disorder (1/4-8 million), is considered a segmental premature aging syndrome. It is a sporadic autosomal dominant disease uniformly fatal at an average age of 13 y. Cardiovascular disease causes most of the morbidity and mortality, but the disease is multisystemic. Affected children generally lack clinical findings at birth, but progressively manifest stunningly consistent features. In 1972, DeBusk described the clinical features of HGPS, but no comprehensive evaluation of the clinical course has been performed. In February 2005, we initiated a natural history protocol at the NIH to document the clinical progression of HGPS. We have evaluated 15 children, ages 1 to 16 y. Sclerodermatous dermatologic changes, one of the first findings noted by parents, occurred in 14/15 children before 1 y of age. Resting echocardiograms were normal in 13/15 children. All 15 children had radiographic findings of clavicular blunting, acroosteolysis, and coxa valga. Joint involvement, with contractures, was present in all 15 children, but the pattern and extent of joint involvement was highly individualized. Mean hip flexion was 101.5 $\pm$ 17 degrees (nl 120), mean ankle total range of motion (ROM) was 36 $\pm$ 20 degrees (nl 70), and mean knee total ROM was 101.5 $\pm$ 28 degrees (nl 135). Percent body fat as measured by DXA showed a linear decrease with increasing age. Children under age 2 y had, on average, 24% body fat, but by 10 y, the average was 10% body fat. Z-scores of bone density were uniformly low, ranging from -1.5 to -6.6. Insulin resistance was seen in 6 of 13 children studied. The phenotypic presentation of children with HGPS is remarkably constant and involves multiple organ systems. Longitudinal studies will elucidate the disease progression and provide objective clinical markers to monitor the efficacy of potential treatments.

Morphological abnormalities of the mitochondrial network in fibroblasts of patients affected with autosomal dominant and recessive optic neuropathies. *N. Kadhom, S. Gerber, F. Barbet, A. Munnich, J. Kaplan, J.M. Rozet*
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Inherited non syndromic optic atrophy (OPA) are frequently inherited as an autosomal dominant trait. The most common of these autosomal dominant disorders are related to mutations in the OPA1 gene on chromosome 3q28. On the other hand, a second probably uncommon locus was reported on chromosome 18q12 (OPA4) and we recently mapped a third OPA locus (OPA5) on chromosome 22q12.3. Besides, autosomal recessive non syndromic OPA are very rare. Until now, only one locus has been reported on chromosome 8q23 (ROA1). Fibroblasts of patients related to OPA1 (n=1), OPA5 (n = 2) and ROA1 (n=2) have been labelled using MitoTracker Red CMXRos dye, a fluorescent mitochondrial marker that binds free sulfhydryls within the cells. Fibroblasts of age-matched controls were studied at the same time for each genetic subtype. Morphological abnormalities of the mitochondrial network were observed in the five patients compared to the respective controls. The cellular distribution of mitochondria in fibroblasts of patients related to OPA5 (n=2) and OPA1 (n=1) was superimposable: the labelling of the MitoTracker Red CMXRos dye appeared fragmented and highly concentrated around the nucleus of cells (ring-like aspect). Interestingly, the distribution of mitochondria in the fibroblasts of the two patients related to ROA1 was also superimposable but clearly different from that of patients related to dominant optic atrophies. The labelling of the MitoTracker Red CMXRos dye was highly heterogeneous and testified a fragmentation of the mitochondrial network but no nuclear ring-like aspect was noted. In conclusion, this study suggests that a selective vulnerability of the optic nerve to perturbations in mitochondrial function may underlie a final common pathway in all inherited optic atrophies. These findings provide a rationale in order to select candidate genes as genes encoding proteins with high mitochondrial targeting probability for both the OPA5 and ROA1 loci.

Array-CGH further defines constitutional supernumerary marker chromosomes (SMCs). *C.M. Higgins, D.L. Pickering, M. Wiggins, D. Zaleski, A.H. Olney, G.B. Schaefer, B.J. Dave, W.G. Sanger* Munroe Meyer Institute, Omaha, NE.

Microarray comparative genomic hybridization (aCGH) technology in the clinical cytogenetics laboratory not only allows for rapid and efficient characterization of supernumerary marker chromosomes but also detects the specific chromosomal region that defines the marker chromosome. This facilitates the precise identification of the abnormality which in turn helps genotype/phenotype correlation. We present two cases of males less than 2 months of age who were referred for cytogenetic studies. Karyotypic analysis revealed 47,XY,+mar in both cases. Case 1 was a 7 week male with large hands and feet and was greater than the 95th percentile for height and weight. He had thin, deep-set nails, retromicrognathia, downslanting palpebral fissures, a long philtrum and an umbilical hernia. Case 2 was a 2 week male with complex congenital heart disease, dysmorphic facial features and renal failure. Whole chromosome paint and centromere FISH studies determined that the origin of the SMCs were chromosome 5 (case 1) and chromosome 10 (case 2). Microarray studies utilizing the Spectral Chip 2600 further characterized the SMCs at 1MB resolution. In case 1, aCGH analysis identified a duplication of seven clones in the region of 5p13.3-5p13.1. The duplicated region did not include the most proximal bands of 5p and consequently was interpreted as a complex ring chromosome: arr cgh 5p13.3p13.1(RP11-5N11=>CTD-2142G21)x3. In case 2, aCGH detected a copy number gain of 4 clones in the proximal short arm of chromosome 10 indicating a duplication of 10p11.2-10p11.1. The nomenclature for this finding is as follows: arr cgh 10p11.2p11.1(RP11-174P15=>RP11-393J16)x3. SMCs result in isolated partial trisomies of the involved chromosome segment and may provide an opportunity to evaluate the clinical effects exclusive to the duplicated segment. These cases illustrate the importance of aCGH which, when used in conjunction with classical cytogenetics and FISH studies, helps to accurately define the specific chromosomal segments involved. This information is valuable in the management of patients presenting with SMCs.

Independent family study confirms an association between gap junction alpha 8 gene (GJA8) on chromosome 1q21 and schizophrenia. *X. Ni[1], J. Valente[2], M. Azevedo[2], M. Pato[3], C. Pato[3], J. Kennedy[1]* 1) Neurogenetics Section, Ctr Addiction & Mental Health, Toronto, ON, Canada; 2) Department of Psychiatry, University of Coimbra, Portugal; 3) Veterans Administration Hospital, Washington, D.C., USA.

Gap junction, called electrical synapse, permits the direct intercellular exchange of ions and molecules including glutamate and second messengers, and plays important roles in the central nervous system. Recently, we genotyped four polymorphisms in the GJA8 gene, rs989192, rs4950495, rs3766503 and rs1532399, in 220 DSM-IV schizophrenia patients and 220 matched (sex, age and ethnicity) healthy controls, collected from Toronto and Central Canada. A strong association between GJA8 haplotype, especially based on the first two markers, and schizophrenia (LRS=33.13, df=3, p=0.0000003) was found. To confirm this dramatic finding we studied an independent Portuguese families, 163 trios and small families with DSM-IV schizophrenia probands. Two polymorphic markers, rs989192 and rs4950495, were genotyped and the program TDTPHASE in the UNPHASE (v2.404) was used for individual marker and haplotype analyses. The G allele of rs989192 (T:UT=61:36.5, LRS=4.9, p=0.027) and the T allele of rs4950495 (T:UT=73:43.8, LRS=5.95, p=0.015) transmitted much often than the opposite alleles. G-T haplotype also showed a significant association with schizophrenia (T:UT=57.97:33.74, X2=5.07, p=0.024). These results replicate our previous finding in Toronto cases and matched controls, and indicate that the GJA8 gene may play a role in the genetic susceptibility to schizophrenia. Due to rs989192 and rs4950495 are both in the intron, we searched for polymorphic markers in the coding and promoter regions in the GJA8 gene in public SNP databases, and found only one, rs7536277, in the promoter region. Unfortunately, we did not detect any variant of rs7536277 in our samples. To screen and identify functional variants in GJA8 gene is warranted. This project is supported by the Young Investigator Award of NARSAD (X.N.).

The association of GABA related genes with autism. *D.Q. Ma[1], A.L. Collins[1], P.L. Whitehead[1], E. Martin[1], R.K. Abramson[2], H.H. Wright[2], J.P. Hussman[3], J.L. Haines[4], M.L. Cuccaro[1], J.R. Gilbert[1], M.A. Pericak-Vance[1]* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) University of South Carolina School of Medicine, Columbia, SC; 3) The Hussman Foundation, Ellicott City, MD; 4) Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

GABA and gabaergic pathways are the major neural inhibitory system in human brain. It is considered one of the most important neurotransmitters and developmental signals and may relate to onset of autism and related developmental disorders. Our previous studies have confirmed association of the $GABA_A$ receptor (GABRA) subunit genes with autism and the interaction of GABRA4 with GABRB1 as contributing to increased autism risk. To test the possibility of a more general role for gabaergic pathways in autism, we examined 13 additional GABA related genes. An average 4-5 SNPs from different LD blocks within each gene were analyzed in 470 Caucasian autistic families using the family-based pedigree disequilibrium test (PDT) for allelic and genotypic association analysis and using extended multifactor dimensionality reduction (EMDR) for gene-gene interaction analysis. The only nominally significant associations were found at rs1054899 (PDT: p=0.037) on chromosome 6 within the ALDH5A1 gene; RS499368 (geno-PDT: p=0.036) on chromosome 12 within the SLC6A12 gene and RS1640989 (PDT: p=0.039) on chromosome 16 within the ABAT gene. Surprisingly, EMDR analysis did not identify any significant interactions among these markers as well between the previously reported GABRA4 and GABRB1 genes. These results do not suggest a major role for these GABA related genes in autism risk.

Genome-wide association analyses for Metabolic Syndrome on the Micronesian island of Kosrae. *J.K. Lowe[1,2,3], I. Pe'er[2,3], J. Salit[1], M.L. Blundell[1], M.L. Sullivan[1], H.E. Lee[1], R. Tewhey[2], A. Havens[1], W. Ji[5], J.-N. Foo[5], L. Garcia[1], P.E. Bonnen[1], N.P. Burtt[2], K.W. Jones[4], R.P. Lifton[5,7], J.L. Breslow[1], M.J. Daly[2,3,6], J.M. Friedman[1,7], D.M. Altshuler[2,3,6], M. Stoffel[1]* 1) The Rockefeller University, New York, NY; 2) The Broad Institute of MIT and Harvard, Cambridge, MA; 3) Massachusetts General Hopsital, Boston, MA; 4) Affymetrix, Inc., Santa Clara, CA; 5) Yale University School of Medicine, New Haven, CT; 6) Harvard Medical School, Boston, MA; 7) Howard Hughes Medical Institute.

The island of Kosrae in the Federated States of Micronesia was founded ~2000 years ago by Polynesians. Geographic isolation and multiple population bottlenecks reduced genetic diversity on Kosrae, producing an order of magnitude more extensive linkage disequilibrium in this population compared to Asians or Caucasians, with genetic markers being more effective in capturing population variation. This makes Kosrae an attractive population for mapping genetic components of complex diseases, even with marker density that is insufficient elsewhere.

We ascertained 3200 Kosraean adults in screenings carried out in 1994 and 2001. Patients were characterized for traits related to Metabolic Syndrome, including type 2 diabetes, obesity, dyslipidemia and hypertension. The high prevalence of Metabolic Syndrome in this population is illustrated by the diabetic cohort. Type 2 diabetics comprise 35% of Kosraean adults age 40-60, compared to 12-15% incidence in western European populations of similar age. A single extended pedigree with 4431 total individuals was reconstructed using both genetic and self-reported family data. Subjects were genotyped using the Affymetrix 100k Mapping Assay. We developed automated analysis tools to accommodate this large, population-based cohort. Signals of association from pedigree founders are combined with family-based transmission distortion tests and evaluated with empirical significance testing. We will present results from genome-wide analyses for components of Metabolic Syndrome, including type 2 diabetes, obesity, dyslipidemia and hypertension, as well as attempts to replicate the most promising loci observed in the Kosraean population.

DNA methylation at the human *IGF2* locus is highly heritable and associated with SNPs *in cis*. *B.T. Heijmans[1], D. Kremer[1], E. Tobi[1], D.I. Boomsma[2], P.E. Slagboom[1]* 1) Molecular Epidemiology, Leiden University Medical Centre, Leiden, The Netherlands; 2) Biological Psychology, Vrije Universiteit, Amsterdam, The Netherlands.

Little is known about epigenetic variation between individuals and the causes of this variation. Therefore, we studied DNA methylation of the insulin-like growth factor II (*IGF2*) locus in 196 adolescent (172 y) and 176 adult (457 y) twins. The *IGF2* locus is maternally imprinted and one of the best characterized genomic regions under epigenetic control. We assayed DNA methylation of two sequences associated with *IGF2* imprinting: the promoter of the *H19* transcript (10 CpGs/413 bp) and the *IGF2* DMR (6 CpGs/338 bp). To quantitatively measure DNA methylation of individual CpG sites we used a new mass spectrometry based method (Sequenom). The mean methylation of CpGs in the *H19* region varied between 23-30% and in the *IGF2* DMR region between 38-62%. Considerable inter-individual variation was observed, which was particularly apparent for two CpGs that displayed a bi- and trimodal distribution. To assess the influence of genetic and environmental factors on this variation, variance components analysis was performed. The heritability of methylation was substantial but lower for *H19* (.20-.74) than for *IGF2* DMR CpGs (.57-.97). Of note, the influence of environmental factors was not greater in the older twins. Next, SNPs were genotyped to trace the genetic variation underlying the heritability. The *MTHFR* Ala/Val variant, which is associated with genome-wide hypomethylation, did not affect methylation of the *IGF2* locus. In contrast, SNPs in the *IGF2* region did. Whereas modest associations were observed for *H19* methylation ($P<.05$), SNPs *in cis* strongly contributed to *IGF2* DMR methylation. Strikingly, a single SNP explained the bimodal distribution observed for one of the CpGs ($P<10^{-19}$). The trimodal distribution observed could be attributed to another SNP ($P<10^{-12}$). The associated SNPs were not located within the sequences assayed and the mechanism underlying our results is as yet unclear. In conclusion, methylation of the *IGF2* locus is highly heritable and strongly associated with genetic variation *in cis*.

Detection of aneuploidic mosaicism by a molecular method, co-amplification of artificial SNPs. *K.M. Hong[1], Y.B. Choi[1], M.H. Ko[1], S.H. Han[2]* 1) Research Institute, National Cancer Center, Goyang, Korea; 2) Seoul Clinical Laboratories (SCL), Seoul Medical Science Institute (SMSI), Dongbinggo-dong, Yongsan-gu, Seoul, Korea.

We showed that co-amplification of artificial SNP sequences (Co-aSNPs) is an accurate and rapid method for the detection of gene copy number changes. The SD values were only 1/2-1/3 of those from MLPA (multiplex ligation-dependent probe amplification) or MAPH (multiplex amplifiable probe hybridization) suggesting that this method might detect mosaic aneuploidies reliably. We tested this possibility by mixing EB-virus transformed cell lines of Downs syndrome and control samples. The cell lines were labeled with different tracking dyes and the ratio of two cell lines was monitored by confocal and fluorescent microscopy. DNAs were prepared from the mixed cell lines and were used for Co-aSNPs assay. The normalized relative ratios (nRRs) were 1.00 0.04 and 1.54 0.06 in control and trisomy 21 samples. In 50% mosaic samples, the nRR was 1.24 0.022 (nRRab) or 1.28 0.044 (nRRac). Our result shows that the Co-aSNP method can reliably detect 25-30% mosaic aneuploidy samples in 95% confidence.

The Clinical Utility of MLPA in Waardenburg Syndrome. *J.M. Milunsky*[1, 2, 3], *T.A. Maher*[1], *A. Milunsky*[1, 2] 1) Ctr Human Genetics, Boston Univ Sch Medicine, Boston, MA; 2) Department of Pediatrics, Boston Univ Sch Medicine, Boston, MA; 3) Department of Genetics and Genomics, Boston Univ Sch Medicine, Boston, MA.

Waardenburg syndrome (WS) is an autosomal-dominant neurocristopathy characterized by sensorineural hearing loss, pigmentary abnormalities of the iris, hair, and skin, and is responsible for about 3 percent of congenital hearing loss. Point mutations in *PAX3* have been identified in more than 90 percent of affected individuals with WS1/WS3. *MITF* point mutations have been identified in 10-15 percent of individuals affected with WS2 (lacking dystopia canthorum). Multiplex Ligation-dependant Probe Amplification (MLPA) is now a standard technology in the molecular laboratory to detect copy number changes in targeted genes. We employed MLPA for *PAX3* and *MITF* in a cohort of 109 patients with a clinical diagnosis of WS1, 2, and 3 who were sequence negative for *PAX3* and/or *MITF*. All coding exons of *PAX3* and exons 1, 2, 3, and 10 of *MITF* were included in the MLPA assay. MLPA on 48 patients with WS1/3 revealed 3 *PAX3* whole gene deletions (2 WS1; 1 WS3), 2 *PAX3* partial gene deletions (WS1: exon 1; WS1: exons 5-9) and 1 partial *MITF* deletion (WS1: exons 3-10)(6/48 12.5%). MLPA on 41 patients with WS2 and 20 patients submitted with a diagnosis of either WS1 or WS2 revealed no copy number changes. *MITF* MLPA may be underestimating the frequency of copy number changes as only 4 of the 10 exons are included in the assay. The detection of both partial and whole gene deletions of *PAX3*/*MITF* in this clinical cohort increases the mutation detection yield by at least 6% and supports integrating MLPA into clinical molecular testing for patients with WS1, 2, and 3.

Statistical methods for modelling extended haplotypes. *P. McKeigue* Conway Inst, Univ Col Dublin, Dublin, Ireland.

Exploiting the new chip-based platforms for scoring several hundred thousand tag SNPs depends on being able to model the underlying haplotype structure for typed and untyped SNPs. As in the program FASTPHASE, the haplotype structure of the human genome can be modelled as generated by K independent Poisson arrival processes corresponding to K modal haplotype states, with block-like structure represented by allowing arrival rates to vary between intervals. We have adapted an existing program for modelling genetic admixture (ADMIXMAP) to this purpose. The posterior distribution of model parameters, hidden states and missing genotypes are generated by MCMC simulation, and score tests are obtained from this posterior distribution. We demonstrate the approach with HapMap data on 51000 SNPs on chromosome 22 typed in 60 unrelated individuals, and a tag SNP panel based on the SNPs included in the 300K chip. Tests for lack of fit show that a model with K = 6 modal states is sufficient to explain residual LD between adjacent pairs of loci in the HapMap. The efficiency of the tag SNP panel (with other genotypes missing), measured by the ratio of observed to complete information in the score tests, was 91%. Thus given any sample of cases and controls typed for a genome-wide panel of tag SNPs, we can test all loci in the HapMap for association with the disease or trait, and evaluate the adequacy with which the tag SNP panel extracts information about association with a locus or region of interest. We are developing a parallel version that will allow this to be scaled up to large-scale asssociation studies.

Non-parametric linkage analysis incorporating unaffected siblings. *A.G. Matthews*[1,2], *E. Feingold*[1] 1) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA.

Traditional non-parametric linkage analysis relies on affected individuals, but there are circumstances under which unaffected individuals may add a non-trivial amount of information. We discuss a class of allele-sharing statistics for sibships that incorporate phenotype information from unaffected individuals, and we evaluate the power of several different statistics in this class. We also discuss the portion of the genetic model space for which these statistics might be useful.

Whole-genome association in Alzheimer's disease using high-density SNP genotyping microarrays: A comparison of the Affymetrix DM and BRLMM genotype-calling algorithms. *D.H. Lince[1], K.L. Ohlsen[1], X. Wang[1], K.J. Elliot[1], M. Ryder[1], L. Bertram[1], R.E. Tanzi[2,3], K.D. Becker[1]* 1) TorreyPines Therapeutics, La Jolla, CA; 2) Genetics and Aging Research Unit-Massachusetts General Hospital, Boston, MA; 3) Harvard Medical School, Boston, MA.

We are completing a genome-wide association study in Alzheimers disease (AD) using a family-based approach and 500,000 SNP markers (Affymetrix). Average SNP call rates of accepted high quality data obtained for the Nsp 250 and Sty 250 arrays were 95.8% and 95.4%, respectively, using the Dynamic Modeling genotype-calling algorithm (DM: Di et al, 2005. Bioinfo. 21:1958-63). While genotyping is still in progress, approximately 97% of all samples genotyped to date are above a 93% call rate threshold. Analysis of performance in a test data set demonstrated high reproducibility (~99%) and low error (0.3% inheritance errors). Filtering out poorly performing SNPs based upon missing genotypes, Hardy-Weinberg equilibrium and inheritance errors will further improve the quality of data for genetic analyses. Application of the Bayesian Robust Linear Modeling using Mahalanobis distance algorithm (BRLMM: Rabbee and Speed, 2006. Bioinfo. 22:7-12) increased the average SNP call rates by ~3% (98.8% for Nsp and 98.6% for Sty). We will present an analysis of SNP genotype quality before and after SNP filtering, as well as a comparison of the Affymetrix DM and BRLMM genotype-calling algorithms with respect to genotype reproducibility, call rates, and accuracy.

Case report of Zimmermann-Laband syndrome: patient affected with inter-auricular communications, and severe hand and feet compromise. *Z. Martinez*[1], *F. Suárez*[2] 1) Departamento de Pediatria, Pontificia Universidad Javeriana, Bogotá D.C., Colombia; 2) Instituto de Genética Humana, Pontificia Universidad Javeriana, Bogotá D.C., Colombia.

Zimmermann-Laband syndrome is a rare genetic disorder characterized by gingival fibromatosis, abnormalities of soft cartilages of nose and ears, hypoplastic or absent nails and terminal phalanges, joint hypermobility, hepatosplenomegaly, mild hirsutism and learning difficulties. Less than 40 cases have been reported in the literature. We present a 9 years old affected, with severe compromise of distal hand and foot phalanges with absent of all nails, hirsutism mental retardation and congenital heart defect.

Determining Evidence for Association Using an LR: Implications for Multiple Test Adjustments. *S.E. Hodge*[1,2], *L.J. Strug*[2] 1) NYSPI, Unit 24, Columbia Univ, New York, NY; 2) Department of Biostatistics, Columbia Univ, New York, NY.

Recently, we have shown that the likelihood ratio (LR) for two simple hypotheses provides a valid measure of evidence in linkage studies (Strug & Hodge, Hum Hered, In Press). We presented alternative error probabilities to Type I and Type II error rates used for planning linkage studies, and showed how one can *decouple* the error probabilities from the measure of evidence, the LR. This approach yields a more practical way of dealing with the multiple testing problem: two-stage designs of linkage genome screens serve as the adjustment for conducting multiple tests of linkage across the genome. Here we extend these results to association studies. An experimenter can choose to construct the LR from profile likelihoods, conditional likelihoods, etc. As an example, we present the LR for the odds ratio=$\exp(_1{}^*)$ for two independent binomial samples, comparing H_1: $_1 = 0$ and H_2: $_1=_1{}^*$, constructed from conditional likelihoods. Define $A(u)=(n_1Cu)[n_2C(t-u)]$, where nCr is the combinatoric coefficient. Then $LR_c = \exp(_1{}^*t_1)A(u) / [A(u)\exp(_1{}^*u)]$ where n_1 and n_2 are the numbers of individuals with and without the trait of interest, respectively; t_0 is the total number of individuals with the genotype of interest; and t_1 is the number of diseased individuals with the gentoype of interest. The summations are defined over u from $\max(t_0-n_2, 0)$ to $\min(t_0, n_1)$. We show that when one uses this conditional LR as the measure of evidence about association for two simple hypotheses, a replication study or two-stage genome-wide association study itself provides the adjustment for conducting multiple tests of association along the genome.

Simultaneous discovery and testing of small segregating deletions for association with disease in SNP genotyping studies. *J.R. Kohler, D.J. Cutler* McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.

There are a number of forms of copy number variation (CNV) including aneuploidies, somatic chromosomal changes, duplications and microdeletions that have long been known to play a crucial, causative role in disease such as Down syndrome, cancer, thalassemia and DiGeorge syndrome. Common techniques to detect large-scale CNV are expensive and time-consuming relative to modern SNP genotyping, which we show can be used to detect small CNV. Deletions have at least three signatures detectable in SNP genotype data: departures from Hardy-Weinberg ratios, Mendelian inconsistencies and unusual patterns of missing data. In this study, we show how to use all three forms of information in a single modeling framework, not only to detect the presence of the deletion, but also to accurately estimate the deletion frequency, to infer who carries the deletion in both heterozygous and homozygous states and finally to test the imputed deletion for potential association with disease using standard association tests (TDT).

We have implemented this approach in a freely available computer program (microdel), and demonstrate its effectiveness on both simulated and real data. In realistic coalescent simulations with a 500 K SNP density, containing both genotyping error and missing data, we show that in studies of 100 trios, a 20 kb deletion at frequency 0.05 can be detected with greater than 75 % power. Power increases with increasing sample size, deletion frequency and deletion length. A 100 kb deletion at frequency 0.20 can be found with ~100 % power. We further show that estimated deletion frequencies are highly accurate, and that even with imperfect sensitivity, TDT tests on the imputed deletion maintain nominal false positive rates. Furthermore, false positive deletions are only detected ~1 per 5,000 SNPs and are generally just a single SNP in length. We also report our survey of deletion polymorphism in the HapMap 16c data, where 1882 deletions of length two or more SNPs were inferred, to date 510 of these deletions have been validated by other methods.

Gametic Phase Disequilibrium as a Method to Map Deafness Genes. *W. Nance[1], A. Pandya[1], S.H. Blanton[2], K.M. Dodson[1], X.J. Xia[1], K.O. Welch[3], K.A. Arnos[3]* 1) Virginia Commonwealth University, Richmond, VA; 2) Duke University, NC; 3) Gallaudet University, Washington DC.

By aggregating mutations for non-allelic genocopies within individuals, assortative mating leads to a non-random distribution of genes known as Gametic Phase Disequilibrium (GPD).Each generation, deaf offspring of hearing parents join the deaf mating pool. Similarly, hearing children of deaf adults (CODA) who are frequently carriers of multiple non-allelic genes for recessive deafness carry these genes into the hearing mating pool. We have collected family histories and CX typing on 105 deaf probands with deaf parents and 995 with hearing parents. We assigned each proband an ancestral deafness index score (I) based on the probability each deaf ancestor transmitted a recessive deafness gene to the proband, summed over all deaf ancestors in the pedigree. The frequency of CX26 heterozygotes increased progressively from 7% in probands with hearing parents (I= 1), to 18.6% in those with deaf parents but no other deaf ancestors (I= 2), to 29 & 40% in probands with I= 2-3 and I> 3 respectively. The hearing loss in these subjects must be caused by at least one additional non-allelic gene for deafness. These data therefore provide clear evidence for GPD and its association with the duration and intensity of assortative mating. Probands in the most extreme group had a CX26 carrier frequency that exceeded the 3.5% rate in the general population by an order of magnitude. The striking aggregation of genes for deafness in this mating group offers a unique opportunity to map them either by case control SNP frequency comparisons of high-density genome scans using conditional logistic regression analysis, or by GPD mapping, an iterative, orthogonalized analysis of non-syntenic disequilibrium beginning with known genes for deafness. For the latter we are currently typing SNPs in and around GJB2, to identify the region showing the greatest SNP frequency differences by case-control comparison. This process will be reiterated until all identifiable known deafness genes in the sample have been detected. Remaining sites of disequilibrium represent candidate regions for new deafness loci.

Characterizing genomic rearrangements in oligodendroglioma using whole genome tilepath hrCGH arrays. *N.V. Johnson[1], J.J. Connelly[1], J. Virgadamo[1], R.E. McLendon[2], J.M. Vance[1], D.D. Bigner[2], S.G. Gregory[1]* 1) Duke Center for Human Genetics, Durham, NC; 2) Duke Comprehensive Cancer Center, Durham, NC.

Primary brain tumors account for 1% of the new cancer cases in the US which, given the mortality of 4.1% per 100,000 persons, accounted for 13,100 deaths in 2001 alone. These tumors have a broad histopathology, variable sensitivity to treatment and, therefore, have unpredictable progression and survival times. In general, the molecular mechanisms underlying all these variables are poorly understood. For many years it has been contended that genetic instability leads to cancer development via non-random chromosome losses and gains that contribute to tumor malignancy. To identify these underlying molecular mechanisms we have generated high-resolution comparative genomic hybridization (hrCGH) data using our whole genome tilepath microarrays to identify chromosomal rearrangements associated from 110 oligodendroglioma (OD) tumors. We have generated hrCGH data at 100kb resolution from 45 tumors histopathologically determined to be well differentiated oligodendroglioma (WD); 40 anaplastic oligodendroglioma (AO) tumors; and 25 tumors with only an OD designation. Analysis of the entire tumor set identified characteristic loss of 1p and 19q within 72% of the tumors analyzed. In addition to gross chromosome rearrangements, amplifications or deletions (>4Mb) throughout other regions of the genome, we observed a number of single clone deletions and amplifications within our tumor set. The novel data associated with our analysis therefore represents either novel copy number polymorphisms or, more likely, genomic loci which contain genes associated with the development and progression of oligodendroglioma. Additionally, we observed an accumulation of genomic rearrangements between stage II and III stages. We will present a detailed analysis of genomic intervals that define minimally deleted and amplified regions between all OD tumors. These regions now form the focus of on-going candidate gene analysis.

Gene copy number variation and the limits of detection using Affymetrix GeneChip Human Mapping 100K and 500K arrays. *A.D. Delaney[1], R.A. Holt[1], H. Li[1], T. Nayar[1], A. Baross[1], A. Ally[1], J. Asano[1], D. Bailey[4], P. Birch[2], M. Brown-John[1], M. Cao[4], S. Chan[1], P. Eydoux[3], N. Fernandes[2], S. Flibotte[1], A. Go[1], G. Kennedy[4], S. Langlois[2], J.M. Friedman[2], M.A. Marra[1]* 1) Genome Sciences Centre, British Columbia Cancer Agency, Vancouver, BC, Canada; 2) Dept. of Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 3) Dept. of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada; 4) Affymetrix Inc., Santa Clara, CA.

Genomic amplifications and deletions are known to be important in disease and have recently been shown to occur frequently as normal polymorphisms. It has recently been shown that the frequency of such polymorphism increases as the size of the aberration decreases. In our study, we used Affymetrix GeneChip Human Mapping 100K and 500K arrays in combination with new informatics techniques to detect such copy number variation(CNV) in a large set of unaffected parents (214) and their children (107) with mental retardation. This presentation focuses on the large set of newly discovered presumably benign CNVs in the unaffected parents. Detection of CNVs depends on recognition of a set of adjacent SNPs that show a concordant change in copy number. As the minimal number of SNPs chosen to define an amplification or deletion decreases, the rate of false positive discovery rises. We have quantified this dependency and present the lower limit of detection of the Mapping 100K and 500K arrays as a means for CNV discovery. We also present several approaches to data normalization, as well as treatment of non-random variation and noise. This includes comparison of four software packages currently available for normalization and CNV detection from Mapping 100K and 500K array data, as well as novel approaches being developed by our group.

Retrospective association analysis of case-control data using clustered haplotypes. *M.L. Jones[1], M.P. Epstein[2], J.Y. Tzeng[1]* 1) Department of Statistics, North Carolina State Univ, Raleigh, NC; 2) Department of Human Genetics, Emory University, Atlanta, GA.

Many regression-based methods exist for conducting haplotype analysis in case-control studies of disease. Such methods generally are based on either a prospective framework (modeling the probability of disease conditional on haplotypes and covariates) or a retrospective framework (modeling the probability of haplotypes and covariates conditional on disease). For haplotype analysis, both theoretical and simulation work have demonstrated that a retrospective framework can be more efficient than a prospective framework in this context. Given this result, we propose to further improve the performance of the retrospective haplotype framework by allowing for haplotype clustering. Our previous work to enhance the efficacy of haplotype analysis suggested that clustering haplotypes under the prospective framework can increase the power of haplotype-based association analysis. With the aim of seeing an even greater improvement from clustering for case-control data, this work extends the clustering idea to the retrospective framework. Specifically, we construct a retrospective likelihood using a design matrix that does not require pre-specification of the haplotype of interest and derive generalized scores statistics to test for haplotype effects at the global and individual levels. Through simulation, we assess the validity of the retrospective clustering method and compare the power of the method with the power of the full-dimensional analysis. We also examine the power of the retrospective method relative to the prospective clustering method. In addition, we will apply our proposed approach to real data from a genetic study of Type 2 Diabetes.

Evaluating life scientists' and geneticists perceptions and understanding of research ethics. *J. McCormick, A. Boyce, R. Garg, M. Cho* Center for Biomedical Ethics, Stanford University, Palo Alto, CA.

As research on fundamental biological processes and genetics moves forward, significant questions are raised about ethical, legal, social, and policy (ELSP) issues, , as well as how to ethically conduct research. It is important for scientists to have a good understanding of the issues and to be proactive in addressing these concerns. The questions are then, how much do scientists know about ELSP implications related to their research, and how capable and willing are they to participate in addressing these concerns? The goal of this study was to evaluate the perceptions life science and genetics researchers have of the ELSP concerns related to their work and to determine the role they see themselves playing in the policymaking process. In additional, we assessed why and when scientists might use a service providing assistance in resolving ethical and social quandaries related to their research. This service, called the Benchside Ethics Consultation Service (BECS), has recently been established at Stanford University. We conducted interviews (N=20), focus groups (N=28), and surveys of graduate students, postdoctoral fellows, research staff, and faculty at Stanford University (N=500) and 6 other research universities (N=1500) across the United States. Genetics departments at all institutions were included in the sample. Our preliminary findings suggest that researchers do think about ELSP issues but often not in the context of their specific research. The term research ethics conjured up thoughts on scientific misconduct, data falsification, and author recognition. Informed consent issues were also recognized by study participants as important issues. Some researchers using human subjects seem to recognize concerns about reporting research results and incidental findings. Confidentiality and privacy of their own consultations are some of the main concerns for potential users of BECS or a service like it. Our analyses suggest topics about which we should be more proactive in informing the life science community and support the need for a service like BECS.

Association study of 7 ALOX5AP gene polimorphisms in patients with myocardial infarction or coronary artery disease. *G. Malerba[1], U. Cavallari[1], L. Xumerle[1], E. Trabetti[1], M. Biscuola[1], N. Martinelli[2], O. Olivieri[2], D. Girelli[2], R. Corrocher[2], P.F. Pignatti[1]* 1) Department of Mother and Child and of Biology-Genetics, Section of Biology and Genetics, University of Verona, Verona, Italy; 2) Department of Clinical and Experimental Medicine, University of Verona, Verona, Italy.

Recently, variants of the gene ALOX5AP (also known as FLAP) encoding arachidonate 5-lipoxygenase activating protein have been associated with risk of atherosclerosis, myocardial infarction (MI) or stroke. The present study aimed to validate those findings in an Italian sample. To assess the contribution of the ALOX5AP variants to cardiovascular diseases, 7 single-nucleotide polymorphisms (SNPs, rs17222814, rs17216473, rs17222772, rs17216522, rs17222828, rs17222877, rs17222842), tagging the previously reported high risk haplotypes in Icelandic (HapA) and British (HapB) populations (Helgadottir et al., Nat. Genet. 2004), were genotyped from 1171 patients with coronary artery disease (CAD) and 407 CAD-free assessed by coronary angiography. Single marker, 2 or 3 marker SNP haplotype analyses did not show any significant difference in the frequencies between the control and patient groups for any of the investigated phenotypes (CAD and MI). The hypothesis that ALOX5AP contributes to susceptibility for MI was not confirmed in the Italian population.

Chromosome Analysis of Single Cells by High Resolution Comparative Genomic Hybridization after Whole Genome Amplification using A Random Fragmentation Approach. *B. Levy[1], O. Nahum[2], K. Hirschhorn[2]* 1) Dept Pathology, Columbia Univ, New York, NY; 2) Dept Human Genetics & Pediatrics, Mount Sinai School of Medicine, New York, NY.

We used a random fragmentation whole genome amplification (WGA) approach combined with high resolution comparative genomic hybridization (CGH) to detect the chromosomal status of single cells. Single cells were extracted from 32 random specimen cultures that were initially obtained for clinical cytogenetic analysis. All cultures were de-identified and blinded (with respect to the cytogenetic diagnosis) before being transferred from the clinical laboratory to the research laboratory. Single cells were lysed and then subject to WGA using a random fragmentation approach (GenomePlex - Sigma-Aldrich). The resulting DNA was labeled using Nick-translation and high resolution CGH was performed to determine DNA copy number and thus the chromosomal status of the single cells. CGH results were compared to the karyotype as determined by conventional cytogenetic analysis. The study was approved by the IRBs of Columbia University and the Mount Sinai School of Medicine. The specimens analyzed included normal males, normal females, various trisomies (4, 10, 11, 13, 14, 16 and 21), an unbalanced translocation and an iso-22 chromosome. There were no false negatives and the correct diagnosis was made in 32/32 cases at the 99.9% CI and 31/32 cases at the 99.99% CI. Certain artifactual abnormalities were observed in addition to the true abnormality in some cases and the impact of these still needs to be determined in a larger test series. This approach offers significant benefits over current FISH-based preimplantation genetic diagnosis as the entire genome is scanned for chromosomal imbalances. Further assessment of sensitivity and specificity is required before such methods can be targetted for clinical application.

A locus for an autosomal dominant paroxysmal abdominal pain maps to 8q13.2-22.3. *B. Kremeyer[1], N.G. Pineda-Trujillo[1,2], M.W. Burley[1], F. Lopera[2], G. Bedoya[2], A. Ruiz-Linares[1,2]* 1) Department of Biology, University College London, London, United Kingdom; 2) Medical School, Universidad de Antioquia, Medelllin, Colombia.

We identified an extended family in Antioquia (Colombia) with multiple individuals presenting abrupt crises of severe abdominal pain. These episodes occur from infancy and are characterised by very intense spasmodic pain, predominantly in the abdomen, but occasionally generalised. Episodes are associated with stiffness of the abdominal wall, profuse sweating but not loss of consciousness. One episode can last for 30 to 60 min, the pain gradually becoming more severe as the crisis progresses. We carried out a whole genome linkage scan using ~550 microsatellite markers (deCODE Genetics Genotyping Service) with an average spacing of 6 cM. Parametric linkage analysis was carried out using a model of autosomal dominant inheritance with high penetrance (0.985). Two-point LOD scores 2, suggesting genetic linkage, were found on chromosomes 2p, 3p, 8q, 9q, and 13q. A single two-point LOD score 3 was obtained on chromosome 8q12.3, for marker D8S512 (Z=4.18 at =0). Multipoint LOD score analysis of chromosome 8 yielded a maximum LOD score of Z=4.42 in the interval D8S512-D8S279, notably in the same region that had also shown the most significant LOD score in the two-point analysis. Haplotype analysis confirmed the segregation of a candidate region in the family. We therefore conclude that we identified a region on chromosome 8q13.2-22.3 that harbours a predisposing gene for familial paroxysmal abdominal pain. Fine-mapping and sequencing of candidate genes is under way.

Familial Borjeson-Forssman-Lehmann syndrome not associated with PHF6 mutation. *M. Ottaviani[1], A. Zeffiri[1], L. Di Medio[1], L. Carosi[1], S. Toccafondi[1], S. Guarducci[2], L. Giunti[2], E. Lapi[2], M.L. Giovannucci Uzielli[1]* 1) Department of Paediatrics, Genetics, University of Florence, Florence, Italy; 2) Childrens Hospital A. Meyer, Florence, Italy.

Borjeson-Forssman-Lehmann syndrome (BFLS; OMIM 301900) belongs to the syndromic forms of XLMR disorders. BFLS is now best described as a mental deficiency-endocrine disorder. This rare disorder was described for the first time in 1962. It is characterised by moderate to severe intellectual disability, epilepsy, microcephaly, coarse facial features, long ears, short stature, obesity, gynecomastia, hypogonadism, tapering fingers, and shortened toes. Although BFLS is an X chromosomal recessively inherited disorder, it may also be seen in females, perhaps as a consequence of X inactivation skewing as has been confirmed by methylation specific PCR. Four years ago, mutations have been identified in the PHF6 gene, a novel widely expressed zinc-finger [Plant Homeodomain (PHD)-like finger] gene, mapped to the human Xq26 region, by Lower et al., Nat Genet 2002;32:661.5 We report a two-generation family with clinical phenotype of the Borjeson-Forssman-Lehmann syndrome in a 11 years old girl, and in her maternal uncle. Both the phenotype, severely expressed in the uncle and also in the proposita, the natural history, and the X chromosomal mode of inheritance, prompted the diagnosis of BFLS. The differential diagnosis included several rare and less rare disorders. Molecular analysis performed in the proposita, by direct sequencing of the coding exons 2-10 and of the flanking intronic sequences (50 base pairs) of the PHF6 gene, in genomic DNA, revealed no mutations. This study does not rule out a mutation in the uncharacterised gene regulatory elements or in the intronic sequence outside the splice donor and acceptor sites. X inactivation and UPD studies are now in progress as well. However, it seems to us too early to speak about genetic heterogeneity of BFLS.

Exploring the role of maternal age and the location of recombination in chromosome 21 nondisjunction. *T.R. Oliver[1], E. Feingold[2], S.L. Sherman[1], K. Yu[3]* 1) Human Genetics, Emory University, Atlanta, GA; 2) Human Genetics, University of Pittsburg, PA; 3) Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD.

Human chromosome nondisjunction (NDJ) occurs at a high frequency in humans and is known to be influenced by maternal age and recombination. We examined the association of these two known risk factors among maternal chromosome 21 NDJ stratified by meiosis I (MI, n=637) and meiosis II (MII, n=278) errors. We found intriguing patterns of association that were specific to the type of NDJ error. For MI errors, the frequency of a single exchange near the telomere of chromosome 21 is highest among young mothers and decreases significantly with age. In fact, the pattern of exchanges amongst our eldest group of women mimics the pattern observed among normally disjoining chromosomes 21. Recombination among MII errors reveals a different relationship with maternal age: single pericentromeric exchanges occur most often among the oldest maternal age group. These results suggest that multiple mechanisms that lead to NDJ. For example, single telomeric exchanges appear to predispose to NDJ irrespective of maternal age. However, for MII errors, recombination-related factors appear to interact with maternal age related factors.

2DE-DIGE proteomic analysis in mesial temporal lobe epilepsy. *M.J. Murai[1], R. Horiuchi[2], D. Martins[2], C.V. Maurer-Morelli[1], J.C. Novello[2], F. Cendes[3], I. Lopes-Cendes[1]* 1) Medical Genetics Department, Unicamp, Campinas, São Paulo, Brazil; 2) Biochemical Department, Unicamp, Campinas, São Paulo, Brazil; 3) Neurological Department, Unicamp, Campinas, São Paulo, Brazil.

Epilepsy affects 1% to 2% of the general population; therefore, it is considered a public health problem by the World Health Organization. Mesial temporal lobe epilepsy (MTLE) is the most common and severe type of partial epilepsy, representing ~50% of all adult epilepsy patients and frequently associated with pharmaco-resistance. The relationship between MTLE and hippocampal sclerosis (HS) is well established. However, the precise pathogenesis of HS and its relationship with MTLE is not completely clarified. Two-dimensional electrophoresis (2DE) is a powerful fractionation method for complex protein mixtures. In difference gel electrophoresis (DIGE) based proteomics, the experimental and control samples are labeled with different fluorophores and are run in the same gel, thereby minimizing gel preparation variation. DIGE is one of the few techniques that is capable to perform quantitative proteomics, generating statistical data to differences in protein abundances. We analyzed the proteome of three hippocampus removed from patients with refratory MTLE who underwent epilepsy surgery. 2DE-DIGE identified 2 up-regulated and 10 down-regulated proteins as determined by Students T-test (p0.01). The observed molecular weight of the spots ranged from 28 to 93 kDa. The identity of these spots will be elucidated by mass spectrometry in order to gain additional information in a global scale about the mechanism of epileptogenesis in MTLE.

Comparison between BAC and oligo array platforms in detecting submicroscopic genomic rearrangements. *P. Hixson[1], E. Laritsky[1], X. Wang[2], T. Jiang[1], S. Cheung[1], I. Van Den Veyver[1,2], W. Cai[1]* 1) Dept of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Dept of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX.

Array-based comparative genomic hybridization (array CGH) has emerged as a powerful diagnostic technique for high resolution analysis of the human genome. It is a specific, sensitive and rapid technique enabling detection of genomic arrangements and copy number changes. A variety of array CGH platforms are currently available, both commercially and in academic institutions. The choice of platform may depend on the type of data sought, however, the price, reproducibility and standardization are crucial factors that need to be considered. Our goal was to determine the ability of our in-house prepared BAC array to identify submicroscopic genomic rearrangements. We constructed a 39,000 human whole genome (HWG39K) BAC clone microarray from the RPCI-11 human library with a resolution of approximately 12 BACs/Mb using standard alkaline lysis method. This platform was used, in a masked fashion, to analyze genetic aberrations in five clinical samples showing abnormalities using a targeted array that has been clinically implemented. These samples were also examined using oligo-based platforms in an attempt to compare the performance of our BAC arrays with the oligo arrays. We were able to identify deletions on chromosomes 1 (1 patient) and 22 (2 patients), a deletion/duplication event on chromosome 8 (1 patient) and a duplication on chromosome 12 (1 patient). Our results were consistent with previous cytogenetic and FISH findings and were comparable to those obtained by different oligo array platforms available commercially. The negative rate of detection for our BAC array platform ranges from 0 to 12.4% using 2.5 SD units as the cutoff level. Therefore, this platform provides us with an alternative to characterizing submicroscopic genomic rearrangements on a whole human genome array in a single experiment at an affordable cost.

Characterizing Intronic Sequences by Chromosomal Location: Valleys in the Genomic Terrain. *C.M. Malcom*[1], *G.J. Wyckoff*[2] 1) Department of Anthropology, University of Chicago, Chicago, IL; 2) Department of Molecular Biology and Biochemistry, University of Missouri---Kansas City, Kansas City, Missouri.

Through previous work, we have established that, in mammalian lineages, genes co-located in syntenic blocks (i.e., stretches of coding sequence that have been preserved over evolutionary time and have persisted throughout chromosomal rearrangements) resemble one another in many respects, including length, evolutionary rates, and number and length of domains (Malcom et al., 2003, 2004). This work has allowed us to identify chromosomal families---that is, human chromosomes that cluster together in terms of genic characteristics. These families are relevant to the study and understanding of human disease, as their shared characteristics imply shared ancestry and evolutionary history. Thus, these relationships may give us insight into factors that have worked within biologically bounded units to shape the genome over time. Here, we expand our inquiry into positionally shared characteristics by turning to noncoding sequences. We consider how closely noncoding sequences within a chromosome resemble one another (in terms of length, substitution rates, GC content, and other characteristics) as well as the relationship between coding and noncoding sequences within chromosomes. In keeping with previous research (e.g., Hughes and Yaeger, 1997; Wyckoff et al. 2005; Ko et al., 2006), our work here shows that the relationship between characteristics of coding and noncoding regions is complex and not simply summarized. However, consideration of coding and noncoding regions as two distinct groups that are internally homogenous obscures patterning in the data. Consequently, partitioning both the coding and noncoding parts of the genomes into biologically meaningful subunits (such as syntenic blocks and chromosomes) has considerable potential to enlighten researchers on the mechanisms that control genomic fidelity and change over evolutionary time and the scale on which these mechanisms work. Viewing the genomic terrain from these multiple perspectives may greatly enhance researchers ability to select targets for disease research.

Variants in the Trehalase Gene (TREH) are Associated with Type 2 Diabetes Mellitus and Determine Trehalase Activity in Pima Indians. *Y. Muller, G. Nishimoto, J. Loebel, R. Hanson, S. Kobes, J. Goswami, W. Knowler, C. Bogardus, L. Baier* Epidemiology & Clinical Res, NIDDK/NIH, Phoenix, AZ.

Trehalase is an enzyme which hydrolyzes the disaccharide trehalose into the glucose. Trehalase is encoded by the trehalase gene (TREH) which is located on Chr.11q23, within a region linked to both body mass index (BMI) and type 2 diabetes mellitus (T2DM) in diverse populations including Pima Indians. Association studies of single nucleotide polymorphisms (SNPs) for fine mapping of linkage in the Pima Indians identified several SNPs near/within the TREH gene that were associated with BMI and/or T2DM. The coding region and ~2 kb of the promoter region of the TREH gene was sequenced in 39 Pima Indians, and 12 SNPs were identified. These 12 SNPs, and 14 additional database SNPs were genotyped in 1037 subjects that gave rise to the linkage peak. 21 SNPs were associated with T2DM (p=0.01 to 0.0001), and 5 SNPs were associated with BMI (p=0.01-0.001). These analyses were adjusted for age, sex, birth year, heritage and family membership. In addition, serum trehalase activity was measured in 570 non-diabetic subjects, and trehalase activity was highly associated with a nonsynomous SNP Arg486Trp and a promoter SNP located at -100 bp ($p=10^{-12}$, $p=10^{-15}$, respectively, adjusted for age, sex, heritage and family membership), and several other SNPs that were in perfect LD with these SNPs. Haplotype analysis showed that subjects who were homozygous for 3 variants (Trp486, Ala389 and C at -100 bp) had the lowest trehalase activity, and carried the lowest risk of T2DM. Furthermore, a higher trehalase activity was associated with higher BMI in 564 subjects (r=0.11, p=0.01), where the association was more prominent in 269 male subjects (r=0.24, p<0.0001) than in 295 female subjects. However, mean trehalase activity measured in non-diabetic subjects was not significantly associated with subsequent development of diabetes in 570 subjects (diabetic=250, non-diabetic=320, p=0.57). These results indicate that the genetic variants in the TREH gene highly influence the serum trehalase activity, and a specific haplotype of TREH is associated with both low levels of TREH activity and risk of T2DM.

Novel mutation in *DSPP* underlying DI type II. *S. Hart[1], J. Atkinson[2], S.J. Choi[2], M.D. Ramaswami[2], T.C. Hart[2]* 1) Office of the Clinical Director, NHGRI, Bethesda, MD; 2) Section on Human and Craniofacial Genetics, NIDCR, Bethesda MD.

Dentinogenesis imperfecta (DI) is an autosomal dominant condition characterized by tooth discoloration and by enamel that fractures easily from the underlying dentine. Radiographically, bulbous crowns, narrow roots, and small or obliterated pulp chambers and root canals are seen. Three subtypes have been recognized, with types II and III the non-OI forms. In this report, we report mutational analysis of a 16 year old male from the Brandywine triracial isolate segregating DI and his unaffected mother. A sample on the affected father was not available. Sequencing of the *DSPP* gene revealed that the mother was heterozygous for a T>C transition that is predicted to substitute threonine for isoleucine at position 131, but she did not transmit this alteration to her son The son was heterozygous for two coding alterations, neither of which was present in the mother. The first, a T>C transition predicted to change a highly conserved proline residue to a serine (p.P17S), was not present in Caucasian or African American controls. Mutation of this same residue (p.P17T) was previously reported in a Chinese kindred segregating DI and bilateral high frequency sensorineural hearing loss. The proband in this study nor his father reported any hearing problems. The second coding alteration, p.R68W, was reported as disease-causing in a Swedish kindred. We detected the p.R68W mutation in control individuals and thus this change is most likely a polymorphism. Site directed mutagenesis was used to introduce the mutations into human DSP cDNA, subcloned into the pcDNA3 expression vector that was then transfected into mouse odontoblast cells. Supernatants and cellular extracts were analyzed for DSP protein. The p.P17T mutation was associated with significant decrease in DSP excretion from cells, while the p.R68W alteration displayed normal excretion. The p.P17S alteration is currently being evaluated in this system. Analysis of DI families supports that the term DI type II is appropriate to describe the non-OI forms of DI. In conclusion, we report a novel mutation, p.P17S, that causes DI type II.

Discordance for ovarian dysgenesis in a pair of monozygotic twins. *L. Matyakhina[1], J. Meck[1], A.L.A. Martin[2], M.M. Martin[2]* 1) Obstetrics and Gynecology, Georgetown University Hospital, Washington; 2) Pediatrics, Georgetown University Medical Center, Washington.

An unusual case of monozygotic (MZ) female twins is presented, one normal and one with congenital anomalies including ovarian agenesis. The twins were born at 29 weeks gestation with birth weights and lengths at the 5th percentile. They were monochorionic; monozygosity was confirmed by molecular VNTR analysis. At birth, Twin A had a single umbilical artery (SUA) and a displaced and dysplastic left thumb. She also had a patent ductus which required surgical correction, as well as an ASD and VSD. Elevated serum gonadotropins suggested ovarian dysfunction. Laparoscopy revealed no left ovary and a streak on the right. A biopsy showed fibrovascular connective tissue, but no epithelial cells or follicles. Sonography showed an infantile uterus. At age 12, when twin B entered puberty, estrogen replacement therapy was initiated in twin A with estradiol 20 ng/kg po. This dose was increased over several years guided by clinical, cytological and hormonal findings, using twin B as the model. At age 17, 125 ng/kg po estradiol provided adequate estrogen replacement. Cyclic hormonal therapy was started and regular periods followed. Growth and development proceeded appropriately. Twin A reached an adult height of 156.4 cm, close to twin Bs height of 159.7 cm. Gonadal dysgenesis is associated with X-chromosome anomalies. Karyotype analyses on lymphocytes from both twins and on fibroblasts from twin A were normal 46, XX. FISH on lymphocytes from both twins with X and Y centromere probes showed no sex chromosome mosaicism or Y signals. Discordance associated with SUA is common in MZ twins. 25% of babies with SUA have chromosome anomalies. Somatic anomalies; not genital tract abnormalities, have also been reported. Post zygotic, epigenetic and environmental events are often suggested to explain MZ twin discordance. 20% of MZ twins differ in X chromosome inactivation patterns. Highly skewed X inactivation may explain the findings in Twin A. X-linked mutations may be unmasked in her but not Twin B. X chromosome inactivation testing may assist in understanding their discordance.

Unbiased Evaluation of TagSNP Panels Genetic Coverage. *K. Hao, S. Cawley* Algorithm & Data Analysis, Affymetrix, Inc, Santa Clara, CA.

Common genetic polymorphism explains a portion of the heritable risk for at least some and possibly many common diseases. Because of linkage disequilibrium (LD), it is possible to catalog many polymorphisms by genotyping only a relatively small collection of tag SNPs. Various tag SNP selection algorithms and genotyping panels have been developed, facilitating whole-genome association studies. Genetic coverage and average r2 are often used as benchmarks evaluating the performance and power of whole genome SNP panels and tag SNP selection algorithms. Since SNP panels are typically designed using a specific collection of subjects and SNPs, both independent subjects and SNPs are necessary to provide accurate estimates of genetic coverage. Here, we conduct an unbiased evaluation of the genetic coverage of tag SNP panels on a genome-wide scale. First we apply Affymetrix GeneChip technology and genotype >100K SNPs which are not included in the HapMap project (as of HapMap release 20). We find a sizable over-fitting effect, where the genetic coverage of a 300~500K tag SNP panel is typically overestimated by 10~15% if evaluated on the training SNP set itself (e.g., HapMap SNPs) rather than an independent testing set. This finding is consistent to our simulation studies. Secondly, employing independent subjects, we also observe ~10% overestimation of genetic coverage due to over-fitting on samples (also known as the tag SNP transferability problems). In addition, simulations show the SNP and sample over-fitting effects are additive and our results indicate that caution is necessary in evaluating such tag SNP panels. Furthermore, we propose improvements in tag SNP selection algorithms to reduce sample and SNP over-fitting risks. Keywords: TagSNP, Genetic Coverage, Linkage Disequilibrium (LD), Over-fitting and Independent Validation.

Hereditary colorectal cancer: the French Canadian mutation spectrum. *J. Jarry[1], G. Chong[1,2], I. Thiffault[2], S. McVety[2], S. Winocour[1], P.H. Gordon[1], G. Ouellette[3], I. Gorska[4], D. Farber[1,2], V. Marcus[2], R. Fodde[5], P. Hutter[6], W.D. Foulkes[2]* 1) SMBD-Jewish General Hospital, Montreal, Canada; 2) McGill University, Montreal, Canada; 3) CHUS, Sherbrooke, Canada; 4) CHUM, Montreal, Canada; 5) Dept Pathology, Erasmus Medical Center, Rotterdam, the Netherlands; 6) Institut Central des Hôpitaux Valaisans, Sion, Switzerland.

Colorectal cancer (CRC) ranks amongst the most frequent cancers worldwide and is the second leading cause of cancer-related deaths in the Western world. Inherited forms of CRC include hereditary non-polyposis colorectal cancer (HNPCC), caused by mutations in the mismatch repair genes MLH1, MSH2, MSH6, and PMS2; familial adenomatous polyposis (FAP), caused by mutations in APC; and MYH-associated polyposis (MAP). The CRC mutation spectrum in the French Canadian (FC) population, a recently founded population in which many founder effects have been reported, remains poorly documented. We report our experience on the mutation screening of FC patients with hereditary CRC. Using a multimodal approach that includes testing for both DNA and RNA, we have successfully identified mutations in MLH1, MSH2, and MSH6 in FC families fulfilling the Amsterdam or Bethesda criteria for HNPCC. A total of 22 mutations have been identified of which half were caused by exon deletions. Four of these mutations were detected more than once, but pedigree analysis indicates the families are unrelated. Haplotype analysis indicates the presence of a founder effect for these recurrent mutations. An additional number of FC families were tested for FAP and a total of 16 distinct mutations were identified in the APC gene. Families, in which probands exhibited a phenotype compatible with the attenuated form of FAP but for which no APC mutation could be found, were screened for the two common European mutations in the gene MYH. Four individuals were identified as biallelic carriers of mutations in MYH. While the spectrum of CRC mutations is wide in French Canadians, an abundance of exon deletions and the presence of founder effects for HNPCC patients can be singled out as characteristics of this population at the molecular level.

Chromosome 6q21: Positional candidate genes for psychosis in Alzheimers and Schizophrenia. *V. Kodavali[1], R.A. Sweet[1], M.I. Kamboh[2], V.L. Nimgaonkar[1,2]* 1) Dept Psychiatry, Univ Pittsburgh, Pittsburgh, PA; 2) Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA.

Psychotic symptoms, i.e., hallucinations and delusions, are common among patients with late onset Alzheimers Disease (LOAD) and Schizophrenia. Our group is working on the genetic causes of psychosis in these patients. We have found LOAD+P (LOAD with Psychosis) to be familial, with a heritability of psychosis during LOAD of 61-70%. We performed linkage analysis among families having two or more first-degree relatives with LOAD+P, using data from the NIMH Alzheimer Disease Genetics Initiative. We found suggestive linkage at chromosome 6q near marker D6S1021 (112.2 cM, MLS = 2.32; p = 0.001) a region that may also harbor psychosis liability genes for schizophrenia and bipolar illness. We conducted follow-up association studies of the chr 6q region in DNA pools of 180 subjects with LOAD+P, 180 unrelated individuals with LOAD-P (LOAD without psychosis), 180 subjects with schizophrenia and 180 unscreened neonatal controls. We conducted our studies in three stages, a) Identification of tagged SNPs, b) Allele-frequency estimations using pooled DNA method and c) Individual genotyping by SNPlex and Illumina assay methods. We analyzed information on 3500 SNPs from the Hapmap project, covering 10Mbp genomic region near DNA marker D6S1021. We obtained 679 tagged SNPs (r2=0.8) generated by using the H-clust method, covering 41 genes in this region. We estimated allele frequencies in these DNA pools for 679 tagged SNPs using DNA pooling method [Chowdari KV et al, Genes, Brain and Behavior, 2006. (In press)]. Thus we selected 75 SNPs, which showed p value of <0.2 by comparing LOAD+P, LOAD-P, schizophrenia and neonatal control pools, for further individual genotyping. We found significant associations at several SNPs from NR2E1, SNX3, ARMC2, SESN1 and c6orf182. We show here that the pooled DNA method is a convenient and rapid method for efficiently screening case-control differences for genetic association studies. We are currently evaluating these findings in additional replication samples.

A High Density SNP Genome Screen For Age Related Macular Degeneration Reveals Novel Loci. *M.A. Hauser[1], S. Schmidt[1], R.R. Allingham[2], P. Gallins[1], W.K. Scott[1], A. Argawal[2], E. Postel[2], K.L. Spencer[3], J.L. Haines[3], M.A. Pericak-Vance[1]* 1) Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Ophthalmology, Duke Univ Medical Ctr, Durham, NC; 3) Center for Human Genetics Research, Vanderbilt, Nashville, TN.

Age-related macular degeneration (AMD) is the leading cause of blindness in older Americans. Here we report a SNP-based genome-wide screen on a large AMD family dataset. A genome-wide screen was performed on a dataset of 364 individuals from 127 multiplex AMD families. The Illumina Bead Station platform (Linkage Panel IV) was used to genotype 5253 single nucleotide polymorphisms (SNPs). For multipoint analyses a single SNP was selected from each bin of SNPs in linkage disequilibrium (r2 0.16). Both parametric and non parametric analyses were performed. This analysis replicates previous linkages, including the regions on 1q containing the Complement Factor H gene, and on 10q containing the LOC388715 gene, both of which play a major role in the genetic susceptibility to AMD. Linkage to chromosome 9 replicates findings from multiple other groups. We have previously identified linkage to chromosomes 14 and 16 by using ordered subsets analysis (OSA) to stratify a microsatellite genome scan by clinical measures including intraocular pressure, systolic blood pressure and body mass index. Application of OSA to the new linkage data significantly increases the lod score on chromsome 14 from 1.55 to 4.1 in a clinically distinct subset of 61 families (p=0.017). In addition, this new genome scan identifies novel linkage peaks with multipoint lod scores greater than 1.0 on chromosomes 3, 6, 13, and 22. The most interesting novel linkage is on chromosome 20 with a lod 3.0. These linkage regions are being further examined using both family based and case/control association analyses. This work continues the rapid dissection of the genetic underpinnings of AMD, which will lead to new therapeutic interventions to help prevent blindness in millions of AMD patients.

Genome-wide association analysis of obesity using 304,968 SNPs in 869 Finnish normal glucose tolerant individuals. *K.L. Mohlke[1], A.U. Jackson[2], L.J. Scott[2], K.N. Conneely[2], A. Swift[3], K.F. Doheny[4], E.W. Pugh[4], R.M. Watanabe[5], G.R. Abecasis[2], T.T. Valle[6], J. Tuomilehto[6], R.N. Bergman[5], F.S. Collins[3], M. Boehnke[2]* 1) U North Carolina, Chapel Hill, NC; 2) U Michigan, Ann Arbor, MI; 3) NHGRI, Bethesda, MD; 4) CIDR, JHU, Baltimore, MD; 5) U Southern California, Los Angeles, CA; 6) National Public Health Institute, Helsinki, Finland.

Obesity is a major cause of morbidity and mortality in the USA and worldwide. Although obesity shows strong heritability, the underlying genes are not well understood. Waist to hip ratio (WHR) is a measure of fat distribution that strongly predicts cardiovascular disease. In the context of a case-control association study for type 2 diabetes, we performed preliminary association analysis with WHR using the first 869 of a planned 1186 normal glucose tolerant individuals in stage 1 of our 2-stage study. All individuals are participants in the Finland United States Investigation of NIDDM Genetics (FUSION) and/or Finrisk 2002 studies. These 869 individuals have an average WHR of .900 .088 (range .67-1.12) and an average BMI of 27.1 3.7 (range 17.5-39.1). Prior to association analysis the trait values were adjusted for age, gender, and birthplace within Finland. 304,968 SNPs genotyped on the Illumina HumanHap300 BeadChip passed initial quality control using criteria of >95% success, HWE p>.001 and <2 duplicate discrepancies. For each SNP analyzed we calculated p-SNP, an overall p-value for the SNP corrected for 3 genetic models tested. The lowest p-SNP of $2.4x10^{-6}$ was observed for rs966935, a SNP on chromosome 10 located 7.5 kb from a putative processed transcript; the median WHR values for 77 GG, 384 AG, and 407 AA individuals were .918, .906 and .890, respectively. Slightly more p-SNP values exceed the significance threshold of .005 than expected by chance (p=.01). These results suggest that the genetic variants responsible for susceptibility to obesity have modest effects not easily distinguished from chance results in 869 samples. Analysis of all stage 1 samples for WHR, waist circumference, and BMI will further clarify the role of these SNPs in obesity susceptibility.

Prader-Willi syndrome (PWS) in Taiwan. *S.-P. Lin[1, 2, 3], H.-Y. Lin[1], J.-L. Yen[4], M.-C. Chao[5], D.-M. Niu[6], P.-L. Kuo[7]*
1) Department of Pediatrics & Medical Research, Mackay Memorial Hospital, Taipei, Taiwan; 2) Mackay Medicine, Nursing and Management College, Taipei, Taiwan; 3) Department of Infant & Child Care, National Taipei College of Nursing, Taipei, Taiwan; 4) Department of Pediatrics, Branch for Women & Children, Taipei City Hospital, Taipei, Taiwan; 5) Department of Pediatrics, Kaohsiung Medical University Chung-Ho Memorial Hospital, Kaohsiung, Taiwan; 6) Department of Pediatrics, Taipei Veterans General Hospital, Taipei, Taiwan; 7) Department of Obstetrics & Gynecology, National Cheng-Kung University Hospital, Tainan, Taiwan.

The information concerning the clinical features of PWS is rather fragmentary in Taiwan. We report retrospective analysis of 70 PWS cases (M:F=39:31; age one month to 23 years) seen in 4 major medical centers in Taiwan from 01/88 to 05/06. All cases were confirmed by methylation-specific PCR. Complete genetic study was performed in 52 patients. The abnormalities found included deletions in 45 (87%), maternal UPD in 5 (10%), and probable imprinting center deletion or imprinting defect in 2 (4%). The average weight at birth was 2588540 gm. Bone age delay > 2 years was detected in 11/40. GH deficiency was noted in 12/20. In 2000, Taiwan instituted Rare Diseases & Orphan Drugs Act and mandated a 3-phase screening protocol for PWS. Of the 41 diagnosed prior to 2000, only 4 (10%) were diagnosed before age of 3 months; in the 29 patients diagnosed after 2000, 15 (52%) were diagnosed before 3 months of age ($p<0.001$). Our finding is in contrast to most of the previous reports that indicated a higher incidence of UPD. It is not clear whether this discrepancy in incidence of UPD arises from under-diagnosis, or because of ethnic differences, or a younger maternal age in our group. This question is worthy of further study. The 3-phase screening program has brought great impact to early diagnosis of PWS in Taiwan. Our Bureau of National Health Insurance agreed to subsidize GH therapy, in May 2005, for all the PWS patients up to the end of puberty. Up to now, 24 patients have received the therapy for more than a year. They all show a remarkable improvement in linear growth, weight control, and quality of life.

Molecular profiling of idiopathic hirsutism for markers of androgenic metabolism and insulin endothelial regulating genes. *D. Minella[1], F. Amati[1], M. Biancolella[1], G. Grassi[1], F. Gullotta[1], A.M. Nardone[1], P. Spitalieri[1], A. Farcomeni[2], S. Bueno[2], G. Chillemi[2], D. Lauro[3], A. Desideri[4], C. Moretti[3], G. Novelli[1]* 1) Dept. of Biopathology and Diagnostic Imagining, Tor Vergata University, Roma, Italy; 2) CASPUR, Roma, Italy; 3) Dept. of Internal Medicine, Tor Vergata University, Roma, Italy; 4) Dept. of Biology, Tor Vergata University, Roma, Italy.

Hirsutism is the presence of terminal hairs in females in a male-like pattern, and affects between 5% and 15% of women surveyed. Hirsutism results from the interaction between the androgen level and the sensitivity of the hair follicle to the androgen. However the severity of hirsutism does not correlate well with the level of androgen, because the response of the androgen-dependent follicle to androgen excess varies considerably within and among persons. In fact some women with excess androgen have no skin manifestations; other women develop hirsutism also with normal androgen levels (idiopathic hirsutism IH). The pathophysiology of IH is presumed to be associated to SRD5A activity, probably both isoenzyme types (SRD5A1 and SRD5A2) and possibly an alteration in androgen receptor function. However, hirsutism is also related to obesity and insulin resistance. With the aim of identify gene involved in the pathogenesis of this disease we have developed a specific oligonucleotide low-density microarray (called AndroChip). We used the microarray technology to study the expression profiles of 204 genes involved in the androgen biosynthesis and metabolism, and other genes coding for products active in the insulin pathway, in skin genital fibroblasts of four idiopathic hirsute women and 4 related controls. Expression level filtering and statistical analysis identified 6 differentially expressed genes, 4 up-regulated and 2 down-regulated. Differentially expressed genes included products involved in the insulin signalling and none was found to be related to the androgen pattern. This result suggests the misregulation of genes related to insulin pathway occur in hirsutism.

Long-term wide-spread somatic correction in MPS II mice using a self-complimentary AAV2 vector. *L.K. Kang*[1], *H.F. Fu*[2], *D.M.M. McCarty*[2], *J.M. Muenzer*[1] 1) Department of Pediatrics, University of North Carolina, Chapel Hill, NC; 2) Center for Gene Therapy, Columbus Childrens Research Institute, Department of Pediatrics, Ohio State University, Columbus, OH.

Mucopolysaccharidosis II (MPS II) is an X-linked lysosomal storage disorder due to the deficiency of the iduronate sulfatase (Id-S). No definite treatment is available for MPS II patients. AAV gene therapy is a promising treatment for MPS disorders. Traditional AAV vectors deliver a single-stranded DNA genome (ssAAV), which must be converted by host-cell-mediated DNA synthesis to double-stranded DNA for active expression. The recently developed self-complementary AAV (scAAV) vector allows more efficient expression by delivering a duplex genome and bypassing second strand DNA synthesis which may be the rate limiting step in AAV transduction. In this study, scAAV2 vector expressing human Id-S was compared to traditional ssAAV in MPS II mice. Both scAAV2 and ssAAV2 were administered to the adult MPS II mice intravenously (5 X 1011 viral particles) and intracisternally (5 X 1010 viral particles) after pretreatment with mannitol (1-2mg/gm body weight). Four scAAV treated MPS II mice were scarified at 20-22 months of age when they developed severe neurological symptoms. Our result demonstrated that complete correction of glycosaminoglycan storage in multiple tissues were observed in the scAAV treatment group, including liver, spleen, kidney, heart, lung, intestine, and muscle, compared to the nontreated mice ($p<0.05$). The ssAAV2 treated MPS II mice group shows complete correction in liver, partial correction in spleen, heart, lung, intestine and muscle and no correction in the kidney. Id-S enzyme activity was > normal levels in liver and spleen of the scAAV2 treated group, and about 10-100% of the normal activity in the kidney. In contrast, Id-S enzyme activity can only be detected in the livers, but not in the spleens or kidneys of the ssAAV2 treatment group. These results suggest scAAV2 mediated gene delivery results in significant wider distribution of Id-S expression compared to ssAAV. These preliminary results suggest that scAAV vector is a promising candidate for the treatment of MPS II.

Identification of multiple-gene associations from SLC6A4, ITGB3 and GABA receptor subunit genes in autism by MDR-Phenomics. *H. Mei[1,2], E. Martin[1], M. Pericak-Vance[1], M. Cuccaro[1]* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Bioinformatics Research Center, NCSU, Raleigh, NC.

We present an application of a novel method, MDR-Phenomics, to identify multiple gene effects by analysis of autism (AUT) candidate genesserotonin transporter gene [SLC6A4] and integrin beta 3 [ITGB3] on Chr17 and -aminobutyric acid (GABA) receptor subunit genes (GABRB3, GABRB5 and GABRG3) on Chr15. In complex diseases like AUT, multiple genes may increase disease risk through weak additive effects or gene-gene interactions when only weak single-gene effects exist. Traditional methods based on tests of main gene effects often fail to detect disease-risk genes. While the MDR (Multifactor Dimensionality Reduction) and its extensions, EMDR and MDR-PDT, are designed to detect multiple gene effects, they may not detect joint effects when genetic heterogeneity exists. Genetic heterogeneity may be expressed as phenotypic heterogeneity (different genetic variants may lead to different constellations of clinical characteristics). To improve power in the presence of heterogeneity, we developed MDR-Phenomics, which detects single- or multi-locus associations with disease in families by using phenotypic covariates (PC). AUT is more frequent in males and displays broad variation in core features including social interaction, language, and repetitive behaviors. Using sex and severity of repetitive behaviors as PC for MDR-Phenomics we studied candidate genes in the serotonergic and GABA-ergic systems. We analyzed 3 markers in SLC6A4 and 4 markers in ITGB3 in 489 family triads with AUT using sex of the proband as a PC. We also examined eight markers in GABRB3, GABRB5 and GABRG3 in 148 family triads with severity of repetitive behaviors as a PC. The first analysis showed a significant 4-locus model with two loci from SLC6A4 and two loci from ITGB3. Statistical modeling is ongoing to understand this 4-locus effect. Results from the second analysis showed that a 2-locus model with a locus from GABRB5 and GABRG3 was marginally significant after adjusting for multiple tests. The results suggest that gene-gene interactions may exist between SLC6A4 and ITGB3, and between GABRB5 and GABRG3.

Association of toll-like receptor polymorphisms and pulmonary tuberculosis. *C.D. Hamilton[1], W.F. Hulme[1], J.B. Rimmler[1], S.G. Patillo[1], A.W. Mosher[1], M.E. Stryjewski[2], E.H. Abbate[3], R. Estevan[3], J.R. Gilbert[1], W.K. Scott[1]* 1) Department of Medicine, Duke University Medical Center, Durham, NC; 2) CEMIC, Buenos Aires, Argentina; 3) F.J. Muñiz Hospital, Buenos Aires, Argentina.

Tuberculosis (TB) is a significant cause of premature mortality worldwide. Ten percent of exposed individuals develop pulmonary TB, suggesting that host factors, partly under genetic control, determine development of active TB. Pattern recognition molecules such as the toll-like receptors (TLRs) are important contributors to innate immunity. In particular, TLR2, TLR4, and TLR9 have been shown to play a role in response to infection with *Mycobacterium tuberculosis* in mouse models. We collected samples from 171 African-American and 107 white families with at least one case of pulmonary TB. Individuals older than 14 years with culture-confirmed pulmonary TB and children younger than 14 years with culture- or clinically-confirmed TB were recruited from the North and South Carolina TB control programs and the outpatient population of F.J. Muñiz Hospital, Buenos Aires, Argentina and enrolled along with unaffected sibling or parental controls. We examined tag single nucleotide polymorphisms (tagSNPs) in each of these genes for association with TB in a family-based study. tagSNPs were selected from HapMap Phase II data using the tagger function of HaploView. tagSNPs captured the common variation (minor allele frequency (MAF) 5% in each gene with r^2 0.8. Other coding SNPs (cSNPs) with MAF 1% were genotyped as well. Nine SNPs in TLR2, 25 in TLR4, and 12 in TLR9 were genotyped using TaqMan. The association in the presence of linkage (APL) test was used to analyze marker-disease associations. Families were analyzed stratified by self-reported race to limit confounding by ethnic background. No significant associations were detected in the white family sample. Three SNPs in TLR2 and two SNPs in TLR9 were nominally associated with TB in the African-American sample (0.01p0.05) in single point analysis. These results suggest that genetic susceptibility to TB in individuals of African descent might be influenced by variation in the TLR2 and TLR9 genes.

Direct sequencing quality control: a novel software approach to reducing researcher labor. *K. Hunkapiller, E. Vennemeyer* Applied Biosystems, Foster City, CA.

With the completion of the Human Genome Project, the shift from de novo sequencing to direct sequencing (resequencing) has created the need for more accurate variant detection for medical research and clinical diagnostics. The bottleneck in the workflow (from DNA extraction to result data analysis) has been cited as taking up to 70% of researchers time per project, due to manual review of individual nucleotide bases. This review has been required due to the necessity of having confidence in the variant result. Increasing confidence can come from applying diligent quality control metrics, including use of Quality Values for DNA trace value and confidence values for variant validity. Based on Applied Biosystems experience, this system will both filter out bad data and alert users to potential resequencing problems. The software will then direct users to review only low confidence variants. A flexible workflow based system is being built to enable researchers to obtain their high confidence results in less time. Methods for filtering low quality data based on optimal settings and quality visualization tools will be integrated into the system along with simpler variant review and reporting tools to allow researchers to quickly analyze their data.

Mutations in TMEM76 are associated with N-acetyltransferase deficiency in mucopolysaccharidosis type III C patients. *M. Hrebicek[1], L. Mrazova[1], J. Majewski[3], L. Noskova[1], H. Hartmannova[1], R. Ivanek[2], A. Cizkova[2], H. Poupetova[1], J. Sikora[1], J. Urinovska[1], V. Stranecky[1,2], J. Zeman[1,2], S. Kmoch[1,2]* 1) Institute of Inherited Metabolic Disorders, Charles University, Prague, Czech Republic; 2) Center for Applied Genomics,Charles University, Prague, Czech Republic; 3) Department of Human Genetics and Genome Quebec Innovation Centre, McGill University, Montréal, Canada.

Mucopolysaccharidosis type IIIC (MPS IIIC) is caused by a deficiency of the acetyl-coenzyme A: -glucosaminide N-acetyltransferase (N-acetyltransferase), leading to impaired degradation and lysosomal storage of heparan sulfate. To identify the MPSIIIC gene we assessed 54 individuals from four Czech MPS IIIC families. Measurement of N-acetyltransferase levels in leukocytes unambiguously classified affected, carriers, and non-carriers and allowed us to performed linkage analysis under an autosomal codominant model. We genotyped 18 microsatellite markers located in a recently identified MPS IIIC locus on chromosome 8. In all families we confirmed the linkage to MPS IIIC region with a maximum LOD score of 7.8 at D8S531 and identified several recombination events that narrowed the candidate region between markers D8S1115 and D8S1831 and physical coordinates 42655724-51968859 Mbp of chromosome 8. Bioinformatic search and gene expression analysis of all the genes located within the candidate region on oligonucleotide microarrays pinpointed a single gene *TMEM76* with significantly decreased expression in patient samples. Sequence analysis of the *TMEM76* showed that all five patients from analyzed families are compound heterozygotes for eight different mutant alleles. Five mutations are predicted to result in truncated products (L321X, R384X and R506X, 16 bp deletion I345fs, and splicing mutation IVS9+5G>A). The rest are missense mutations R344H, M482K and P571L affecting the conserved residues. All the mutations completely segregated in the families with the phenotype based on the enzyme assay, which provides support that *TMEM76* is the gene encoding N-acetyltransferase deficient in MPSIIIC patient. Support: NR/8361-3, 1A/8239-3 from IGA MZ-CR.

Activity-dependent PP2A dephosphorylation of FMRP suggests a novel, dynamic and immediate phase of translational regulation in neurons. *U. Narayanan[1], M. Nakamoto[1], D. Pallas[2], S.T. Warren[1,2,3]* 1) Department of Human Genetics, Emory University, Atlanta, GA; 2) Department of Biochemistry, Emory University, Atlanta, GA; 3) Department of Pediatrics, Emory University, Atlanta, GA.

Fragile X syndrome is a frequently inherited form of cognitive impairment caused by transcriptional silencing of the *FMR1* gene. Thus, there is a functional absence of the encoded protein, FMRP, a selective RNA-binding protein that suppresses translation of target messages when phosphorylated at serine 499. We have identified protein phosphatase 2A (PP2A) as a FMRP phosphatase in both primary neuron cultures and non-neuronal cells. Here, we present biochemical studies demonstrating that PP2A enzymatic activity mediates a rapid (< 1 min) FMRP dephosphorylation following mGluR5 stimulation in primary hippocampal neurons. However, longer mGluR5 stimulation (> 5 min) resulted in FMRP phosphorylation. These activity dependent changes in FMRP phosphorylation correlated with translational changes of a predominantly dendritic target transcript, *SAPAP3*. Thus, these data link mGluR5 activation and PP2A enzymatic activity with FMRP phosphorylation suggesting a novel, temporally restricted phase of local protein synthesis that may represent an immediate effect of synaptic activation.

Population Stratification Adjustment Based on Assessment of "Spurious" Linkage Disequilibrium. *D. Hu, E. Ziv*
Center for Human Genetics, Department of Medicine, University of California San Francisco, San Francisco, CA.

Population substructure or recent admixture may confound the results of genetic association studies among unrelated individuals. Several methods exist for assessing and adjusting for population stratification using additional control loci. These methods can be classified as genomic control and structured association. Both approaches generally work well, but have certain limitations. We have developed a novel approach to assess and adjust for substructure and recent admixture in association studies. The approach is based on the concept that allelic association between physically unlinked markers (spurious linkage disequilibrium) is the basis for the false positive and false negative results. We derive a summary measure of spurious LD (SLD) for each candidate marker with the rest of the genome. The estimate of SLD is then used to weight the association statistic for that particular marker. We simulate the method under a variety of conditions using data on allele frequency from the HapMap. We show that the method works well under a variety of discrete and admixed population scenarios. For example, to eliminate the bias in an association study that includes individuals of both African and European ancestry, approximately 50 random SNP markers are required. In contrast, for a population consisting of groups as similar as Chinese and Japanese approximately 250-500 random SNP markers are required. Our approach is similar to the structured association approach which identifies subpopulations based on (SLD). However, our approach does not require explicit identification of subpopulations and therefore may be more useful in studies where substructure is subtle and the number of subpopulations is difficult to detect.

100 Robust Dosage PCR (RD-PCR) assays: easy to develop and highly accurate dosage assays can robustly detect germline deletion mosaicism at a level of 1.4 copies per genome. *V.Q. Nguyen[1], J. Han[2], C.H. Buzin[2], C. Kasper[3], S.S. Sommer[1,2]* 1) Dept Molecular Genetics, City of Hope Medical Ctr, Duarte, CA 91010; 2) Clinical Molecular Diagnostics Laboratory, City of Hope Medical Ctr, Duarte, CA 91010; 3) Orthopaedic Hospital of Los Angeles, Los Angeles, CA 90007.

Recently, Multiplex Ligation PCR Amplification (MLPA) has become widely used for dosage analyses. Though multiplex dosage analyses can be performed, new assay development can be time consuming and significant false-positive and false-negatives can occur (1-3). In contrast, RD-PCR assays are easy to develop, reproducible and highly accurate, but each region has to be analyzed individually. Wild type male and female samples serve as dosage controls, even in the absence of known deletions. We have developed over 100 RD-PCR assays, which are routinely validated by blinded analyses. To illustrate the accuracy of RD-PCR, we describe a mosaic for a large deletion in hemophilia B. The mother is a heterozygous carrier while the maternal grandmother is mosaic for the deletion (average of 1.4 copies per white blood cell). The average standard deviation across three exons from 48 samples assayed by two individuals for wild type males, wild type females, and the mosaic person are 0.009, 0.016 and 0.012, respectively. RD-PCR assays are flexible and rapidly developed with an accuracy sufficient to detect mosaicism as well as heterozygous deletions and duplications. 1) Walsh et al., (2006) JAMA 295(12):1379-1388; 2) Monfort et al., (2006) J Lab Clin Med, 147:295-300; 3) Warshawsky et al. (2006) Clin Chem 52:7.

Analysis of a de novo complex chromosomal rearrangement: t(4q;9q) with ins(Xq;4q) identified by FISH and defined by array-based comparative genomic hybridization in a patient with dysmorphic features. *K.S. Hwang[1], Y.J. Chen[2], M.H. Tseng[3], D.C. Ding[4], Y.S. Yuh[3], J.S. Chang[5], S.W. Cheung[6]* 1) Department of Obstetrics and Gynecology, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan; 2) Institute of Genome Sciences, School of Life Sciences, National Yang-Ming University, Taipei, Taiwan; 3) Department of Pediatrics, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan; 4) Department of Obstetrics and Gynecology, Buddhist Tzu Chi Medical Center, Hualien City, Taiwan; 5) Youthgen Biomedical Laboratory, Taipei, Taiwan; 6) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

The development of molecular cytogenetics techniques has made it possible to identify complex and cryptic chromosome rearrangements in mentally retarded and dysmorphic individuals who could not be diagnosed by conventional cytogenetics analysis. We report on a 9-months-old girl who presented with growth deficiency, developmental delay, and dysmorphic features including hypertelorism, low-set ears, simian crease, and low saddle nose. The G-banding analysis revealed a de novo complex chromosomal rearrangement (CCR) involving the distal long arm of one chromosome 4, 9, and X. FISH analysis using whole chromosome painting revealed not only a translocation between the long arms of chromosomes 4 and 9 but also an interstitial deletion from chromosome 4q that became inserted at the translocational breakpoints on derivative chromosome X. Comparative genomic hybridization (CGH) on metaphase chromosomes showed no apparent loss and gain. Subsequent array-based CGH will be performed to identify the abnormality. Molecular cytogenetics characterization of the CCR with these methodologies and follow up studies will be presented. This case further demonstrates the diagnostic usefulness of combining conventional cytogenetics with new molecular chromosome analysis methods for clinical interpretation of complex chromosome abnormalities.

Phenotype-association based ranking of human disease protein complexes. *K. Lage[1], E.O. Karlberg[1], Z.M. Størling[1], P.I. Olasson[1], O. Rigina[1], A. Gorm[1], Z. Tumer[2], F. Poicot[3], N. Tommerup[2], S. Brunak[1]* 1) CBS, DTU, Lyngby,Denmark; 2) WJC, IMBG, University of Copenhagen, København; 3) Steno Diabetes Center, Gentofte.

Efforts are ongoing to search for novel global aspects of human disease and recent studies hint at the general existence of human disease protein complexes. Here we make a systematic analysis of such complexes hereby showing they exist in all categories of human disease. By making a phenotype association based ranking of putative human disease protein complexes, we develop a novel Bayesian predictor which in 298 out of 669 linkage intervals correctly ranks the known disease causing protein as the best candidate, hereby outperforming other computational methods for disease gene identification. Based on this approach we prioritize candidate genes in ~850 linkage intervals, with no identified disease gene, leading to likely novel candidates in disorders such as Alzheimer disease, amyotrophic lateral sclerosis, inflammatory bowel disease and type 2 diabetes. By constructing a quality controlled human protein interaction network and implementing an computational phenotype association scheme, we show here that disease complexes exist in essentially all categories of human disease. We use this approach to identify a large number of potential human protein disease complexes, and train a Bayesian predictor to prioritize candidates in around 850 linkage intervals associated with various specific diseases, based on these complexes. We present a thorough benchmark and a number of case studies to show how the proposed disease complexes can be exploited to generate novel hypotheses which point directly at specific validation experiments and specific patient material. Thus, integrated data underlying these predictions provide a first draft of ~90 potential human disease protein complexes in many different categories of human disease at a level of completeness determined by the current amount of data.

Subclinical orbicularis oris muscle (OO) defects are increased in relatives of individuals with nonsyndromic cleft lip with or without cleft palate (CL/P). *K. Neiswanger[1], S.M. Weinberg[1], C.R. Rogers[1], C.A. Brandon[1], M.E. Cooper[1], K.M. Bardi[1], M.P. Mooney[1], J.M. Resick[1], F.W.B. Deleyiannis[1], A. Bowen[2], J.E. De Salamanca[3], M.L. Marazita[1]* 1) Univ Pittsburgh, Pittsburgh, PA; 2) Ch Hosp, Pittsburgh, PA; 3) Niño Jésus Univ Ch Hosp, Madrid, Spain.

Nonsyndromic CL/P is a common birth defect that occurs more frequently in males than females. We hypothesized that defects in the OO muscle are a subclinical form of cleft lip. To test this, upper lip ultrasound images were taken for 782 people with no overt cleft (525 unaffected relatives of individuals with CL/P and 257 controls). The images were scored and staffed by three independent raters as normal (a continuous OO muscle), affected (discontinuous at some point), or unknown. Study subjects were ascertained from three populations: Guatemalans (285 relatives, 136 controls), U.S. Caucasians (147 relatives, 121 controls), and Spanish Caucasians (93 relatives only). In the overall sample, 10.3% (54/525) of the relatives had an OO defect, significantly higher than the 5.8% (15/257) observed in controls ($p = 0.04$). Although not statistically significant, the same trend was seen in the Caucasian and Guatemalan samples separately. When the sample was stratified by gender, significantly more male relatives had OO defects than male controls [12.0% (28/234) vs 3.2% (3/94); $p = 0.01$]; similarly, more female relatives than female controls had OO defects, but this difference was not statistically significant [8.9% (26/291) vs 7.4% (12/163); $p = 0.56$]. Inclusion of OO data in candidate gene SNP association tests added 29 new affected individuals to the analysis, and increased significance for association with IRF6 from $p = 0.007$ to 0.006, with FGF10 from $p = 0.01$ to 0.003, and for EGFR from $p = 0.006$ to 0.003. Our results suggest that, as with overt clefting, males tend to demonstrate a lower threshold for subclinical forms, such as OO defects, and that OO defects are likely to be part of the phenotypic spectrum of CL/P. Supported by NIH grants R01-DE016148, P50-DE016215, R21-DE016930, M01-RR00084, SNP genotyping: CIDR NIH contract N01-HG-65403.

Initial Discovery and Replication in Whole Genome Association Scans: A Sequential Analysis Approach. *B.S. Maher, R.J. Weyant, T. McHenry, M.L. Marazita* Univ Pittsburgh, PA.

A major issue in whole genome association studies is replication. To address this issue, many two stage designs have been proposed. However, for many investigators, sample splitting will result in a sample size lacking power to detect an initial discovery. Thus, many investigators are faced with the a priori decision of a two-stage design, sacrificing the power of the full sample for initial discovery; or a single stage design, sacrificing the ability to replicate an initial discovery. We propose a sequential analytic design that maintains the power of a single stage design while providing the opportunity for replication in a single sample. The method follows the approach proposed by OBrien and Fleming (1979) for sequential clinical trials. Under the sequential design, samples can be sequentially added in a planned number of interim analyses, while preserving a subset of the sample for replication and maintaining the study-wise type I error rate. To simulate a large scale genetic association study, we simulated 10,000 replicates of 10,000 SNPs (1 disease, 9999 null) in a sample of 1000 case-control (c-c) pairs under a variety of genetic models. The samples were analyzed and compared under three analytic approaches: two-stage (500 initial and 500 replication c-c pairs), the single stage with 1000 c-c pairs, and the sequential design (performing 3 interim analyses while reserving the remaining sample for replication). We demonstrate that the sequential design outperforms the two-stage design and maintains the power of the complete sample (single stage) for initial discovery under every tested model. For example, at an odds ratio of 1.4, the power to detect initial allelic association is 90.3% and 89.8% in the sequential and single stage designs respectively while it is only 19.0% for the two-stage design. The sequential design also has greater study-wise power to detect and replicate an allelic association. At an odds ratio of 1.4, the power to detect and replicate a finding was 57.4% for the sequential design and only 18.8% for the two-stage design. Lastly, we demonstrate that the sequential design maintains the proper study-wise type I error rate.

BAX gene polymorphisms increase risk and severity of multiple sclerosis in Caucasians. *J.D. Hart[1], J.M. van der Walt[1], A.M. Prokop[1], S. Schmidt[1], S.G. Gregory[1], N. Schnetz-Boutaud[2], J.L. McCauley[2], J.L. Haines[2], M.A. Pericak-Vance[1]* 1) Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Ctr Human Genetics Research and Dept of Molecular Physiology and Biophysics, Vanderbilt Univ Medical Ctr, Nashville, TN.

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system characterized by demyelination and axonal damage. Mechanisms which underlie MS etiology are not well defined; however, disregulation of the apoptotic pathway may play an important role. Therefore, we examined eight polymorphisms within the pro-apoptotic gene, BAX, in a large family-based study including 842 families and 636 discordant sib-pairs for association with MS risk. BAX is located within a region of genetic linkage in MS on chromosome 19q. Family-based tests (PDT, APL) were used to test whether SNPs in BAX influence risk of MS. We observed significant association of markers rs1009316 (p-value=0.007), rs1805419 (p-value=0.013) and rs2387583 (p-value=0.006) with increased risk of MS. All makers are located within intronic regions and are in high linkage disequilibrium. We also calculated Multiple Sclerosis Severity Scores (MSSS) for each affected individual in order to define disease progression (severity). MS severity can be evaluated by comparing a patient's EDSS score (Expanded Disability Status Scale) for a given disease duration against the Global MSSS distribution calculated for a large Caucasian reference population (Roxburgh et al. 2005). Our analysis demonstrated that rs1009316 significantly effects disease severity (p-value = 0.01). Our preliminary results suggest that BAX influences both risk and severity of MS. We are conducting comprehensive resequencing of BAX to identify causal variants that may be associated with MS risk.

Investigating the origins of the BRCA1 mutation c.5385dupC. *N. Hamel[1], L. Foretova[2], S.A. Narod[3], L. Tihomirova[4], V. Zajac[5], S. Ciernikova[5], S. Armaou[6], D. Yannoukakos[6], C. Greenwood[7], W.D. Foulkes[1]* 1) Dept Medicine, McGill Univ Health Ctr, Montreal, Canada; 2) Dept Cancer Epidemiology & Genetics, Masaryk Memorial Cancer Inst, Brno, Czech Republic; 3) Ctr for Research on Women's Health, Univ Toronto, Canada; 4) BMC, Riga LV-1067, Latvia; 5) Dept Cancer Genetics, Cancer Res Inst, Slovak Academy of Sciences, Bratislava, Slovak Republic; 6) Mol Diag Lab, Natl Ctr Scientific Research, Athens, Greece; 7) Prog in Genetics & Genomic Biol, Hospital for Sick Children Res Inst, Toronto, Canada.

The BRCA1 mutation c.5385dupC is a well-known cancer susceptibility founder in the Ashkenazi Jewish (AJ) population, but it is also found in several non Jewish populations throughout Europe and elsewhere. We wanted to know whether the mutation originated from a single common ancestor or whether it arose many times in human history. Since c.5385dupC consists of a C inserted among three consecutive Cs, this site may represent a hotspot for replication error. We collected 135 AJ and non-AJ mutation carriers from Latvian, Greek, Slovak, Czech, Polish, Brazilian and Canadian populations. All carriers were genotyped for 15 microsatellite markers spanning 12 Mb on chr 17 including BRCA1. When available, close relatives of the carriers were also genotyped. Preliminary data showed 100% linkage between the mutation and a rare allele (frequency < 1% in controls) at marker D17S1327, 268 kb from the mutation, suggesting c.5385dupC is identical by descent in all populations studied. Based on our preliminary data, we genotyped 36 additional SNP markers within a 2.9 Mb maximum region of conservation observed around BRCA1. We are currently reconstructing haplotypes from all genotypes using the program PHASE. These haplotypes will be used to estimate the age of the mutation based on progressive decay of linkage disequilibrium as a function of distance from the mutation using the Bayesian linkage analysis program DMLE. Neuhausen et al (1996) previously estimated the age of this mutation to be 36 generations (720 years). Our larger study should refine this preliminary estimate and help us gain insight into the place of origin of c.5385dupC and how it spread to the populations where it is found today.

The medical sequencing pipeline at the Washington University GSC. *Y. Kasai, L. Ding, E. Mardis, R. Wilson, GSC Medical Sequencing Team* Genome Sequencing Center, Washington University School of Medicine, 4444 Forest Park Ave. St. Louis, MO 63108.

With the completion of the reference human genome sequence, researchers have begun to answer questions about individual human genomic variation, both in the context of normal variation and of disease onset, progression and prognosis. Over the past year, the GSC at Washington University has developed a high-throughput pipeline for PCR-based genomic re-sequencing, including infrastructure to design and test primer pairs, automation to achieve 384-well plate-based sample processing, and barcode-based sample tracking. We also have developed robust procedures and QC checks throughout the pipeline. At present, human re-sequencing data is actively being produced with this pipeline for multiple genes using DNA from a variety of patient-derived tissues. Our corresponding sequence analysis pipeline utilizes a suite of informatics tools for mutation detection of the assembled sequence reads. A GBrowse-based viewer that illustrates polymorphisms and somatic mutations in the context of relevant genome annotation are under development. Results from some ongoing projects (including a cancer genomics effort) will be presented.

The relationship between Glucokinase -30 G/A variant, early growth and adult metabolic phenotypes in a Finnish birth cohort. *M-R. Jarvelin[1,5], A.J. Bennett[2], U. Sovio[1], A. Ruokonen[3], H. Martikainen[4], A. Pouta[5], A-L. Hartikainen[4,5], S. Franks[6], P. Elliott[1], M.I. McCarthy[2,7]* 1) Dept of Epidemiology and Public Health, Imperial College London, London, UK; 2) DRL OCDEM, Oxford, UK; 3) Dept of Clinical Chemistry, University of Oulu, Finland; 4) Dept of Obstetrics and Gynaecology, University of Oulu, Finland; 5) Dept of Public Health Science and General Practice, University of Oulu, Finland; 6) Institute of Reproductive and Developmental Biology, Imperial College London, UK; 7) Wellcome Trust Centre for Human Genetics, University of Oxford, UK.

Rare mutations in the glucokinase gene (GCK) have been associated with type-2-diabetes and low birth weight. More common variants, such as the -30 promoter variant (G/A rs1799884) have also been associated with glucose intolerance, reduced beta-cell function and abnormal early growth. To determine the ability to generalize the findings, we genotyped this variant in 5141 subjects from the Northern Finland Birth Cohort of 1966 using an Amplifluor assay. We used linear regression to analyse associations with early growth (e.g. birth weight, ponderal index) and adult metabolic phenotypes (e.g. fasting glucose, BMI, beta-cell function [HOMA%B]) measured at age 31y. Genotype frequencies were in Hardy-Weinberg equilibrium (p=0.62). No significant associations were observed for measures of early growth. However, fasting glucose at age 31 was significantly associated with an increasing number of copies of the minor A allele (p=0.003, adjusted additive model). When the individuals with fasting glucose levels >6mmol/l were excluded (to remove outliers with frank diabetes), the per-allele increase in glucose remained significant (p=0.001). HOMA%B showed non-significant reductions with increasing copies of the A allele (p=0.066). In this large birth cohort, we confirmed that variation in the GCK promoter modifies adult fasting glucose levels, but were unable to confirm associations with early growth measures such as birth weight. This may reflect the competing effects of maternal GCK variation (causing gestational diabetes and macrosomia) and variation in the fetus which compromises insulin secretion and growth.

Genetic and Cellular Factors Influencing the Frequency of Nonallelic Recombination Among Interspersed Repetitive Elements. *D.J. Hedges, P.L. Deininger* Tulane Cancer Center, Tulane University Health Sciences Center, New Orleans, LA.

A substantial fraction of the human genome is comprised of parasitic L1, Alu, and SVA repetitive elements. While a number of genetic diseases have been directly attributed to disruptions caused by mobile element insertions, still more appear to be the consequence of subsequent genetic instability resulting from nonallelic recombinations among interspersed element copies. The various factors governing the relative stability/instability of repetitive elements residing in diverse genetic contexts remain poorly understood. Additional empirical data concerning when and where instability most frequently occurs may aid in predicting regions in the genome that have the highest likelihood to destabilize in the germline or in the soma during an organism's lifespan. Identifying such regions of "cryptic genomic instability" may help in further narrowing candidate regions, not only for specific genetic disorders, but also those related to tumorgenesis, metastatic evolution, and genetic deterioration associated with aging individuals. In order to gain further insight into the influence of various genetic and cellular factors on the propensity for nonallelic recombinations among mobile elements, we have constructed a series of cell culture-based reporter assays. With these assays, we have the ability to place repetitive elements within diverse genetic contexts and cellular conditions in order to observe the resultant impact on nonallelic homologous recombination frequencies. Here we report on our initial results from these experiments. Our preliminary data indicate that increases in the ambient level of mobile element expression itself significantly impacts the observed recombination frequency. This result suggests that, in addition to providing ideal substrates for nonallelic recombinations, mobile element activity also actively instigates these instability events, probably through introduction of DNA double-strand breaks. These results further illustrate the potential epidemiological risks associated with inter-individual variation in human mobile element activity.

GENOME: a rapid coalescent-based whole genome simulator. *L. Liang, S. Zöllner, G.R. Abecasis* Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, MI.

Computer simulations can provide important information to help plan genome-wide association studies. Nevertheless, present simulation methods do not allow efficient simulation of whole genomes. We present GENOME, a computer program that uses a rapid coalescent approach to sample whole genome data (>3000 Mb) from a population simulated using the Wright-Fisher neutral model. The program assumes an infinite-site mutation model and allows for recombination and migration among subpopulations. Past demographic events such as population bottlenecks or expansion and population merges and splits can also be simulated. In addition to uniform recombination rates, it is possible to allow recombination rates to vary so as to mimic the pattern of hotspots along the genome. Within small regions, we have compared our simulated samples with those generated by other coalescent simulators and theoretical predictions and show that GENOME provides the expected LD patterns and frequency spectra. To simulate 1200 sequences, each 150Megabases long, GENOME requires ~44 minutes, compared to >12 hours for Hudson's ms. The program can be used to study the sampling properties of any statistic for a whole genome study under the neutral model. We evaluate the properties of several statistics that may be useful for genome wide association scans by simulating regions of different lengths.

N-Acetylmannosamine treatment rescues a mouse model of Hereditary Inclusion Body Myopathy. *M. Huizing[1], E. Klootwijk[1], B. Galeano[1], I. Manoli[1], M-S. Sun[1], C. Cicone[1], D. Darvish[2], D. Krasnewich[1], W.A. Gahl[1]* 1) MGB,NHGRI, NIH, Bethesda, MD; 2) HIBM Research Group, Encino, CA.

Hereditary Inclusion Body Myopathy (HIBM) is an adult onset autosomal recessive neuromuscular disorder characterized by slowly progressive distal and proximal muscle atrophy and weakness. This myopathy is caused by deficiency of UDP-GlcNAc 2-epimerase/ManNAc kinase (GNE), the rate-limiting, bifunctional enzyme catalyzing the first two committed steps of sialic acid biosynthesis. Decreases in GNE activity impair sialic acid production and interfere with sialylation of muscle glycoproteins such as -dystroglycan. Levels of polysialic acid on neural cell adhesion molecule (PSA-NCAM) may also be affected. To study the pathogenesis and treatment of HIBM, we created a GNE knock-in mouse mimicking the human Persian-Jewish GNE founder mutation, M712T. Homozygous (-/-) mutant mice were born in a Mendelian distribution, but did not survive beyond postnatal day 3 (P3). GNE enzyme activity in skeletal muscle tissues of -/- mice at age P2 was 20% of normal. However, histological examination did not reveal any muscle pathology. Rather, the kidneys of -/- mice showed petechial hemorrhages, proteinuria, and signs of glomerular disease. As a treatment option, we administered to pregnant females ManNAc (1 g/kg/d), an uncharged sugar intermediate located within the sialic acid pathway after the GNE feedback inhibition step. Upon feeding +/- matings ManNAc in their drinking water, 44% of the -/- pups survived beyond P3. At P2, -/- mice that received ManNAc had less severe kidney hemorrhages, and their muscle GNE activities increased to 50% of normal mouse levels, suggesting that ManNAc might stabilize the mutant enzyme. Surviving -/- mice were smaller than their littermates, but appeared healthy otherwise, even after weaning (P21), when they no longer received ManNAc. It remains to be determined if the surviving -/- mice (now ages 3 and 6 mo) will develop a muscular pathology later in life. Taken together, survival of -/- mice, improved kidney pathology and increased GNE activity after ManNAc administration strongly support consideration of a clinical trial of ManNAc for the myopathy of HIBM.

Genomewide SNP linkage scan of schizophrenia in a large multicenter sample. *D.F. Levinson[1], P.V. Gejman[2], C. Laurent[3], M.J. Owen[4], A.E. Pulver[5], B. Riley[6], D.B. Wildenauer[7], K.S. Kendler[6], J. Mallet[3], B.J. Mowry[8], G. Nestadt[5], M.C. O'Donovan[4], A.R. Sanders[2], S.G. Schwab[7], V.K. Lasseter[5], I. Nikolov[4], R. Segurado[4], P.A. Holmans[4], Schizophrenia Linkage Collaborative Group* 1) Stanford University, Palo Alto, CA; 2) ENH/Northwestern University, Evanston, IL; 3) LGN/CNRS, Paris, France; 4) Cardiff University, Cardiff, UK; 5) Johns Hopkins University, Baltimore, MD; 6) Virginia Commonwealth University, Richmond, VA; 7) University of W. Australia, Perth, Australia; 8) University of Queensland, Brisbane, Australia.

A genomewide linkage scan was carried out in pedigrees from 8 clinical samples, including 971 families with 4,540 genotyped subjects, 2,120 affected under a narrow diagnostic model (schizophrenia or schizoaffective disorder). Of 5,992 autosomal, X and XY SNP markers (Illumina v4 map), 5,903 met quality control criteria. We are analyzing marker-marker linkage disequilibrium (Haploview), constructing maps that maximize marker heterozygosities while constraining marker-marker r2 values to 0.05 or less, and carrying out multipoint linkage analyses (ALLEGRO, exponential model). Analyses of six chromosomes have been completed for the primary analysis of 755 informative European-ancestry pedigrees (3,479 genotyped individuals, 1,727 affected). Suggestive evidence for linkage (pointwise $p < 0.0005$, information content 0.90) was observed on chromosome 8p at 46.8 deCode cM. This peak is consistent with findings in other samples, and it is of interest that the well-known candidate gene NRG1 lies outside the 1-lod support interval (42.6-50.5 cM), but it is not yet known whether NRG1 and/or other loci account for the linkage evidence observed in these studies. Notably, on the chromosomes analyzed so far, 25-30% of SNPs had to be removed to reduce LD, otherwise very large spurious linkage peaks were observed in regions with multiple high-LD marker pairs. Ongoing are analyses of all chromosomes for the European-ancestry families, of all families, of linkage taking cross-site heterogeneity into account, and of the effect of these and other recent results on Genome Scan Meta-Analysis results for schizophrenia.

SCREENING TO FIND LOCI ASSOCIATED WITH CLEFT LIP AND PALATE. *K. Osoegawa[1], G.M. Vessere[1], R. Pfundt[2], E.F.P.M. Schoenmakers[2], J. Staaf[3], A. Borg[3], M.A. Mansilla[4], B.C. Schutte[4], E. Lammer[1], J.C. Murray[4], P.J. de Jong[1]* 1) Children's Hospital Oakland Research Institute, Oakland, CA; 2) Dept. Human Genetics, University Medical Centre Nijmegen, The Netherlands; 3) Dept. Oncology, Lund University, Sweden; 4) Pediatrics and Biological Sciences University of Iowa, IA.

Human bacterial artificial chromosome (BAC) libraries played a critical role for construction of physical maps and genome sequencing. We previously developed a high-resolution overlapping-BAC collection of >32,000 well-characterized clones, spanning the entire human genome. We subsequently used PCR-amplified DNAs from these BACs to create array comparative genomic hybridization (CGH) slides with a detection resolution for copy number variation of about 70 kbp. Cleft lip with or without cleft palate (CL/P) are common structural birth defects. We are surveying the genome for candidate gene regions by searching for micro-deletions/duplications using DNA samples derived from 48 cases of syndromic CL/P (20 of Van der Woude syndrome). The patient and reference DNA samples were labeled with Cy3 and Cy5, respectively, and co-hybridized onto 32k array-CGH slides. We identified several deletions and duplications with sizes exceeding 500 kb. Interestingly we have identified a novel large duplication on 8p21.3-8p12 (8.8 Mb) along with a deletion of 8q23.1-8q24.12 (11.2 Mb) in one of the samples tentatively diagnosed with Kabuki syndrome. We also identified a 2.7 Mb deletion on 22q11.21 from a patient having cleft palate. The region is overlapping with known DiGeorge syndrome region. We also focused on the chromosome 1q32.2 region that includes the interferon regulatory factor 6 (IRF6) gene and found 100 kb - 1Mb deletions for five patients, including two previously reported deletion cases. Mutation in the IRF6 gene has been reported to cause Van der Woude Syndrome, the most common form of syndromic cleft lip and palate. While these 1q32.2 mutations do not indicate a new genetic or congenetic source for CL/P, the findings reveal the sensitivity of our detection approach. We plan to look at parents for evidence of de novo deletions.

Improved long-term survival of the mucopolysaccharidosis II mouse after combined intravenous and intracisternal administration of an AAV2 vector expressing iduronate sulfatase. *J. Muenzer[1], L. Kang[1], S. Moy[2], H. Fu[1,3]* 1) Dept Pediatrics, University North Carolina, Chapel Hill, NC; 2) Dept Psychiatry, University North Carolina, Chapel Hill, NC; 3) Center for Gene Therapy, Columbus Children's Research Institue, Dept of Pediatrics, Ohio State University, Columbus,OH.

Mucopolysaccharidosis II (MPS II) is an X-linked disease due to the deficiency of the lysosomal enzyme iduronate sulfatase (Id-S). Deficiency of Id-S results in lysosomal accumulation of glycosaminoglycans (GAG), with progressive tissue and organ dysfunction, and premature death. No definite treatment is available for MPS II patients. Adeno-associated virus (AAV) mediated gene delivery is a promising treatment for lysosomal storage disorders. We studied the therapeutic effects of combined intravenous (IV) and intracisternal (IC) injections of AAV2 vector expressing the human Id-S cDNA driven by a CMV promoter after pretreatment with mannitol in the MPS II mouse. We injected 2 X 10^{11} viral particles IV and 2 X 10^{10} viral particles IC after 1-2 mg of mannitol/gm body weight in 22 MPS II mice at 4 to 6 weeks of age. AAV2 treated MPS II mice were scarified when they developed severe neurological symptoms between 15 to 23 months of age. The AAV treated mice had complete correction of GAG accumulation in liver ($P<0.01$) and partial correction in spleen, heart, muscle, and lung ($P<0.05$). Id-S enzyme activity was detected in the liver of treated MPS II mice from 50% to 120% of that in normal mice. Histopathology and transmission electron microscopy studies show clearance of lysosomal storage in liver. Decreased CNS lysosomal storage was shown by histopathology in the brain. Id-S enzyme was detected in the brain of the AAV injected mice (1%-7% of normal). MPS II mice after AAV treatment had improved physical features and rotarod performance. The life span of the MPS II mice after the AAV gene therapy was prolonged (means 17.8 months), compared with the life span of the non-treated group (means 13.4 months) ($p<0.01$). These results suggest that IV combined with IC injections of AAV2 vector following mannitol pretreatment is a promising approach for treating both somatic and CNS disease in lysosomal storage disorders.

Acute presentation of beta ketothiolase deficiency- Should PALS take into consideration metabolic disorders? *L. Lukose*[1], *T. Markello*[2] 1) Medical Genetics, National Human Genome Research Institute, Bethesda, MD; 2) Division of Genetics and Metabolism Children's National Medical Center 111 Michigan Avenue NW Washington DC.

We present a case of beta ketothiolase deficiency with significant morbidity. A previously healthy 12-month-old female infant developed malaise from a viral illness. Newborn screening (not extended) was negative. She presented to a rural ER with vomiting, listlessness, fever, poor oral intake, and labored breathing. Arterial blood gas analysis and electrolytes were suggestive of metabolic acidosis with an anion gap. Serum ammonia was minimally elevated. Urinalysis revealed ketones. There was tachycardia, but normal oxygen saturation. The patient was managed for shock by current Pediatric Advanced Life Support (PALS) protocol. Urine organic acid analysis later revealed elevated tiglylglycine levels and elevated 2- methyl 3- hydroxybutyric acid. During initial management, she did not receive glucose in the intravenous fluids. Intravenous bicarbonate was given. She was treated for persistent acidosis with hemodialysis. During dialysis she had a cardiopulmonary arrest and later developed a femoral artery thrombosis. After resuscitation she was transferred to a tertiary care facility where she had an unremarkable clinical course. Beta ketothiolase deficiency was later confirmed by enzyme analysis on skin fibroblasts. This case illustrates a typical presentation of beta ketothiolase deficiency. This patient may have benefited from earlier consideration of metabolic disorders in the differential diagnosis. As expanded newborn screening identifies more infants with metabolic disorders, consideration to include an alternative management in PALS might avoid inappropriate care. PALS guidelines do include a consideration of reversible causes (including metabolic disorders). Subsequent steps in the algorithm, including a recommendation for the co-administration of intravenous glucose will improve the outcome of patients with metabolic disorders. While rapid consultation with metabolic specialists is always desirable, initial training of first responders may also contribute to improved care.

Inter-population linkage disequilibrium (LD) patterns of *GABRA2* and *GABRG1* genes at the GABA cluster locus on human chromosome 4. *C. Ittiwut[1,2,3], J. Listman[4], R. Hirunsatit[1,2,3], A. Mutirangura[1], R. Malison[2], J. Covault[5], H. Kranzler[5], A. Sughondhabirom[6], N. Thavichachart[6], J. Gelernter[2,3]* 1) Inter-Dept. Program of Biomedical Science, Chulalongkorn U., Thailand; 2) Dept. of Psychiatry, Yale School of Medicine, CT; 3) VAMC, West Haven, CT; 4) Dept. of Anthropology, New York U., NY; 5) Dept. of Psychiatry, U. of Connecticut School of Medicine, CT; 6) Dept. of Psychiatry, Chulalongkorn U., Thailand.

The *GABRA2* locus has been found to be associated with alcohol dependence (AD) in several studies, but no functional variant that can account for this association has been identified to date. In order to understand the reported associations, it is important to understand LD patterns and haplotype structure of this gene; extending this understanding to multiple populations would allow further inferences about the evolutionary history of a variant that increases AD risk. The *GABRA2* and *GABRG1* genes, encoding subunits of the GABA-A receptor, are located within the GABA cluster on chromosome 4p. As these genes are close to one another (intergenic distance, ~90 kb), it was anticipated some markers might show LD between them. To study LD architecture in 5 different populations (European American, African American, Chinese (Han and Thai), Thai, and Hmong), we genotyped 13 SNPs, 7 of which map to *GABRA2* and the other 6 of which are located within and flanking *GABRG1*. Haplotype structures were determined by Haploview v3.2 software and compared between populations. Variations in haplotype block structure were observed between populations; the most extreme variation being seen in the Hmong, which showed considerably higher LD than any other population. In Hmong, high LD (D>0.85) extended from rs1497571 in intron 7 of *GABRG1* to rs279837 in intron 3 of *GABRA2*, a 280 kb sequence, whereas in other populations, there were two or more LD blocks across this region. This LD break observed in other populations corresponds to lower D between rs10033451 and rs567926, which are located 89 kb apart in an intergenic region. These findings may aid in understanding genetic association of this locus with alcohol and drug dependence in several populations.

Recurrence of a multiple congenital anomaly syndrome: value of fetal MRI in gaining patient acceptance. *M. McCoy[1], S. Bhatt[1], D. Krakow[2], R. Sinow[3]* 1) Genetics, Genzyme, Orange , California; 2) Cedars-Sinai Medical center, Los Angeles, California; 3) Magella Medical, Torrance, California.

We present a case of a 29 year old G4P2012 female, who was referred for genetic counseling and ultrasound at 17 3/7 because of a prior son with multiple anomalies: developmental delay, seizure disorder, polymicrogyria, agenesis of the corpus callosum, VSD, bilateral hearing loss, GU anomalies, dysmorphic ears, and eleven ribs. Karyotype analysis was normal and no diagnosis had yet been made. 2D and 3D ultrasound of the current pregnancy revealed a male fetus with a VSD, absent cavum septum pellucidum, ventriculomegally (11 mm), small, posteriorly rotated ears, flat nasal bridge, and micrognathia. Amniocentesis was performed and revealed a 46, XY karyotype and normal FISH for 22q11 deletion. Because of the presence of fetal brain abnormality and similar structural malformations observed in this fetus, the family was counseled regarding a recurrence of the syndrome affecting their older child. The couple had difficulty accepting the fetal findings. To confirm the findings, a fetal MRI was then performed and which confirmed dysgenesis of the corpus callosum and ventriculomegaly. After review of the MRI with the parents they were more accepting of the recurrence and chose not to continue the pregnancy. The family elected against fetal autopsy. The experience with this case suggests that fetal MRI can be an important adjunct to ultrasound in helping parents accept the presence of major congenital anomalies and aid in critical decision making.

Genome Wide Association (GWA) Studies: Performance experience with the Illumina HumanHap300 Genotyping Beadchips in a high-throughput setting. *K. Hetrick[1], C. Bark[1], E. Kwasnik[1], J. Gearhart[1], J. Romm[1], M. Zilka[1], C. Ongaco[1], A. Robinson[1], J. Goldstein[1], R. King[1], L. Watkins[1], M. Barnhart[1], B. Craig[1], M. Boehnke[2], E. Pugh[1], K. Doheny[1]* 1) Center for Inherited Disease Research (CIDR), IGM, JHUSOM, Baltimore, MD; 2) Dept. of Biostatistics, Ann Arbor, MI.

CIDR is a centralized facility established to provide genotyping and statistical genetics services for investigators seeking to identify genes that contribute to human disease. Finland-United States Investigation of NIDDM Genetics (FUSION) case-control samples were assayed using Illumina Infinium II HumanHap300 Beadchips as part of our evaluation process for choosing a GWA platform for CIDR. Here we present quality metrics of genotypes generated with NO MANUAL data review using the Illumina-provided cluster definition file and BeadStudio v. 2.3.25. Of the 2,608 samples attempted, 2,576 (99%) were successful (call rate >98%) after an initial attempt. 25 (78%) of the initial failures succeeded when repeated. Average call rate was 99.7%, within-dataset replicate reproducibility rate for 52 independent pairs was 99.994%, and the parent-parent-child Mendelian consistency rate for 15 independent trios was 99.98%. For 41 CEPH samples (17 unique), the concordance rate with HapMap genotypes was 99.83%. Sample processing was tracked with a LIMS developed at CIDR which assured correct pairing of DNA sample to BeadChip and validated workflow and reagent use at each step. In addition to using BeadLab Tecan robots for assay automation, existing Beckman Coulter Biomek 2000 and Perkin Elmer Multiprobe II PLUS robots automated portions of the assay, increasing throughput over currently available Illumina protocols. An Illumina BeadScan software modification resulted in a 38% decreased scan time of version 1.0 BeadChips with a 99.998% reproducibility rate for chips scanned in both modes and a reduction in average call rate of 0.13%. Tools developed for daily data QC include Spotfire DecisionSite 8.1 templates for trend monitoring and a gender check program. This pilot projects success allowed us to offer the Infinium products as a GWA genotyping service to investigators to research the genetic basis of complex human diseases.

Genomic analysis of sentinel lymph nodes: in search of predictive markers of breast cancer metastasis. *B.R. Haddad[1], S.L. Santos[2], E.M. Ribeiro[2], J.D. Rone[1], C.A. Urban[3], R.S. Lima[3], I.J. Cavalli[2], L.R. Cavalli[1]* 1) Georgetown Univ. Medical Ctr., Washington, DC; 2) Federal University of Parana, Curitiba, Brazil; 3) Hospital Nossa Senhora das Gracas, Curitiba, Brazil.

Despite significant improvement in our understanding of the mechanisms of breast cancer development and progression, accurate prediction of the potential for future metastasis of non-metastatic primary tumors remains illusive. We set out to determine new predictive markers to augment the current criteria used to assess prognosis. The sentinel lymph node (SLN) is the first node to harbor malignant cells in breast tumors with metastasis. Because genetic alterations in the SLN lesions are likely to represent early and significant changes in the process of metastasis, characterization of such changes may aid in the discovery of new prognostic markers. Towards this end, we initiated this study to compare genomic alterations between primary breast tumors and their corresponding sentinel lymph node metastatic lesions using CGH analysis. Here, we report our results from the analysis of the 1st fourteen cases (paired samples). Although the number of cases analyzed so far is too small for statistical analysis, an interesting trend emerged: i) chromosomal abnormalities were observed in all 14 cases studied in both the primary tumors and the SLN lesions; ii) despite the diversity in the alterations detected in each case, both the primary and metastatic lesions shared a small number of alterations [gains (+) and losses (-)], specifically: -1p31~p21, +17, +19 and +20; iii) the above four alterations were also the most commonly observed in the whole set; iv) gain on chromosome 20 was more frequently observed in the primary tumors, whereas losses on 1p31~p21 and gains on 17 and 19 were equally observed in both groups. This study will allow assessment of clonal divergence and genetic heterogeneity that characterize the metastatic process and identification of a subset of relevant genetic alterations associated with metastasis. These can potentially be used as additional markers to predict metastasis thus reducing the need for invasive surgical procedures (e.g. axillary lymph node dissection).

Detecting signals of selection on standing variation. *S. Kudaravalli, X. Wen, J.K. Pritchard* Department of Human Genetics, University of Chicago, Chicago, IL.

A benefit of large scale genotyping efforts such as HapMap is that they allow us to explore evolutionary patterns across the entire genome, including recent positive selection. We have identified several regions that are putative targets of natural selection[1]. Several other reports have also explored the human genome for evidence for recent positive selection. An assumption underlying such studies is that an episode of selection is initiated when a selectively advantageous allele arises due to mutation and sweeps to high frequency. Statistics that are currently used to characterize signatures of selection are most powerful when a new allele is immediately advantageous. However, an allele that is already segregating in the population (standing variation) may also be a target of selection [2,3,4,5]. But standard tests for selection do not fare well in detecting such episodes[4,5]. By examining unusual patterns of linkage disequilibrium we aim to identify regions that are targets of selection on standing variation in the human genome. Analysis of simulations shows that our method has appreciable power in detecting such episodes of selection.

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Localization of psoriasis susceptibility gene PSORS4 to the LEP/SPRR cluster of genes within the epidermal differentiation complex (EDC) on chromosome 1q21. *C. Helms[1], J. Robarge[1], K. Gold[1], S. Duan[1], R. Donaldson[1], P. Stuart[2], R. Nair[2], J. Elder[2], A. Bowcock[1]* 1) Genetics, Washington Univ Sch Medicine, St Louis, MO; 2) Dermatology, Univ Michigan Med Sch, Ann Arbor, MI.

Psoriasis is a complex inflammatory disease of the skin affecting 1-2% of the Caucasian population. It is accompanied by an increase in epidermal proliferation, recruitment of inflammatory cells to the dermis and epidermis, and a disruption of the cornified envelope; a structure at the surface of the skin that forms a barrier to the environment, preventing water loss and guarding against infection by pathogens. Although a major determinant for psoriasis lies within the histocompatibility locus antigen cluster (HLA) on chromosome 6p21, additional loci have been identified with genome-wide linkage scans. One of these maps to the epidermal differentiation complex (EDC); a cluster of over 80 genes on chromosome 1q21 (PSORS4). We have evaluated 250 psoriasis trios for association to this region, with a set of 136 tag SNPs spanning the interval. Two peaks of association were identified where $p < 0.001$ with both single and multilocus transmission disequilibrium (TDT) analyses. These associations were confirmed in a second sample of 535 psoriasis families of various structures, where $p < 0.01$ was observed for both peaks with single locus pedigree disequilibrium (PDT) analysis. One is in a ~75kb interval harboring the late envelope proteins (LEPs). A second is a ~75kb region harboring the small proline rich proteins (SPRRs). Genes encoding these proteins are expressed late in epidermal differentiation and are involved in the formation of the cornified envelope. Resequencing of the associated intervals in individuals with risk- and non-risk haplotypes revealed variants that are being considered for their role in psoriasis susceptibility. Some variants are those typically seen in clusters of gene family members and include gene deletion and gene conversion events. The mapping of PSORS4 to a genomic interval involved in epidermal barrier formation suggests that an alteration in terminal differentiation of keratinocytes may be triggering increased proliferation in the basal layer.

Mutations in the leucine zipper domain of ORF1p adversely affect L1 reverse transcription. *A.E. Hulme, D.A. Kulpa, J.N. Athanikar, J.V. Moran* Dept. of Human Genetics, University of Michigan, Ann Arbor, MI.

The non-LTR retrotransposon LINE-1 (L1) comprises ~17% of human DNA. A retrotransposition competent L1 contains a 5' UTR, two non-overlapping reading frames (ORF1 and ORF2), and a 3' UTR that ends in a poly (A) tail. ORF2 encodes a protein (ORF2p) with endonuclease (EN) and reverse transcriptase (RT) activities that are required for retrotransposition. ORF1 encodes a protein (p40 or ORF1p) that co-localizes with L1 RNA and ORF2p in cytoplasmic ribonucleoprotein particles (RNPs), which are likely retrotransposition intermediates. The N-terminus of ORF1p mediates multimer formation and contains a predicted coiled coil domain, whereas the C-terminus contains conserved amino acids needed for RNA binding. Human ORF1p also has a putative leucine zipper (LZ) domain near its N-terminus that is required for L1 retrotransposition, but is dispensable for ORF1-ORF1 protein interaction.

Here, we have characterized the LZ domain with respect to L1 RNP formation and function. Mutations in the LZ domain did not affect overall L1 RNA levels, but resulted in less ORF1p in cytoplasmic RNPs when compared to a wild type L1. ORF2p translation was not affected by LZ domain mutation, as assayed in a trans-complementation assay. Therefore, we next examined LZ mutant RNPs for ORF2p RT activity, using a biochemical assay. Surprisingly, mutation of the LZ domain decreased RT activity and seemed to adversely affect the initiation of reverse transcription. This decrease in activity could not be attributed to simply less ORF1p as an ORF1 RNA binding mutant that does not localize to RNPs has robust RT activity. Thus, our data suggest that the LZ domain is needed for proper RNP formation and robust reverse transcription, both of which are critical for L1 retrotransposition.

Gene Expression Profiling in CD4+CD25+high T cells from a recent-onset Type 1 Diabetic subject reveals reduced expression of HLA genes. *P. Jailwala, S. Glisic-Milosavljevic, J. Waukau, S. Jana, J. Rovensky, L. Meyer, M. Hessner, S. Ghosh, V. Magnuson* Department of Pediatrics, Medical College of Wisconsin, Wauwatosa, WI.

Type 1 diabetes (T1D) is a T cell-mediated autoimmune disease leading to the destruction of pancreatic insulin-producing beta cells. Genetic studies suggest T1D is also an oligogenic disease, the major predisposing region mapping to the HLA locus, that harbors over 125 expressed genes. Naturally occurring CD4+CD25+high T cells, which reduce effector CD4+CD25- T cell function both in vitro (known as suppression) and in vivo, may be defective at controlling autoimmunity in T1D. FoxP3, a transcription factor, is highly expressed in CD4+CD25+high T cells. We hypothesized that HLA and other susceptibility genes could act at the level of CD4+CD25+high T cells. We carried out gene expression profiling of CD4+CD25+high cells isolated by Fluorescence Activated Cell Sorting (FACS) from a recent-onset T1D patient and a Control subject, using Affymetrix human genome U133 plus 2.0 GeneChip. Data were filtered and normalized using standard software. The most striking difference was in expression of HLA genes. Of the 30 HLA Class II genes, 24 had reduced expression, while all of the 32 HLA-Class I genes had reduced expression in the T1D subject. The recent-onset T1D subject showed weak CD4+CD25+high T cells suppressive activity (48%) compared to the control subject (78%). Intracellular staining for FoxP3 protein was also reduced (11.8% vs 28.6%). An overall reduction in HLA gene expression along with reduced function of the CD4+CD25+high T cells could potentially implicate abnormal antigen-specific interaction between effector CD4+CD25- and regulatory CD4+CD25+high T cells in the pathogenesis of disease. After confirming gene expression results in 5 more T1D-control pairs, we plan to find a polymorphism in a transcription factor that regulates overall HLA gene expression and is associated with T1D in over 230 families.

Investigating the Role of the Transcription Factor FEV in Response to the Antidepressant Citalopram Utilizing a Large Clinical Sample. *J. Kraft[1], E. Peters[1], S. Slager[2], G. Jenkins[2], M. Reinalda[2], P. McGrath[3], S. Hamilton[1]* 1) Dept Psychiatry/Biopharm Sci, University of California, San Francisco, San Francisco, CA; 2) Division of Biostatistics, Mayo Clinic College of Medicine, Rochester, MN; 3) New York State Psychiatric Institute and Columbia University.

The large inter-individual variability seen in patient response to antidepressant medication is thought to be influenced at least in part by DNA variation. Selective Serotonin Reuptake Inhibitors (SSRIs) act by antagonizing the serotonin transporter; however, the delay in therapeutic response suggests other mechanisms or regulatory events are necessary for a response phenotype to occur. Specifically, we are interested in the role that the transcription factors FEV plays in antidepressant response. Recent studies done on the mouse homolog, Pet-1, suggest this transcription factor plays a large role in the expression of several genes in the serotonin signaling pathway (TPH, MAOA, SERT, etc.).

In order to uncover unknown variants and select tagging SNPs, we resequenced all exons, intron-exon boundaries, and 5 conserved non-coding sequence of the gene FEV in a subset of 95 individuals from our larger dataset. We discovered 5 previously undiscovered SNPs in FEV. Then from all of our identified SNPs, we chose tagging SNPs, which are currently being genotyped in a larger sample (N=1,953) of patients with major depression who took the SSRI citalopram. This sample is a subset of a study investigating treatment options for patients with major depression (Sequenced Treatment Alternatives to Relieve Depression, STARD). Sequencing and association results from our study will be presented.

Association between genes on Chr 4p16 and Nonsyndromic Oral Clefts in 4 Populations. *R.G. Ingersoll[1,2], J.B. Hetmanski[1], J.W. Park[1], M.D. Fallin[1], I. MacIntosh[3], E.W. Jabs[2], C. VanderKolk[2], Y. H. Wu-Chou[4], P.K. Chen[4], V. Yeow[6], S.S. Chong[5], F. Cheah[5], J.W. Sull[7], S.H. Jee[7], A.F. Scott[2], T.H. Beaty[1]* 1) Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 2) Johns Hopkins School of Medicine, Baltimore, MD; 3) American University of the Caribbean, St. Maarten, Netherlands Antilles; 4) Chang Gung Memorial Hosp, Taoyuan, Taiwan; 5) KK Women's & Children's Hosp, Singapore, Singapore; 6) National University of Singapore, Singapore, Singapore; 7) Yonsei University, Seoul, Korea.

Isolated cleft lip with/without palate (CL/P) and cleft palate (CP) are among the most common human birth defects. Linkage studies have consistently found a relationship between CL/P and chromosome 4p16. One gene in the region, MSX1, has been found to contain different mutations and may account for a small percentage of non-syndromic CL/P. These mutations, however, cannot explain the strength and consistency of the signal in this region. To map this region, 393 SNPs were selected across a 2 Mb region surrounding MSX1, with an average spacing of 1 SNP per 5 kb. The region spans 18 genes in NCBI genome build 35. These SNPs were genotyped using the Illumina platform, on 383 case-parent trios from 4 populations (Taiwan, Korea, Singapore and Maryland). The 67 cases of CP trios from Taiwan, Singapore and Maryland were analyzed separately. TDT analysis using both individual SNPs and sliding haplotype windows of size 2-5 SNPs each showed 19 genes or intergenic regions yielding suggestive evidence of linkage and disequilibrium among CL/P trios in >1 populations, as defined by empirical p-values. Two genes (STK32B and EVC) and an intergenic region yielded consistent evidence in all 4 populations, while EVC2 and STX18 were statistically significant in 3 of 4 populations. Eighteen genes or intergenic regions gave suggestive evidence from the CP trios in >1 populations. Three genes (STK32B, EVC2 and EVC), 2 putative genes (LOC391613 & LOC402162), and 2 intergenic regions were suggestive in CP trios from all 3 populations. This analysis confirms the etiologic importance of genes in this region, but indicates MSX1 may not be the only influential gene here.

Genetic regulation of vitamin D in a cohort of multiple sclerosis twins. *S. Orton[1], B. Herrera[1], M. Lincoln[1], S. Ramagopolon[1], M. Chao[1], K. Morrison[1], R. Vieth[2], G.C. Ebers[1], Canadian Collaborative Project on Genetic Susceptibility to MS (CCPGSMS)* 1) Clinical Neurology, University of Oxford,UK; 2) Pathology and Laboratory Medicine, University of Toronto, Canada.

Vitamin D deficiency has been implicated in several autoimmune diseases, including multiple sclerosis (MS). Levels of 25-hydroxy-vitaminD (25OHD) are deemed to be the best measure of vitamin D health and are believed to be determined predominantly by environmental factors, primarily UV exposure. However, recent evidence suggests that genetic factors are also important and their effects would be most evident when environmental influence is marginal, such as wintertime. Published studies considering the genetic regulation of vitamin D metabolism are scarce and more systematic studies are needed. Twin study designs offer one route for assessing relative contributions of genetic factors affecting a trait. The first objective was to consider the relative genetic contribution to variance of serum 25OHD levels. Secondly, as our dataset consisted of MS patients, we asked whether a relationship between 25OHD and MS risk could be observed. The sample was drawn from the CCPGSMS and included 39 monozygotic (MZ) and 61 dizygotic (DZ) twin pairs, with a mean age of 53 years and 83 pairs discordant for MS. We collected serum at the end of winter to minimize environmental impact on levels, namely UV exposure, and collected data on use of supplements, tanning beds and recent vacations. Radioimmunoassay was used to measure serum 25OHD (overall mean = 78 nmol/L). Applying logistic regression with robust variance estimators, we did not find any significant association between 25OHD and MS risk. Gender and age were also not associated with levels in our sample. The correlation coefficient for 25OHD in MZ twins ($r=0.72$, $p<0.000$) was significantly higher than that of DZ twins($r=0.34$, $p=0.003$) and heritability was estimated at 0.77. Although there was no significant association between 25OHD levels and MS status observed, we can not rule out the possibility that such a relationship exists. The findings suggest that 25OHD levels are under genetic regulation and further investigation in family-based datasets is warranted.

Single Cell Aneuploidy Analysis by High Resolution Comparative Genomic Hybridization (CGH) after Whole Genome Amplification reveals Maternal Cell Contamination in the Original Products of Conception Culture. *O. Nahum[1], V. Jobanputra[2], K. Hirschhorn[1], B. Levy[2]* 1) Depts of Human Genetics and Pediatrics, Mount Sinai School of Medicine, New York, NY; 2) Dept of Pathology, Columbia University Medical Center, New York, NY.

We performed high resolution CGH analysis on a single cell extracted from a culture set up from tissue obtained from the products of conception (POC) of a spontaneously aborted pregnancy. Whole genome amplification (WGA) using a random fragmentation approach (GenomePlex - Sigma-Aldrich) was utilized in order to obtain a sufficient amount of DNA for the CGH analysis. The culture was de-identified and blinded (with respect to the cytogenetic diagnosis) before being transferred from the clinical laboratory to the research laboratory. The POC culture was part of a larger IRB approved study assessing aneuploidy analysis of single cells. Single cells were lysed and then subject to WGA. The resulting DNA was labeled using nick-translation and high resolution CGH was performed to determine the chromosomal status of the single cells. CGH results were compared to the karyotype as determined by conventional cytogenetic analysis. Following CGH analysis, a normal female karyotype was predicted. However, when compared back to the original karyotype (47,XY,+16), both the sex and diagnosis were incorrect. Our recent experience with single cell aneuploidy analysis has been very favorable and we therefore considered maternal cell contamination as a feasible explanation for the discrepancy. Interphase FISH using probes specific for the X and Y chromosomes was performed on the original POC culture and, in addition to a small number of XY cells, revealed a high number of XX cells consistent with our hypothesis. Also, DNA extracted from the original culture confirmed the presence of maternal cell contamination. This case illustrates the potential of single cell aneuploidy analysis but highlights the dangers of a diagnosis based on a single cell. This is particularly applicable to preimplantation genetic diagnosis where mosaicism is an issue and fetal cells from maternal circulation where maternal cell contamination is a problem.

Null evidence for linkage of familial breast and colon cancer to CHEK2. *H. Ochs-Balcom[1,2], D. Daley[1, 3], R. Elston[1,2], G. Wiesner[2,4]* 1) Department of Epidemiology & Biostatistics, Case Western Reserve University, Cleveland, OH, USA; 2) Ireland Comprehensive Cancer Center at University Hospitals of Cleveland and CWRU, Cleveland, OH, USA; 3) The James Hogg-iCAPTURE Centre for Cardiovascular and Pulmonary Research, University of British Columbia, St. Pauls Hospital, Vancouver, British Columbia, Canada; 4) Department of Genetics and Center for Human Genetics, CWRU, Cleveland, OH, USA.

CHEK2, a gene important in the cell cycle and DNA repair, is hypothesized to be important in breast cancer etiology and perhaps also in other cancers. Families with both breast and colon cancer have been reported to have mutations in the CHEK2 gene, leading to the hypothesis that this gene may be a susceptibility factor for both of these cancers. A total of 159 sibling pairs in 33 nuclear families, each containing two cases of advanced polyps or colon cancer and at least one primary breast cancer in a sibling or mother, were genotyped for 6 markers on chromosome 22. Model-free linkage analysis was performed to determine whether the CHEK2 region is linked to familial breast and colon cancer. Allele sharing identical by descent for concordantly affected sibling pairs at D22S689, 0.02 cM centromeric of CHEK2, was 0.47 (SE 0.05; p=0.76), and at D22S685, 3.74 cM telomeric of CHEK2, was 0.46 (SE 0.05; p=0.77). Similarly, there was no statistically significant evidence for linkage using Haseman-Elston regression. The study had 90% power at the 5% level to detect a locus-specific sibling relative recurrence risk of 1.4 and therefore suggests that CHEK2 is an unlikely susceptibility locus in kindreds with breast and colon neoplasia. We further plan to investigate epistatic interaction of CHEK2 with BRCA1 and 2 because both are candidate genes for breast cancer that may jointly influence risk.

Feasibility of neonatal screening of lysosomal tripeptidyl peptidase 1 (TPP1) for classical late-infantile neuronal ceroid lipofuscinosis (NCL2). *Y. Huang[1,2], W. Ju[1], N. Zhong[1,2]* 1) New York State Institute for Basic Research , Staten Island , NY; 2) Peking University Center of Medical Gnetics.

Neuronal ceroid lipofuscinosis (NCL) affects 1 per 20,000 to 100,000 in the general population with an incidence of 1 per 12,500 births in homogenous population. About 35% of NCL are late-infantile neuronal ceroid lipofuscinosis (LINCL). Among which, about 80% of LINCL are caused by a deficiency of the lysosomal tripeptidyl peptidase I (TPP I) that is encoded by CLN2 gene. Presently, little is known about the frequency of the affected and carriers in newborn children worldwide. To carry out a large neonatal screening for TPP1 deficiency, we have investigated the feasibility of existing approach of analyzing TPP1 activity. We analyzed a capillary electrophoresis (CE) procedure for TPP1 by using Ala-Ala-Phe-NA as a substrate. We optimized the pH condition to 4.5 and found that the maximal absorption is at wavelength of 380 nm, at which higher product peak with almost no nonspecific noise background can be obtained. The enzyme reaction volume can be scaled down to 25-50 ul, and only 0.5-1.0 ug cellular total protein is needed. Although this procedure is reliable and can be robotic, it is time consuming (at least 2.5 hours for each sample including 2 repeat and 1 blank) and frequently capillary can be easily got clogged by air bubbles. Using the same substrate, we also analyzed TPP1 activity with a microplate reader at 405 nm. Although this approach is efficient and economical, it is less sensitive and less reliable. In our case, the absorption of the digested NA product is quite low. Fluorescent method is much sensitive and reliable, more and more enzyme assays are using fluorescent substrate. We are currently optimizing the conditions using a fluorescent substrate of TPP1, which has been indicated to be suitable for large-scale screening, espacially when bloodspots, which have been used for other neonatal screening, are applied.

Profiling Segmental Polymorphisms in the Human Genome Using High Resolution Fosmid Arrays. *T.J. Jiang[1], W.L. Liu[1], S.S. Scherer[1,2], R.G. Gibbs[1,2], J.B. Belmont[1], P.H. Hixon[1], WW.C Cai[1]* 1) MH Genetics, Baylor college medicine, Houston, TX; 2) Human Genome Sequencing Center, Baylor College Medicine, Houston, Texas.

Segmental copy number variations have been found to be a common type of genome polymorphisms in humans since the introduction of Array-based Comparative Genomic Hybridization (aCGH) in profiling human genomes. Previous studies about segmental polymorphisms have been incomplete and preliminary because of limitations in aCGH resolution, data quality and sample size. Currently, the platforms for aCGH are mainly BAC arrays and oligo arrays. Large scale whole genome oligo arrays usually can only be obtained from commercial sources. The costs of these commercial arrays are still too high for large-scale applications. Whole genome tiling BAC arrays are relatively easier to make in an academic lab. The resolution (about 100-200kb) of BAC tiling path arrays is determined by the size of BAC clones and these arrays cover all euchromatic sequences in the genome. Drawing on our extensive experiences in fabricating whole genome human and mouse BAC arrays, we have developed whole genome human fosmid tiling arrays. Briefly, 85,062 clones were picked from human fosmid library WIBR-2 to form a tiling path human fosmid clone set. Clones were cultured in 96-well blocks, and DNA was extracted by protease-SDS lysis. Fosmid DNAs were chemically modified and spotted onto hydrophobic glass slides using high-precision silicon pins, which can uniformly print high density arrays with each spot size of 50 um in diameter. These whole genome human fosmid arrays contain 85,062 fosmid DNA clones printed on a single slide, with resolution of 40kb based on cloned insert size. We have used this high resolution fosmid array in analysis of 180 human samples from different populations. Novel and concordant segmental copy number variations (SCNV) have been found. More samples will be studied using this fosmid array to establish a more complete database on SCNV in the human genome.

Chromosomal deletion syndrome 1q22-25. *R.R. Lebel*[1, 2], *B.J. Shutt*[2] 1) Greenwood Genetic Center, Greenwood, SC; 2) Clemson University, Clemson, SC.

Prenatal ultrasonographic findings revealed major abnormalities, including apparent schizencephaly and cleft lip/palate. Amniocentesis was performed, and the karyotype reported by a reference laboratory as 46,XY. Multidisciplinary conference was convened for planning delivery and neonatal interventions (genetics, neonatology, neurosurgery, nursing, obstetrics, pastoral care, parents). The newborn was stable in the special care nursery. Dysmorphology examination revealed low birth length, microcephaly, bilateral facial clefting, malformed and low-set ears, cryptorchidism with inguinal hernia, small hands and feet, clinodactyly, hypotonia. These findings did not connote an apparently identifiable hereditary syndrome. Lymphocytes sent to a different reference laboratory revealed an interstitial deletion: del(1q22-q25). Review of the literature showed very few published cases of similar deletions, and that there is a consistent phenotypic pattern of dysmorphic features associated with poor prenatal and postnatal growth, and psychomotor developmental delays. The present case illustrates useful information about long-term survival with this syndrome.

Multi-SNP Genetic Risk Scores Increase Power to Predict Clinical Disease Phenotypes. *B.D. Horne[1,2], J.L. Anderson[1,3], J.F. Carlquist[1,3], J.B. Muhlestein[1,3], N.J. Camp[2]* 1) Cardiovascular Dept, LDS Hospital, Intermountain Medical Center, SLC, UT; 2) Genetic Epidemiology, Univ Utah, SLC, UT; 3) Cardiology, Univ Utah, SLC, UT.

Genetic association studies of common, complex diseases such as coronary artery disease (CAD) and myocardial infarction (MI) have produced unreliable results for both single nucleotide polymorphisms (SNPs) and SNP haplotypes, perhaps due to allelic and locus heterogeneity. We have described a genetic risk score (GRS) method that integrates SNP data with intermediate phenotypes (IPs) to derive a multi-SNP score for association testing with clinical phenotypes. In this study, the GRS method was applied to two data sets: 11 tagging (t) SNPs in the cholesteryl ester transfer protein (CETP) gene, with high-density lipoprotein cholesterol (HDL-C) as the IP, for association with CAD; and 8 SNPs in matrix metalloproteinase (MMP) and MMP inhibitor genes, with high-sensitivity C-reactive protein (hsCRP) as the IP, for association with MI. Association of SNPs with an IP used linear regression and the GRS was calculated from regression coefficients. In 6,285 patients with CETP tSNPs, the CETP-HDL GRS was associated with CAD (OR=1.32 [p=0.033], 1.44 [p=0.008], 1.05 [p=0.69], 1.20 [p=0.17], 1.24 [p=0.10], 1.32 [p=0.034], 1.20 [p=0.17], 1.35 [p=0.023], 1.16 [p=0.27] for deciles 1-9, respectively, vs. decile 10). Individually only a few tSNPs were nominally associated with CAD. Adjustment for age, sex, HDL-C, and CAD risk factors gave similar results. In 3,304 patients with MMP SNPs, the MMP-hsCRP GRS was associated with MI (OR=0.85 [p=0.17], 0.78 [p=0.029], 0.80 [0.061], 0.76 [p=0.017] for quintiles 2-5, respectively, vs. quintile 1). Analyzed individually, only MMP-9 predicted MI. Adjustment (including for hsCRP) marginally improved significance. The GRS method is multi-locus, allowing for sensible combination of SNPs in a single metric based on relevant IPs. In two different data sets the GRS approach increased power to detect association with clinical endpoints, confirming its potential in genomic risk evaluation. Further extension of the GRS approach to the genome-wide association study setting is suggested.

Novel assay for detecting integrated HPV. *P. McGrath, P. Moen, D. Hochberg, E. O'Lear* One Cell Systems, Inc., Cambridge, MA.

There is increasing consensus that integration of oncogenic HPV DNA into the host genome is the primary cause of cervical cancer and an assay that specifically detects viral integration would have high clinical utility. We are developing a method to detect integrated viral DNA after removing interfering episomal DNA. Using a model HPV cell line containing both episomal and integrated HPV-16 DNA, we developed experimental conditions for selectively removing episomal viral DNA without releasing integrated viral or cellular DNAs. Using in situ hybridization, the diffuse HPV DNA signal generated by episomal DNA present in control cells was absent in extracted cells. Conversely, hybridization signal from integrated viral DNA and an endogenous gene remained, indicating that the extraction conditions specifically removed protein-bound episomal DNA. Using a more sensitive PCR assay, we confirmed that, after extraction, the level of HPV DNA decreased in a cell line containing both episomal and integrated virus and remained essentially unchanged in two integrated cell lines. Successful application of this assay format to clinical samples will lead to precise detection of integrated HPV DNA and identification of patients who are likely to develop cervical cancer.

Delineation of a *de novo* 3q deletion in a newborn girl using Comparative Genomic Hybridization (CGH). *M.J. Macera[1], G. Kupchik[2], S. Krinshpun[2], D. Sulllivan[2], A. Babu[1]* 1) Div Molec Medicine & Genetics, Dept of Medicine, Wyckoff Heights Medical Ctr, Brooklyn, NY; 2) Dept Pediatrics, Maimonides Medical Ctr, Brooklyn, NY.

A newborn female was evaluated due to dysmorphic features. The family was known because of an older sibling with Trisomy 21. Amniotic fluid analysis performed elsewhere reported an apparently normal female karyotype. Fetal ultrasound at 17 weeks gestation was within normal limits. A follow-up fetal ultrasound at 32 weeks gestation revealed polyhydramnios, single umbilical artery and dilated ventricles. On physical exam after birth, her head circumference measured above the 90th percentile and she had widely spaced sutures, soft fontanels, hirsutism, a prominent occiput, bulbous nose, low set ears, triangular facies, down slanting palpebral fissures and prominent big toes. Cranial ultrasound indicated ventriculomegaly with extra axial space prominence in the frontoparietal areas. Renal ultrasound and echocardiogram disclosed bilateral hydronephrosis and atrial septal defect, respectively. Because of dysmorphic features, a high resolution chromosome analysis and FISH were preformed on peripheral blood that revealed a karyotype of 46,XX, del(3)(q22q25.1).ish del(3)(wcp3+). No hybridization signal was seen using whole chromosome paint 3 (wcp3), over any other chromosome besides homologues 3. Both parents had normal karyotypes. Comparative genomic hybridization (CGH) analysis revealed that the deleted segment is more distal than that originally designated by GTG-banding. The proximal breakpoint is at band q24 while the distal breakpoint is at the proximal end of band q26.1, therefore the patient is monosomic for 3q24q26.1. A 7-Mb critical region for the Dandy-Walker malformation has been mapped to 3q24 to q25.1. Initial MRI on our patient was not consistent with Dandy-Walker malformation. The 7-Mb Dandy-Walker associated region in our case only accounts for a small portion of the material missing. Accurate delineation of the breakpoints of deletion 3q is important to correlate segmental monosomies with clinical presentation. Such cases are rare in the literature and worth reporting.

Performance of Random Forests in Identifying SNPs Predictive of Phenotype when SNPs are in Linkage Disequilibrium. *Y. Meng[1], L.A. Cupples[1], C.T. Baldwin[1], R. Inzelberg[2], R.P. Friedland[3], L.A. Farrer[1], K.L. Lunetta[1]* 1) Boston University, Boston, MA; 2) Hillel Yaffe Medical Center, Israel; 3) Case Western Reserve University, Cleveland, OH.

Association studies for complex phenotypes produce genotypes on 100s-1000s of single nucleotide polymorphisms (SNPs). One approach to dealing with large numbers of SNPs is to screen the data using some criterion to rank SNPs for follow-up. Random Forests produce a single importance measure (IM) for each SNP that takes into account a SNPs main and interaction effects on an outcome without requiring model specification. Random Forests have been shown to be powerful for detecting independent interacting risk susceptibility SNPs (rSNPs). However, correlation between noise SNPs that do not affect disease risk (nSNPs) and risk SNPs may decrease IM for the rSNPs. We explore the performance of Random Forests in the presence of nSNPs correlated with the rSNPs.

Using simulation under several complex disease models with sib recurrence risk 2 and up to 32 interacting risk-associated SNPs (rSNPs), we show that in general, the Random Forest importance measure IM for rSNPs decreases with increasing number of nSNPs in LD with the rSNPs. The IM for rSNPs also decreases with increasing numbers of nSNPs in LD with each other, but at a slower rate. The ratios of IM(rSNP) to $\max_{nSNP}$ IM(nSNP) also decrease with increasing number of nSNPs in LD with the rSNPs. The probability that IM(rSNPs) exceeds the maximum of independent nSNPs shows a similar trend. The probability that all rSNPs are among the top-ranking SNPs decreases with increasing number of nSNPs in LD with the rSNPs.

We conclude that performance of the Random Forest method in assigning higher ranks to all rSNPs than to independent nSNPs decreases when nSNPs are in LD with the rSNPs. Importantly, the inclusion of nSNPs in LD with the rSNPs actually increases the probability that all rSNPs will be retained for further analysis. We illustrate the application of Random Forests with a data set of Alzheimer cases and unaffected controls genotyped at about 300 SNPs in candidate genes.

A novel bioloop strategy for labeling synthetic oligonucleotide probes. *E. O'Lear, P. Moen, D. Hochberg, J. Trnovsky, P. McGrath* One Cell Systems, Inc., Cambridge, MA.

We have developed a new strategy for labeling synthetic oligonucleotide fluorescence in situ hybridization (FISH) probes. This procedure, termed Bio-Loop labeling, involves synthesizing oligonucleotides (ODNs) to contain multiple labels in a region of the probe not involved in hybridization to a target sequence. Our experimental data shows that BioLoop labeled ODNs generate a 2-fold higher signal to noise ratio than conventional end-labeled oligonucleotide probes and hybridization specificity is identical. Here we present assessment of the optimum BioLoop construct and results using BioLoop labeled ODN probes for several infectious diseases, including HPV, HIV and EBV. This labeling strategy overcomes blocking DNA labeling intellectual property and provides a new approach for FISH as well as other molecular biology technologies that rely on nucleic acid labeling and probing.

Comparison of linkage disequilibrium patterns between a cohort from Cebu, Philippines and the four populations genotyped by HapMap. *A.F. Nave, L.A. Lange, E.M. Lange, Y. Wang, L.S. Adair, K.L. Mohlke* U. North Carolina, Chapel Hill, NC.

Patterns of linkage disequilibrium (LD) differ between populations. Although the International HapMap Project has identified patterns of LD within the human genome in four populations, the similarity of these patterns to other unstudied populations is not fully known. In the current study, we assessed the value of HapMap data to select tag SNPs for a cohort from Metro Cebu, in the central Philippines. We first re-sequenced 800 nucelotides from each of the ten HapMap ENCODE regions in 24 individuals to determine whether common SNPs in Filipinos from Cebu are under-represented in SNP databases. We identified only one new SNP (minor allele frequency (MAF)= .05) not already present in dbSNP or HapMap, suggesting that SNP discovery will not be critical prior to future SNP selection. We then selected polymorphic SNPs from the CHB, JPT, and CEU HapMap data in 40 kilobase regions within each of the ten HapMap ENCODE regions. We successfully genotyped 501 polymorphic SNPs in 80 individuals using the Illumina GoldenGate genotyping assay. Of these, 467 SNPs were also present in the HapMap CHB, JPT, and CHB+JPT samples with an average of 47 SNPs in each ENCODE region (range 26 to 77); 480 and 465 SNPs were also present in the HapMap CEU and YRI samples, respectively. We compared allele frequencies between Filipinos from Cebu and the HapMap samples for SNPs in all ten ENCODE regions and observed average Pearsons correlations of .91, .90, .86, .72, and .60 in CHB, CHB+JPT, JPT, CEU, and YRI, respectively. Differences were observed across the ten regions, however no obvious patterns were detected. We evaluated LD patterns by comparing pairwise r^2 values between Filipinos from Cebu and HapMap samples for SNPs with MAF .1. We observed Spearmans correlations of .81, .82, .81, .66, and .54 in CHB, CHB+JPT, JPT, CEU, and YRI, respectively. The strong correlations between the Filipinos from Cebu, CHB and CHB+JPT analysis panels suggest that using HapMap data will be appropriate and useful for selecting tag SNPs in the Cebu cohort.

Genetic analysis of hypertrophic cardiomyopathy genes in noncompaction cardiomyopathy patients: could NCCM be a phenotypic variant of HCM? *Y.M. Hoedemaekers[1,2], K. Kaliskan[2], F.J. ten Cate[2], D.F. Majoor - Krakauer[1], D. Dooijes[1]* 1) Dept Clinical Genetics, Erasmus Medical Ctr, Rotterdam, Netherlands; 2) Thoraxcenter, Erasmus Medical Ctr, Rotterdam, Netherlands.

Background: Noncompaction cardiomyopathy (NCCM) is characterized by abnormal segmental thickening of the left ventricular wall with deep, prominent intertrabecular recesses, as visualised by two dimensional echocardiography or MRI. Clinical manifestations include heart failure, lethal arrhythmias and/or thrombo-embolic complications, mostly affecting patients at a relatively young age. It is a major cause of paediatric cardiomyopathies. NCCM is genetically heterogeneous and can be inherited as an autosomal dominant (AD) or X-linked disorder in familial cases. DNA variants in familial AD NCCM have been identified in genes coding for cytoskeletal or cell junction proteins: LMNA/C, -dystrobrevin and Cypher/ZASP. In families with X-linked NCCM an association was found with mutations in the G4.5 gene, which is allelic with Barth syndrome. Due to a similarity in morphology, the aetiology of NCCM is thought to be caused by an arrest of normal intrauterine endomyocardial development and a lack of compaction of the loose myocardial meshwork. **Hypothesis:** NCCM may be a phenotypic variant of hypertrophic cardiomyopathy (HCM) in some cases. To investigate this, we performed a genetic analysis of known HCM genes in a group of 23 isolated and familial adult NCCM patients. We report one NCCM family with a mutation in the Myosin Binding Protein C (MYBPC3) gene and one family where NCCM is associated with the -Myosin Heavy Chain (MYH7) gene. Mutations in the MYH7 and the MYBPC3 genes are a well known cause of AD HCM. **Results:** In three patients with NCCM in one family, we identified a D545N/D955N double missense mutation, in cis, in the MYH7 gene. The second family revealed the 2373insG mutation in the MYBPC3 gene, which is a founder mutation in the Dutch population, in a patient with NCCM and in her father, who was diagnosed with dilated HCM. **Conclusion:** Sarcomeric genetic defects in NCCM and the co-occurrence of NCCM and HCM within one family suggest, at least in some cases, a common aetiology.

A novel 300bp Alu insertion in MSH2 causes Lynch Syndrome. *M. Hegde, L.H. Chin, P. Ward, B. Roa, C. Eng* DNA Diagnostic Laboratory,Medical Genetics Laboratories, Baylor Col Medicine, Houston, TX.

Lynch syndrome or hereditary nonpolyposis colorectal cancer (HNPCC) is the most common hereditary cause of colorectal cancer. HNPCC is caused by mutations in DNA mismatch repair genes, with majority of mutations found in the MLH1 and MSH2 genes. Approximately 25% of these mutations can be identified by dosage-sensitive methods for large deletions or duplications, and the remainder are detectable by DNA sequencing. We describe a novel insertion mutation of ~300 bp with Alu repeat homology (1676_1677ins~300bp) in exon 11 of the MSH2 gene. This insertion was detected by PCR and sequencing analysis on a 52 year old Caucasian male with sebaceous adenomas and a family history of gynecological cancer. He was referred to our laboratory for MSH2 gene sequencing and deletion/duplication analysis, subsequent to immunohistochemistry analysis that showed absent MSH2 and MSH6 expression. MSH2 dosage analysis by quantitative Real-Time PCR did not detect any gross gene rearrangements. Concurrently, PCR-based sequence analysis of all 16 coding exons of MSH2 was performed; PCR amplification of exon 11 showed a novel product of ~ 600bp in addition to the 291bp wild type product, which suggested a heterozygous insertion. These bands were individually gel purified and sequenced. The smaller product showed the wild type sequence. The larger product showed an insertion of ~300bp with Alu repeat homology, with 15bp of MSH2 exon 11 flanking the insertion. Our data is consistent with the proposed mechanism for Alu repeats, wherein reverse transcription of an Alu element is followed by insertion at a new site. This process is associated with duplication of 7-21 bp flanking the insertion site. This MSH2 mutation predicts an altered and truncated gene product, and exemplifies an Alu-mediated mutation associated with Lynch syndrome.

Apoptosis as a mechanism for caudal truncation in adrenocortical dysplasia (*acd*) mice. *C.E. Keegan, A.S. Krause, M.J. Morley, T. Else, B.C. O'Connor, G.D. Hammer, D.O. Ferguson* Univ Michigan, Ann Arbor, MI.

Adrenocortical dysplasia (*acd*) is a spontaneous autosomal recessive mouse mutant with developmental defects in organs derived from the urogenital ridge: the kidneys, gonads, and adrenals. In addition, the *acd* mutation exhibits embryonic lethality on certain genetic strains, and analysis of *acd* mutant embryos reveals a striking embryologic phenotype that includes caudal truncation and axial skeletal patterning defects. We have previously characterized the *acd* mutation as a splicing defect in a gene (*Acd*), which encodes a novel component of the telomere cap complex that functions to maintain telomere integrity and regulate telomerase activity. Here, we report widespread expression of *Acd* mRNA in mouse embryos. We observed increased expression in the developing limb buds, tail, and neural tube, which resembles the embryonic expression pattern of the telomerase RNA component (*Terc*) gene and corresponds to the structural defects observed in *acd* mutant embryos. We have also shown that *acd* mouse embryonic fibroblasts exhibit evidence of telomere dysfunction with an increased number of chromosomal end-to-end fusions compared to wildtype cells. To further explore the relationship between telomere dysfunction and caudal dysgenesis, we have investigated potential mechanisms leading to caudal truncation 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 have observed a significant increase number of apoptotic cells in the caudal region of *acd* embryos using TUNEL staining and cleaved caspase-3 immunohistochemistry, but no gross differences in proliferation, suggesting that apoptosis is the predominant mechanism leading to caudal truncation in *acd* mice. We are currently crossing *acd* mice to p53 null mice to determine whether p53 deficiency rescues the caudal dysgenesis phenotype. This work will shed important insights on the role of telomere stability during development. This work was supported by NIH K08-HD42487 and the March of Dimes.

High-throughput genotyping of International HapMap Project Populations with Applied Biosystems TaqMan Drug Metabolism Genotyping Assays: an automated laboratory and analysis pipeline. *K.A. Haque[1], K.D. Lazaruk[2], J.S. Ziegle[2], L.M. Wronka[1], M.B. Beerman[1], R.A. Welch[1]* 1) Core Genotyping Facility, SAIC-Frederick, Inc., NCI-Frederick, Gaithersburg, MD; 2) Applied Biosystems, Foster City, CA.

Although high-density whole genome SNP scans are available for association studies, the tagging SNP approach used to design many of these panels from International HapMap Project data may miss a substantial number of coding functional variation of drug metabolism enzymes (DME). In fact, 40+ DME genes are not covered by the HapMap Project, probably due to the difficulties in assay design for these highly homologous gene families. Additionally, many of these technologies do not provide detection in a high number of known DME genes, leading to further gaps in whole genome scans. Of the polymorphic putative functional DME variants not typed in the HapMap, a large proportion is untagged by any combination of HapMap SNPs. Therefore, to correlate phenotypes to putative functional DME variations in pharmacogenomic drug efficacy and toxicology studies, direct genotyping of these functional SNPs will be necessary. Applied Biosystems has developed a panel of 2,394 TaqMan Drug Metabolism Genotyping Assays to interrogate putative functional variations in 217 DME genes. At the National Cancer Institutes Core Genotyping Facility, an automated, high-throughput pipeline has been created to genotype these assays on the International HapMap Project population. DNA sample preparation and handling, assay set-up, genotype analysis, and data publishing at SNP500 Cancer Database (http://snp500cancer.nci.nih.gov), have all been automated. Using a series of custom-designed methods on two Beckman Coulter Biomek FXs, a Laboratory Information Management System, and analysis software, >650K genotypes have been obtained and analyzed by a single person in ~ 8 weeks. Using this pipeline, a completion rate of >99% and no Mendelian inheritance errors were observed. With this robust set-up for high-throughput genotyping of functional DME variants, we are embarking in larger studies of pharmacogenetics cohorts with similarly high success. Funded by NCI Contract N01-CO-12400.

Current status of thalassemia in minority populations in Guangxi, China. *T. Huang[1,4], H. Pan[1,2], G. Long[3], Q. Li[2], Y. Feng[2], Z. Lei[2], H. Wei[2], Y. Huang[2], J. Huang[2], N. Lin[2], Q. Xu[2], S. Ling[2], X. Chen[2]* 1) Div Human Genetics, Univ California, Irvine, Irvine, CA; 2) Affiliate Hospital of Youjiang Medical College for Nationality, Baise City, Guangxi, 533000, China; 3) Guangxi Medical University, Nanning City, China; 4) Department of Developmental and Cell Biology, University of California, Irvine, CA, USA.

Thalassemia is one of the most common monogenic disorders in the world. A community-based prevention program could be effective. In order to establish a successful prevention program, it is necessary to investigate the genetic status in the endemic area, including the prevalence of the mutation spectrum as well as the available resources. In this study, we screened 12,900 samples for - and -thalassemia in Baise City, Guanxi China with hematological methods and molecular assays. We found that the frequency of carriers in this area for -thalassemia is 15%;. -thalassemia carriers comprise 4.8%; of the population. The majority of - thalassemia carriers in this area have deletion mutationsfive of them account for 98% of -thalassemia. The most common mutations for -thalassemia are the missense mutation. Seven mutations in the -globin gene account for 99%; mutations in this region. The most of -thalassemia major die in the uterus or shortly after birth. We have followed 106 patients with -thalassemia major in our clinic. In general, the patients with -thalassemia major develop clinical symptoms at 3-6 months of age and the majority of them die before 5 years of age. Knowledge surveys about - and -thalassemia were conducted in both the general and medical populations in Baise City. Our results show a severe deficiency in knowledge of thalassemia in both the medical professions and in the general populations. We conclude from our study that thalassemia is a very severe public health issue in minority populations in Baise City, China. Identification of the common mutations will allow us to design cost-effective molecular tests. However, we have a long way to go to educate the general population and the medical community for a successful community-based prevention program.

Identification of regulatory regions nearby COL18A1 gene. *E. Kague[1], L. Armelin-Correa[1], O.T. Suzuki[1], M.C. Sogayar[2], M.R. Passos-Bueno[1]* 1) CEGH-IBUSP, Brazil; 2) IQ-USP, Brazil.

COL18A1 gene contains 43 exons and is transcribed in 3 distinct isoforms using two promoters and an alternative splicing of the third exon. Although collagen is found in all vascular and epithelial basement membranes each of the variants show a characteristic expression pattern, with the highest levels of RNA in liver, kidney and lung. Mutations that lead to inactivation of both the long and short isoforms of COL18A1 gene cause Knobloch syndrome, an autossomal recessive disorder. The collagen XVIII is also involved in other processes through a proteolytic fragment of this collagen called endostatin, an important inhibitor of angiogenesis involved in some pathological processes, such as cancer progression. High sequence conservation among species allows the identification of important gene regulatory regions. Distant regulatory regions of COL18A1 are not yet known. Therefore, the aim of this work is the identification of regulatory regions in the vicinity of the COL18A1 gene (100kb upstream of the first exon, introns and 3UTR). Using available bioinformatics tools (VISTA browser- http://genome.lbl.gov/vista/index.shtml, UCSC browser- http://genome.ucsc.edu/ and, http://enhancer.lbl.gov) we selected 13 conserved noncoding sequence blocks greater than 100pb in length and 75% of sequence identity from orthologous regions of the human and mouse genomes nearby Col18A1. After the *in silico* analysis, we cloned each region in a reporter PGL3-promoter vector (Promega) that carries a luciferase reporter system and transfected these constructs into HEK293T and HeLa cells. These two cell lines were chosen for transfection assays, as we observed through Real-Time quantitative RT-PCR that they have the highest and lowest COL18A1 expression levels, respectively, among five cell lineages tested (HEK293T, SHSY, HeLa, HUVEC, h-FOB). Until now, we observed that two out of 6 tested regions changed the luciferase transcription levels in HEK293T (p0,05). The regions with a positive functional result *in vitro* assay will also be tested *in vivo* models. CEPID / FAPESP erikakague@yahoo.com.br.

Comparisons of genome coverage can be misleading as indicators of the power of SNP sets or chips for association studies. *J. Marchini, C. Spencer, Z. Su, P. Donnelly* Department of Statistics, Oxford University, Oxford, United Kingdom.

When comparing sets of SNPs for use in genome-wide association studies, such as those offered on commercially available chips, it has become standard to compare them on the basis of genome coverage. For example, coverage is often measured by estimating the proportion of common SNPs in the genome which are well correlated (say $r^2$0.8) with at least one SNP on the chip, or sometimes (via multi-marker tagging) with combinations of SNPs on the chip. With the availability of the HapMap data, coverage is straightforward to estimate. Further, coverage is often taken as a surrogate for what really matters, but is much harder to estimate, namely the power to detect causative SNPs in an association study. Here we describe new methods to estimate power and present results which compare the power of different SNP sets for large genome-wide association studies, and contrast these with comparisons of coverage for the same sets. Strikingly, marked differences in genome coverage may well not translate into appreciable differences in power in association studies. As one example, supplementing a commercially available chip with an additional 130K markers specifically chosen to maximise coverage increased coverage (measured pairwise) from 65% to 81%, but resulted in only a slight increase in power, over a range of common allele frequencies and effect sizes. With hindsight, there are several reasons why coverage comparisons can be misleading indicators of relative power.

Role of cytoplasmic DNA in other disease. *M. Houshmand* Medical Genetics Unit, National Institute for Genetic Engineering and Biotechnology, Tehran, Iran.

The hypothesis that mitochondrial DNA (mtDNA) may be implicated in susceptibility to MS is supported by an increasing number of reports. We studied the mitochondrial possible point mutations background for both diseases. In 56 Iranian MS patients we couldn't find any primary LHON point mutations. We also investigated the same mitochondrial haplogroup (J, K) in Iranian patients. We studied 70 MS patients and 149 healthy controls for Haplogroup J and K with PCR RFLP method. Our result showed that 14 out of 70 positive (20%) in MS group and 14/149 (9.4%) in control group for J ($P < 0.05$); and 9/70 (13%) in MS group and 5 out of 70 (7%) in control group positive for Haplogroup K ($P=0.4$). Association between haplogroup J and Optic Neuritis showed; significant ($P< 0.005$). Our haplogroup investigation (J, M, BM and N) in LHON patients normal control group (149 controls for haplogroup J. and 246 controls for haplogroups M, BM and N, from different regions of Iran) showed any relation between LHON and the haplogroups J, M and N. However the results showed a slight relatedness between haplogroup BM and LHON. Conclusion: 1) LHON and MS had not the same haplogroups background as well as point mutations 2) This study showed that haplogroups J may be a risk factor for MS diseases special for MS patient with Optic neuritis but genetic susceptibility factors for these disorders vary between different populations, 3) there is a slight relatedness between haplogroup BM and LHON. Our analysis revealed high proportion of haplogroup W in infertile men (14.2%) compared to normal controls (0%). ($P= 0.02$) Therefore, we hypothesize that mtDNA haplogroup W might constitute a risk factor for infertility and might act as predisposing haplotypes, increasing the risk for infertility.

Long term implications of genetic diagnosis and genetic counseling for families with chromosomal abnormalities.
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We report a case of a three generation family with a balanced translocation in two unaffected females, an affected female with a marker chromosome and an affected male with an unbalanced translocation. The proband was a female infant with microopthalmia, congenital heart defects, and severe dysmorphic features as a result of a maternally inherited marker chromosome [47,XX,+mar.ish der(22)t(17;22)(q25.3;q13.1) (TUPLE1+,ARSA-)mat]. During our interview we discovered that the maternal family had previously had an extensive cytogenetic workup and genetic counseling due to an affected half-uncle. The patients mother was aware that the grandmother was a balanced carrier, but believed that she herself had a normal karyotype. The discovery that she was a carrier of a balanced translocation, and the subsequent feelings of guilt were traumatic for the mother, and were compounded by her daughters subsequent demise. This case reinforces several important concepts: 1.Balanced translocation carriers diagnosed as children may not understand the implications of a balanced translocation, especially as it applies to their own future pregnancies; 2. Follow-up with balanced translocation carriers, especially those who may have children at a later date is vital; 3. Patient memories can become erroneous or faded as time passes. They may also lose their laboratory reports or other clinical documents. Genetic centers must therefore consider the need to maintain records for decades.

Genotype-Phenotype Analysis of the *MGC1203* C430T Mutation in an Ethnically Diverse BBS Cohort. *C. Mok*[1], *J. Bin*[1], *N. Noordeh*[1], *E. Héon*[1,2] 1) Dept. Genetics & Genomic Biol., Sick Kids Hospital, Toronto, ON, Canada; 2) Dept. of Ophthalmology and Vision Sciences, Sick Kids Hospital, Toronto, ON, Canada.

Bardet-Biedl syndrome (BBS) is characterized by features such as retinal degeneration with eventual vision loss, obesity, renal anomalies, frequent digit anomalies and learning disabilities among other less common features. BBS is a genetically heterogeneous, autosomal recessive disease with well-documented cases of inter and intrafamilial phenotypic variability. It has been proposed that genetic modifier(s) may play a role in determining phenotype severity. The C430T change in *MGC1203* (*BBSIP1*) was recently suggested to have such a modifying effect in worsening the phenotype. This C430T mutation causes an increase in aberrant splicing leading to a premature termination codon and therefore a loss of mRNA transcript. We investigated the role of this BBS genetic modifier in 36 unrelated patients affected with BBS. Assessment of phenotype severity included parameters such as the primary BBS mutation, visual acuity, rod and cone function assessed by electroretinography as well as systemic changes (obesity, kidney and liver function, hearing, smell, etc.). A heterozygous C430T sequence change was confirmed using an ARMS assay and direct sequencing in 3 probands (8%). Two of these probands had affected siblings who were also heterozygous for this change. The sequence variant did not explain the intrafamilial phenotype variability of these pedigrees. This study does not support the role of MGC1203 as a major modifier of phenotype severity in the cohort of patients studied.

Toward identification of molecular pathways in dyslexia and related disorders. *J. Kere[1,2,3], M. Peyrard-Janvid[1], I. Tapia-Paez[1], H. Anthoni[1], N. Kaminen[3], S. Massinen[3], M. Zucchelli[1], H. Matsson[1], P.J. Lindsberg[3,4], E. Castren[3], K. Hannula-Jouppi[3]* 1) Karolinska Institutet, Stockholm, Sweden; 2) Karolinska University Hospital, Huddinge, Sweden; 3) University of Helsinki, Finland; 4) Helsinki University Central Hospital, Finland.

Developmental dyslexia (specific reading disability) affects the capacity to learn reading and writing in the presence of normal senses, intelligence and educational opportunity. Specific genes for developmental dyslexia have been identified by genetic studies in recent years, and the first implications for neurodevelopmental processes involved in dyslexia arose from the identification of the axon and dendrite guidance receptor gene ROBO1 and the neuronal migration gene DCDC2 as dyslexia susceptibility genes. The product of the first suggested dyslexia gene, DYX1C1, has been identified as a CHIP-associating protein involved in ubiquitination, and the fourth dyslexia candidate gene KIAA0319 can regulate neuronal migration. Additional candidate genes at the DYX3 locus are being characterized (H. Anthoni et al. this meeting). We are identifying the molecular pathways and interactions of these genes and proteins. We show that DYX1C1 is regulated by SNPs in its promoter region by a transcription factor complex, identified by EMSA and protein sequencing (Tapia-Paez et al. this meeting), and that DYX1C1 protein is rapidly regulated by heat shock to translocate to cytoplasm, interacting there with CHIP and ubiquitin (Kaminen et al. unpublished). Preliminary data suggest that DCDC2 and ROBO1 may be linked in a common pathway, and confirmation of this regulatory pathway may define the first biochemical mechanism involved in dyslexia. We conclude that genes involved in neurodevelopmental processes underlie specific reading disability, and characterization of the biochemical and regulatory pathways may offer first clues to explain dyslexia on the molecular level.

Phenotypic characterisation of alveolar macrophages from atopic asthmatic subjects. *A.-M. Madore[1,2], V. Turmel[3], M. Laviolette[2,3], E. Bissonnette[2,3], C. Laprise[1,4]* 1) University of Montreal Community Genomic Medicine Centre, Saguenay, Canada; 2) Laval University, Quebec, Canada; 3) Institut universitaire de cardiologie et de pneumologie de l'Université Laval, Quebec, Canada; 4) Université du Québec à Chicoutimi, Saguenay, Canada.

Rationale: Environmental and genetic factors play an important role in asthma, particularly with regard to its specific inflammatory process. The alveolar macrophages (AM) are regarded as the major line of defence of lungs. At this time, the literature reports only few genetic determinants and biological ways, which permit to partially define the implication of AM in asthma. **Objectives:** To identify biomarkers specific to the activity of AM in asthma and atopy particularly related to the inflammatory process. The specific objectives are: 1) to study the AM transcriptome using HG-U133A Affymetrix DNA chips, 2) to target new candidate genes for atopic asthma and 3) to select certain genes and carry out a validation by real time RT-PCR. **Methods:** The sample consists of 5 atopic asthmatic individuals and 5 control individuals (without asthma nor allergy). The extraction of AM RNA was followed upon their isolation starting from bronchoalveolar lavages. The analysis of the results of the DNA chips was carried out in order to draw up a list of new candidate genes specific to the activity of AM in atopic asthma. Thereafter, the RNA was used to validate by real time RT-PCR the differences in expression for 5 selected genes. **Preliminary results:** The analysis of chips showed 36 genes, classified in 9 clusters related to inflammation, differently expressed in AM of atopic asthmatic and control subjects. To date, 3 of 5 selected genes were tested by real time RT-PCR of which 2 were validated, the development for other genes being in hand. **Perspectives:** The validation by real time RT-PCR will be completed and the characterization of the protein expression of 5 genes will be done by immunocytochemistry and western blot. Thereafter, functional studies will be carried out for one of the 5 genes starting from cell lines, in order to better characterize the biological way in which it is integrated.

Screening of Nuclear Genes Encoding Proteins Critical For Mitochondrial Genome Replication and Maintenance, and Physiological Function: Retrospective Analysis of Clinical Samples Manifesting Mitochondrial Disorder Phenotypes Without Known Pathogenic Mutations Or Deletions In Mitochondrial DNA. *M. Koul[1], J. Hempel[1], S. Edstrom[1], J. Stoddard[1], B-L. Wu[1,2], S. Lilleberg[1]* 1) Transgenomic Labs, Omaha, NE, United States; 2) Childrens Hospital, Harvard Medical School, Boston, MA, United States.

Disorders associated with mitochondrial respiratory chain dysfunction affect at least 1 in 8000 of the general population. Known defects of mtDNA account for approximately one-half of all primary mitochondrial respiratory disorders. Heterogeneous mitochondrial disorders are caused by gene defects in the either mitochondrial DNA or the nuclear DNA or both. Also, distinct mutations in a single gene may cause strikingly different phenotypes, or conversely, patients with different respiratory chain disorders can have a similar phenotype (phenocopies). To further understand these more complex genotype-phenotype relationships, we initially analyzed the whole mitochondrial genome from 25 clinical samples from patients with various clinical presentations of mitochondrial disorders. For this purpose, we utilized our validated in-depth screening strategy for the detection of mitochondrial DNA alterations by an optimized PCR protocol to cover the entire mtDNA with 40 overlapping amplicons designed specifically for our accurate and sensitive scanning techniques of WAVE DHPLC, SURVEYOR with double stranded DNA cycle sequencing for the detection of both heteroplasmic and homoplasmic variants in mtDNA. Samples with the absence of any known pathogenic mitochondrial DNA alterations, were further analyzed for mutations in the nuclear genes encoding factors needed for mt DNA replication (DNA POLG), followed by genes involved in maintaining the mitochondrial nucleotide pool (DGUOK, TK2), and respiratory function (COXIV assembly genes; SURF1, COX10, SCO1, and SCO2) as part of a tiered screening approach for efficient analysis in a clinical laboratory. Results from this comprehensive analysis of nuclear and mitochondrial genes will be presented with relevant functional information on the mutations/polymorphisms that were detected.

Distribution of clinical data in prostate cancer affected men with early age at onset. *D.M. Mandal*[1], *S.L. Halton*[2], *J.E. Bailey-Wilson*[3], *W. Rayford*[4] 1) Genetics, Louisiana State University Health Sciences Center, New Orleans, LA; 2) Baylor Clinic, Houston, TX; 3) NHGRI/NIH, Baltimore, MD; 4) Department of Preventive Medicine, University of Tennessee, Memphis,TN.

Several risk factors have been identified in prostate cancer, including age, race and family history. In general, incidence rate of prostate cancer in African-American men is twice as high as the Caucasian males. Earlier studies in literature show that African-American men are often diagnosed with advanced stages of prostate cancer and their age-specific PSA is higher than their Caucasian counterparts. We have identified 125 individuals in our prostate cancer study with early age at onset (65 years) from Southern Louisiana. There are 60 African-Americans and 65 Caucasians. Family history was verified for all of them and pathological reports were reviewed. Data were analyzed to observe the distribution of age, PSA and Gleason Scores in two races. A significant difference ($p = 0.0008$) in the age at onset values was observed between African-American (range 38-65 years with median age of 57.5 years) and Caucasian (range 44-65 years with median age of 61 years) males. No statistical significance was observed in PSA and Gleason Score values in two races. In further analyses, data were stratified with respect to sporadic cases and cases with family history of prostate cancer. African-American and Caucasian cases with family history produced a significant difference of $p < 0.01$ in the age at onset values. This result enables us to observe clinical characteristics of prostate cancer in different races in Louisiana males, which may contribute significantly in prostate cancer screening strategies.

New Autosomal Recessive Multiple Congenital Anomalies/Mental Retardation (MCA/MR) Syndrome Consisting of Fronto-Nasal Dysplasia, Hypertelorism, Short Stature, Brachydactily and Speculated Irises. *M. Haimi[1], R. Gershoni-Baruch[1,2]* 1) medical genetics, Rambam medical center, Haifa, Israel; 2) The Ruth and Bruce Rapport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel.

Fronto-nasal dysplasia is a developmental field complex associated with variable degrees of midline facial clefting and singled by wide variability of expression. The clinical findings consist of ocular hypertelorism, median facial clefts, broad nasal root, lack of formation of nasal tip, anterior cranium bifidum occultum and widow's peak hair anomaly. We describe two female sibs born to a consanguineous Arab Muslim couple in northern Israel. Among other clinical findings, both have moderate mental retardation, short stature, leonine facies with hypertelorism, broad nasal root, long philtrum, fronto-nasal dysplasia, speculated irises, brachy-clinodactily, low-set posteriorly rotated ears and a webbed neck. Fronto-nasal dysplasia constitute a salient manifestation of cranio-fronto-nasal dysplasia (CFND) or cranio-fronto-nasal syndrome (CFNS) which is characterized by hypertelorism, coronal synostosis with brachycephaly, downslanting palpebral fissures, clefting of the nasal tip, joint anomalies, longitudinally grooved fingernails and other digital anomalies. Otherwise, the combination of hypertelorism, brachydactyly, ligamentous laxity, pectus excavatum and peculiar penoscrotal relations (shawl scrotum) was reported as an X-linked disorder termed faciogenital dysplasia for which sex-influenced autosomal dominant inheritance was also suggested. This association of anomalies differs from previously reported entities characterized by mid-face clefting and defines a new MCA/MR syndrome. Parental consanguinity and familial occurrence in two sisters suggest autosomal recessive inheritance.

A locus for familial mesial temporal lobe epilepsy mapped on chromosome 18p. *C.V. Maurer-Morelli[1], R. Secolin[1], R.R. Domingues[1], R.B. Marchesini[1], N.F. Santos[1], E. Kobayashi[2], F. Cendes[2], I. Lopes-Cendes[1]* 1) Department of Medical Genetics, FCM/UNICAMP, Campinas, São Paulo, Brazil; 2) Department of Neurology, FCM/UNICAMP, Campinas, São Paulo.

We have reported a large group of families with mesial temporal lobe epilepsy (FMTLE) associated with hippocampal atrophy (HA) and other signs indicative of hippocampal sclerosis (HS) detected by magnetic resonance imaging techniques. Previous pedigree and complex segregation analyses provided evidence for the presence of a major gene predisposing to HA in FMTLE. The aim of this study was to identify the region harboring the main gene associated with HA in FMTLE by linkage analysis. Genome-wide scan was performed using a total of 332 microsatellite markers with a polymorphism information content 0.75% at approximately 12cM intervals. An additional 14 markers were genotyped to fine mapping in the candidate region. Two-point and multipoint LOD scores were calculated with the LINKAGE computer package. We assumed an autosomal dominant inheritance with incomplete penetrance. A locus for FMTLE was identified at chromosome 18p11.3-11.2 with a maximum LOD score of 3.60 at = 0.0 for the D18S976 marker in a single family with 11 affected individuals with HA. Multipoint and haplotype analyses localized the locus within a 6 cM interval flanked by markers D18S976 and D18S452. No other significantly positive LOD score was detected for this family in the entire genome. This is the first conclusive evidence that HA may be caused by genetic factors, which can have major implications in the study of the pathophysiological mechanisms underlying HS and its relationship with temporal lobe epilepsy. Supported by FAPESP.

Pathogenic duplication or variant: Molecular cytogenetic discrimination of proximal 15q and 8p23.1 as models.

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G-banding analysis often reveals structural chromosome variations in regions such as 8p23.1, 9p12, 15q11.2 and 16p11.2, which have been referred to as euchromatic variants. However, as more is learned about the underlying genomic structure, it has become evident that apparently identical imbalances at the G-banding level are not equivalent at the molecular level. Targeted molecular cytogenetic analysis has enabled clarification of these findings to differentiate between copy number variations and pathogenic imbalances. Here, we present data from two such regions, proximal 15q11.2 and 8p23.1, using FISH with genomic clones. The amplification of pseudogenes in proximal 15q has been reported as a normal euchromatic variant, however duplications of unique genes just distal to this region can cause autism. To discriminate this polymorphic variant from clinically significant duplications, we utilized clones corresponding to the NF1 pseudogene and the unique region (SNRPN) of proximal 15q in 6 cases that showed a possible duplication by G-banding. All 6 cases showed an amplification of the pseudogene region on one homolog compared to the other, classifying these cases as polymorphic variants. Likewise, a duplication of 8p23.1 is frequently identified by G-banding. In some cases, this duplication is associated with a normal phenotype, while in others it is associated with congenital anomalies. We have studied two cases with 8p23.1 duplications that look identical by G-banding using FISH with clones mapped to the olfactory receptor (OR) gene cluster and unique genes located between the two OR gene clusters in 8p23.1. In Case 1, one of the homologs showed an amplified hybridization signal for the OR cluster and a normal hybridization pattern for the unique genes. Therefore, this dup(8)(p23.1) is most likely a normal variant. In Case 2, the probes for the unique gene region are duplicated, demonstrating that the dup(8p)(p23.1) is pathogenic. Our data from 8p and 15q demonstrate that molecular cytogenetic characterization of cytogenetically identical imbalances such as these is necessary for an accurate clinical interpretation.

Spurious Evidence for Two Neighboring Disease Loci can be Caused by Different Patterns of Linkage Disequilibrium. *I. Ionita[1], L.J. Strug[2], J. Zhang[2], D.A. Greenberg[2,3]* 1) New York University, New York, NY; 2) Div Stat Genetics, Depts. Biostatistics & Psychiatry, Columbia U; 3) NY State Psychiatric Institute.

Statistical methods exist to detect whether more than one locus in a region is involved with disease susceptibility. We present a situation in which linkage disequilibrium patterns lead to a false conclusion of multiple loci. Linkage analysis of Idiopathic Generalized Epilepsy (IGE) identified a disease-linked region on chromosome 18(Durner et al. 2001) and single-nucleotide polymorphism (SNP) case-control and family-based analysis in that region revealed alleles at several loci strongly associated with disease (Greenberg et al 2005). In the case-control analysis there were two distinct peaks. The first peak was in the malic enzyme 2 (ME2) gene; the second, just telomeric to ME2, could be a second disease locus or perhaps a shadow peak, caused by the pattern of LD in the region. In order to determine the nature of the second peak, we performed a stepwise regression procedure (Cordell & Clayton 2002) to evaluate the relative importance of the SNPs. The puzzling results showed that the case-control analysis did not exclude the possibility of two disease loci, while the family-based data, in a conditional logistic regression, supported the one disease locus model. We performed a simulation study to determine the frequency with which two distinct association peaks would be observed when only one disease locus exists. We simulated both case-control data and family-based data with a single disease locus. We created a pattern of low LD in the disease locus region, followed by a high LD region, a pattern similar to the real data. We observed that the frequency of two significant peaks in the case-control data was 20%. In contrast, in the family-based data, the frequency was less, approx. 6%. The simulation results show that observing two association peaks in the same region can arise by a combination of chance and the fine-grain LD structure of the region. The pattern of LD we used was but one of many possible LD patterns. We anticipate other LD patterns could lead to other types of misleading results in association studies.

Investigating the methylation status of genes which are responsible for the formation of conventional renal cell carcinoma. *H. Onay[1], S. Pehlivan[2], M. Koyuncuoglu[3], Z. Kirkali[4], F. Ozkinay[5]* 1) Medical Genetics, Ege University Faculty of Medicine, Izmir, Turkey; 2) Biology, Ege University Faculty of Science, Izmir, Turkey; 3) Pathology, Dokuz Eylul University, Izmir, Turkey; 4) Urology, Dokuz Eylul University, Izmir, Turkey; 5) Pediatrics,Ege University Faculty of Medicine, Izmir, Turkey.

Renal cell carcinoma (RCC) is the most common malignancy of the kidney. At initial diagnosis of RCC 30% of patients have metastatic disease. Metastatic disease does not respond to most treatment regimes and median survival rate for such patients is less than one year. For this reason early diagnosis of RCC is crucial. Changes in the methylation pattern of the promotor region of tumor supressor genes are important in the development of the cancer. These changes may occur before the clinical manifestations of the disease are recognised. Methylation Specific Polymerase Chain Reaction (MSP) which is frequently used in methylation studies is a sensitive and rapid technique and has a high throughput. In this study, 7 tumor suppressor genes (RASSF1A, ECAD, TIMP3, APC, MGMT, p16, RARB2) which are thouhgt to have a role in the development of RCC were investigated for the methylation status in their promoter region in three different tissue samples (normal, premalign and malign) of 21 conventional RCC patients using MSP technique. The aim of the study was to form a methylation based early detection test comprising of the genes which are detected to be important in the development of RCC. High methylation rates for the genes RASSF1A (76%), p16 (80%), ECAD (42%), TIMP3 (33%) and MGMT (33%) were observed in the patients with RCC. The APC (14%) and RARB2 (19%) genes showed respectively low methylation rates. In conclusion a methylation based early diagnosis gene test which consists of the tumor suppressor genes RASSF1A, ECAD, TIMP3, MGMT and p16 may be used in patients with RCC.

An X;Y translocation in a fertile, phenotypically normal female with a normal daughter and fatal fetal anomaly in a son. *L.C. Layman[1], J.D. Bell[1], S.P.T. Tho[1], A. Kulharya[2], P.G. McDonough[1]* 1) Dept OB/GYN, Sect Reprod Endocrinol, Infertil, & Genet, IMMAG, Medical Colleg of Georgia, Augusta, GA; 2) Dept Pediatrics, Medical Gollege of Georgia, Augusta, GA.

Human X;Y translocations are uncommon and produce considerable variability in progeny outcome. We present the case of a mother with a balanced X;Y translocation ascertained through the analysis of a stillborn male. She is a 25 year-old WF G2P1101 with regular menses who conceived spontaneously without medical intervention. On physical exam, she is 49 but is otherwise phenotypically normal with a normal cycle day #2 FSH level. Her first pregnancy resulted in the birth of a healthy girl who is normal except for short stature (<2.5%), but her second pregnancy ended at 20 weeks with the delivery of a stillborn male with hydrocephalus. The maternal serum unconjugated estriol level was unmeasurable. A karyotype revealed a 46,Y,der(X)t(X;Y)(p?22.3;q?11.2). FISH demonstrated ish der(X)t(X;Y)(wcpY+,wcpX+), with the presence of the KAL1 region and deletion of the steroid sulfatase (STS) locus, defining the breakpoint on the X chromosome. In addition, interphase FISH studies on the probands peripheral lymphocytes demonstrated the the X centromere (DXZ1+) and Yq12 (DYZ1+), but not the Y centromere (DYZ3-), indicating the presence of only Yq on the X;Y derivative chromosome. Both the proband and her daughter carry the 46,X,der(X)t(X;Y)(p22.3;q11.23) balanced translocation with the derivative X lacking p22.3 to the telomere and containing Yq11.2 to the telomere. The patient was counseled to have a cardiac echo to exclude potential aortic abnormalities. Currently, the breakpoints are being determined using STS markers on Yq11.2. Issues of concern for the patient with this translocation include: future fertility, meiotic behavior of the translocation and the risk for congenital anomalies, the risk for gonadoblastoma and aortic root abnormalities, and the challenge of pre-implantation diagnosis.

Can Lmx1a and Lbx1 genes be genetic risk factors for Neural Tube Defects? *T. Jafarov, L. Mehltretter, D. Siegel, T. George, D. Enterline, J. Gilbert, M. Speer, NTD Collaborative Team* Center For Human Genetics, Duke University Medical Center, Durham, NC.

Introduction:Recently published studies on dreher (carrying mutated homeobox gene Lmx1) and Lbx-1- mouse models have demonstrated their important roles in the development of CNS, heart, extremities, roof plate and the rostral part of neural tube. Disruption of normal function of these genes theoretically could lead to complex conditions like neural tube defects (NTDs). Despite of the significant roles these genes can play, there have been no studies of these genes in human NTD patients. We have screened 105 DNA samples of patients with different types of NTDs for mutations in the human homologues of these genes, Lmx1a and Lbx1, with partial 3UTR regions. Materials and methods: Genomic DNA from NTD patients was extracted from blood and was used to amplify the exons and fluorescent direct sequencing was performed afterwards. Sequence data were blasted against the Human Genome Database and were analyzed with different bioinformatics programs. Results: We have found no non-synonymous mutations in coding sequences of Lmx1a and Lbx1 among the studied samples. A number of synonymous mutations were detected in both genes and among them novel ones were: Ser13Ser, Thr344Thr for Lmx1a. Altogether 13 known SNPs and 21 novel SNPs were detected in Lmx1a and Lbx1 genes. SNPs IVS1-35 C>T, IVS4-3 C>T,IVS7-37 G>A, IVS8-30 C>A in Lmx1a gene and SNPs IVS2-38 G>C, IVS2-41 C>A, IVS2-18 A>G and IVS2-21 G>C in Lbx1 gene could be of particular interests due to their position or frequencies. Discussion: Despite extensive search for genetic and environmental factors predisposing to neural tube defects, the progress in understanding the molecular mechanisms in humans has been slow. Although we have not found any mutations altering the protein sequence of Lbx1a and Lmx1a genes among the studied DNA samples, the SNPs found within close proximity to exon-intron boundaries could affect splicing and therefore change their mRNA profiles. The future case-control and mRNA profiles studies could provide supporting evidence for association between these genes and neural tube defects.

Dementia and relatedness in the mid-western Amish. *J.L. McCauley[1], A.E. Crunk[1], J.M. van der Walt[2], L.L. McFarland[1], P.C. Gaskell[2], L. Jiang[1], P.J. Gallins[2], K.A. Welsh-Bohmer[3], W.K. Scott[2], C.E. Jackson[4], M.A. Pericak-Vance[2], J.L. Haines[1]* 1) Center for Human Genetics Research and Dept of Molecular Physiology and Biophysics, Vanderbilt University Medical Center, Nashville, TN; 2) Center for Human Genetics and Dept of Medicine, Duke University Medical Center, Durham, NC; 3) Joseph & Kathleen Bryan ADRC/Division of Neurology, Duke University Medical Center, Durham, NC; 4) Scott & White, Temple, TX.

The genetics of Alzheimers disease (AD) have proven to be quite difficult to understand and elucidate. Although a role for the APOE gene in late-onset AD is apparent, it accounts for less than half of the susceptibility and thus other genetic factors are likely to be involved. Genetic heterogeneity is a major complicating factor hindering further gene identification. To minimize heterogeneity, we have been collecting individuals with dementia from the genetically isolated Amish populations of both Ohio and Indiana. To date we have assessed over 1200 individuals who have consented to participate in our studies (97 of whom have probable or possible AD). Through use of the Anabaptist Genealogy Database (AGDB) and its query software PedHunter, we can interrogate the complete family structure of our sample through the examination of kinship coefficients and the construction of pedigrees based on those coefficients. The average kinship coefficient for our entire sample is 0.012, which is similar to that described for other Amish community samples. These kinship coefficients also demonstrate that the individuals ascertained within Adams County, Indiana are distinctly more related to each other (k.c.=0.041) than to individuals ascertained in the other regions (k.c.=0.0051). The vast majority (>90%) of our sample can be traced back at least 10 generations to a single founding couple. Furthermore, we are tracing both maternal and paternal lineages (using mitochondrial and Y-chromosome markers) to better estimate the number of founders in our sample. These data indicate that the Amish of Adams County, Indiana may represent a separate subsample of the Amish genome, and have helped us to develop 13 complex yet analytically tractable subpedigrees for analysis of dementia.

Preliminary Pharmacogenetic Analysis of Gene Expression in Patients with Major Depressive Disorder Treated with Citalopram. *F. Mamdani[1], P.A. Sequeira[1], J. ffrench-Mullen[2], M. M. Beaulieu[1], G. Turecki[1]* 1) McGill Group for Suicide Studies, Douglas Hospital Research Centre, McGill University, Montréal, Quebec, Canada; 2) GeneLogic Inc., Gaithersburg, Maryland, USA.

Major depression (MD) is a psychiatric disorder that affects 5-10% of the population and is considered the second leading cause of disability by the World Health Organization. Several studies have indicated the involvement of genetic factors in MD, which may also play a role in a patients response to antidepressant treatment. In order to identify gene targets that may mediate response to Citalopram (Cit) treatment, a commonly used SSRI (selective serotonin reuptake inhibitor) antidepressant, we are conducting a large-scale gene expression study of patients being administered CIT for 8 weeks, with Affymetrix U-133 Plus2 microarrays being performed pre- and post-treatment. We are presenting preliminary results on 28 cases. Outlier detection was performed using several quality control variables and principal component analysis. Differential expression analysis resulted in 42 genes having altered expression between responder and non-responder samples and 208 genes showing differential expression in relation to gender. The findings of our preliminary analyses are interesting in that they suggest an interaction between response and gender, not surprisingly given gender differences in MD prevalence rates. Gene ontology analysis of differentially expressed genes revealed several biological processes being affected by treatment; these include regulation, transcription, response to stress, as well as, biosynthesis and metabolism of macromolecules and proteins. Our results suggest that there appears to be differential expression following Cit treatment and these differences may be correlated to treatment response.

Genome-wide linkage study in the NIMH Bipolar data sets of alcohol dependence and correlation with loci significant for psychosis. *B. Kerner, D.L. Brugman, N.B. Freimer* Ctr Neurobehavioral Genetics, Department of Psychiatry and Biobehavioral Sciences, Univ California, Los Angeles, Los Angeles, CA.

Purpose: Alcohol dependence has a high co-morbidity with bipolar disorder and has been shown to be genetically influenced. Linkage signals with this trait have been found on chromosomal regions overlapping with those for schizophrenia or bipolar disorder. In our previous analysis of the NIMH Bipolar data sets a significant linkage signal for psychotic bipolar disorder was found to be located on chromosome 5q33-34. Families in this linkage scan had significant co-morbidity with alcohol dependence. Since this chromosomal region has also been implicated in genome scans for alcohol dependence we performed a linkage scan in the NIMH data sets Waves 1, 2, 3, and 4 and compared the obtained linkage signals with those in our previous linkage scan for psychotic bipolar disorder. Methods: DSMII-R or DSM-IV alcohol dependence was used in this study as the phenotype definition for a whole genome scan of the NIMH Bipolar Genetics Initiative data sets. Wave 1 and 2, as well as Waves 3 and 4 combined, were analyzed using non-parametric linkage analysis in MENDEL. Results: This analysis showed a suggestive linkage signal on chromosome 1q21-1q22 in Wave 1 and 2 in the vicinity of the markers D1S534/D1S1595. This locus has been previously implicated in scans for schizophrenia. A signal in this region was also detected in Wave 3 and 4 near the marker D1S1589. Other interesting results included signals on chromosome 15q15-q21 in Waves 1 and 2 and on chromosome 8q11 and 11q21-q22 in Waves 3 and 4 combined. Conclusion: The phenotype of alcohol dependence in families ascertained for bipolar disorder revealed a suggestive linkage signal on chromosome 1q. The linkage signals were distinct from those that were obtained in a linkage scan for psychotic symptoms in bipolar disorder. The co-morbidity of alcohol dependence should be taken into account when linkage scans for psychiatric disorders are performed since genetic interactions with this trait are likely.

3q29 microduplication: a novel syndrome in a three-generation family. *E. Lisi[1], A. Hamosh[1], B. Jackson[2], R. Galczynski[2], D. Batista[2,3]* 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Kennedy Krieger Institute, Baltimore, MD; 3) Department of Pathology, Johns Hopkins University, Baltimore, MD.

We describe four affected individuals in a three-generation family with a subtle 3q29 microduplication. The proband is an 11 month old male with developmental delay, severe microcephaly, and dysmorphic facies. His affected mother and maternal aunt have mild to moderate mental retardation, microcephaly, dysmorphic facies, and obesity. The affected maternal grandfather has mild mental retardation, obesity and type II diabetes. Previous reports of dup(3q) syndrome, associated with growth retardation, microcephaly, dysmorphic facies, and mental retardation, have mapped the critical region to 3q26.3-27. Additionally, a newly described 3q29 microdeletion syndrome is associated with mental retardation and variable dysmorphic features, but only 1 of 6 patients has microcephaly. Using a combination of microarray CGH and FISH with several BAC clones, we mapped the proximal breakpoint in our family to be between RP11-352G9 and RP11-252K11. The distal breakpoint lies telomeric to RP11-496H1. The maximum size of the microduplication is estimated to be about 2-2.5 Mb. The microdeletion 3q29 syndrome seems to be associated with low-copy repeats that flank the breakpoints and might predispose to nonallelic homologous recombination. In our family with microduplication, the proximal breakpoint lies within the same region as on the microdeletion syndrome, however the distal breakpoint is telomeric. The distal breakpoint in our family is more distal than previously reported. Thus, we postulate that an additional low copy repeat may be present on chromosome 3q, causing a propensity for variable deletions and duplications of this region. Additionally, our family gives evidence that the critical region for phenotypic expression of mental retardation and microcephaly may be more distal than previously described, or additional genes critical to brain growth and development may map to 3q29.

Evaluation of the Risk for Tay-Sachs Disease in Individuals of French Canadian Ancestry Living in New England. *M.R. Natowicz[1], D.C. Martin[2], B.L. Mark[3], B.L. Triggs-Raine[2]* 1) Genomic Medicine Instititute, Cleveland Clinic, Cleveland, OH; 2) Department of Biochemistry and Medical Genetics, University of Manitoba; 3) Department of Microbiology, University of Manitoba.

Background: The assessment of risk for Tay-Sachs disease (TSD) in individuals of French Canadian background living in New England is an important health issue. In preliminary studies of the enzyme-defined carrier frequency for TSD among Franco-Americans in New England, we found frequencies (1:53) higher than predicted from the incidence of infantile TSD in this region. We have now further evaluated the risk for TSD in the Franco-American population of New England.

Methods: The carrier frequencies for TSD in 2783 Franco-Americans were determined using a fluorescence-based assay for -hexosaminidase activity. DNA analysis was used to identify mutations causing enzyme deficiency in TSD carriers.

Results: We determined the enzyme-defined carrier frequency for TSD as 1:65 (95%CI 1:49 to 1:90). DNA-based analysis of 24 of the enzyme-defined carriers revealed 21 with sequence changes: 9 disease-causing, 4 benign, and 8 of unknown significance. Six of the unknowns were identified as c.748G>A p.G250S, a mutation we show by expression analysis to behave similarly to the previously described c.805G>A p.G269S adult-onset TSD mutation. This putative adult-onset TSD c.748G>A p.G250S mutation has a population frequency similar to the common 7.6 Kb deletion mutation that occurs in persons of French Canadian ancestry.

Conclusions: We estimate the frequency of deleterious TSD alleles in Franco-Americans to be 1:73 (95%CI 1:55 to 1:107). These data provide a more complete evidence base from which to formulate policy recommendations regarding TSD heterozygosity screening in individuals of French Canadian background.

A Phase 3 study of the efficacy of sapropterin dihydrochloride (tetrahydrobiopterin, 6R-BH_4) in reducing phe levels in subjects with phenylketonuria. *H. Levy[1], A. Milanowski[2], A. Chakrapani[3], M. Cleary[4], F. Trefz[5], C. Whitley[6], F. Feillet[7], A. Feigenbaum[8], J. Bebchuk[9], H. Christ-Schmidt[9], A. Dorenbaum[10]* 1) Dept Medicine, Children's Hosp, Boston, Boston, MA; 2) Instytut Matki i Dziecka, Warszawa, Poland; 3) Birmingham Childrens Hospital, Birmingham, UK; 4) Great Ormond Street Hospital, London, UK; 5) Klinik fur Kinder und Jugendmedizin Reutlingen, Reutlingen, Germany; 6) University of Minnesota, Minneapolis, MN; 7) Hopital dEnfants - CHU Brabois, Vandoeuvre les Nancy, France; 8) Hospital for Sick Children, Toronto, Ontario, Canada; 9) Statistics Collaborative, Inc, Washington, DC; 10) BioMarin Pharmaceutical,Inc. Novato, CA.

Strict dietary management of Phenylketonuria (PKU) is the only option to prevent mental retardation. Major challenges remain to achieve optimal outcomes. We studied Sapropterin, a synthetic form of BH_4, as a new treatment for PKU that could potentially improve long-term care. Patients previously screened for BH_4 response enrolled in a Phase 3, multicenter, randomized, double-blind, placebo controlled trial. Safety and efficacy in reducing blood phenylalanine (Phe) were compared in PKU patients treated with oral sapropterin 10 mg/kg, or placebo, once daily for 6 weeks. Of the 89 subjects enrolled, 87 completed treatment. Age ranged from 8 to 49 years (mean 209.7). At baseline, mean (SE) blood Phe was 843 (47) M and 888 (47) M in the sapropterin and placebo groups, respectively. After 6 weeks of treatment, sapropterin-treated patients achieved a mean blood Phe decrease of 236 (40) M (-29%) compared with a 3 (35) M (+3%) increase in the placebo group (p0.0001). At week 6, the percentages of subjects with blood Phe levels 600 M were 54% and 23% for the sapropterin and placebo groups, respectively (versus 17% and 19% at baseline). There was a consistent reduction over time in the average mean change in weekly blood Phe levels in the sapropterin group compared to the placebo group (p0.001). The type and incidence of adverse events were similar in the two study arms. Sapropterin was well tolerated and effective in significantly reducing blood Phe levels in PKU patients previously screened for BH4 responsiveness.

SNP-based microarray for the detection of submicroscopic chromosomal abnormalities. *J.M. Kogan, T.A. Smolarek, M. Keddache, H.M. Saal, G.A. Grabowski* Division of Human Genetics, Cincinnati Childrens Hospital Medical Center, Cincinnati, OH.

Aberrations in genomic copy number play a significant part in mental retardation and congenital malformations as well as cancer and infertility. Conventional cytogenetic techniques such as karyotyping and fluorescence in situ hybridization can be useful for diagnosis for these conditions, but they suffer from limitations in resolution and are labor intensive. In addition, they cannot detect copy-neutral abnormalities such as uniparental isodisomy. Recent advances in single-nucleotide polymorphism (SNP) arrays allow high-density genome analysis to be conducted in a relatively short amount of time. SNP-based chromosomal analysis also offers several advantages to other genome-wide analyses such as comparative genomic hybridization including ease of use. Also, with the manufacture of newer high density chips up to 1,000,000 SNPs, greater sensitivity can be achieved.

We used the Illumina Golden Gate assay containing 5,850 SNPs spaced at an average of 0.5 MB across the human genome to detect DNA dosage changes in nine patient samples with known losses or gains of chromosomal material and four samples with no identifiable chromosomal abnormality. Chromosomal abnormalities included trisomies, microdeletions, duplications, and unbalanced translocations. Abnormalities were easily detectable using BeadStudio Genotyping software. Of the chromosomally normal samples, one copy number polymorphism was found.

High resolution SNP-based chromosomal analysis will likely become essential for identifying copy number changes not detectable by conventional cytogenetic methods. Patients with mental retardation and/or having two or more congenital anomalies of unknown etiology are most likely to benefit. One caveat is that an increasing number of large-scale copy number polymorphisms are being identified in the population, and care must be taken when assigning diagnoses. Lack of identification of a similar abnormality in the parents chromosomes increases the likelihood, but does not guarantee that the chromosomal abnormality is causative for the patients phenotype.

Confirmation of Association between Variations in NOS1AP and the QT Interval in a Community-based U.S. Cohort. *W.H.L. Kao[1], D.E. Arking[1], W. Post[1], P.M. Spooner[1], G.F. Tomaselli[1], E. Marban[1], N. Sotoodehnia[2], E. Boerwinkle[3], A. Chakravarti[1]* 1) Johns Hopkins University,Baltimore, MD; 2) University of Washington, Seattle, WA; 3) University of Texas, Houston, TX.

The QT interval is a measure of cardiac repolarization. Through a genome-wide association scan, we previously identified SNPs in the upstream region of NOS1AP to be associated with the QT interval. The association for one of these SNPs, rs10494366, was subsequently replicated in three independent Caucasian cohorts. We now test the importance of genetic variations in NOS1AP in the Atherosclerosis Risk in Communities (ARIC) study, a prospective study of adults aged 45-64 years in 1989-92. Using data from HapMap, a total of 22 SNPs were selected to tag the block containing rs10494366 and rs4657139 (the most significant SNP from fine mapping in our initial study) and its neighboring blocks (criteria of r2>0.65 and MAF>0.05 using Tagger). No putatively functional SNPs were tested. General linear models were constructed to correct for age, sex, heart rate, and history of myocardial infarction. A three-means model was assumed. All analyses were stratified by race. Here, we present results from 3,564 African Americans. Several SNPs were associated with the QT interval in ARIC African Americans. While rs10494366 was marginally associated (p=0.09), an additional SNP, rs7539281, was significantly associated with the QT interval in this group (p=0.04). Genotyping in ~12,000 Caucasians is ongoing. Preliminary results in ~40% of the Caucasians indicate that the magnitude and direction of associations to be similar in the two groups. In summary, variations in NOS1AP are associated with the QT interval in a large cohort of middle-aged African Americans. Differences between findings of this study and our previous findings may be due to differences in populations and potentially inadequate selection of informative tagging SNPs in African Americans. Results from the present study, taken with our previous findings, demonstrate sequence variation in or around NOS1AP influences QT interval in the general population and that additional experiments are needed to identify the causal variant.

Genetic Profiling of Tay Sachs neuroglia cells and delineation of events associated with disease mechanism. *S.A. Igdoura[1,2], B. Trigatti[3], J.P. King[1]* 1) Dept Biology, McMaster Univ, Hamilton, ON, Canada; 2) Dept Pathology & Molecular Medicine, McMaster Univ, Hamilton, ON, Canada; 3) Dept Biochemistry, McMaster Univ, Hamilton, ON, Canada.

Tay Sachs disease is a lysosomal storage disorder characterized by lysosomal accumulations of GM2 ganglioside. The events that follow the initial increase of ganglioside storage and eventually lead to disease pathogenesis in this disorder remain largely undetermined. In order to examine the impact of GM2 accumulation on gene expression in Tay Sachs disease, we characterized global gene expression in cultured human cerebellum neuroglia cells isolated from normal and Tay Sachs embryos using the CodeLink microarray technology (GE-Healthcare). We report that several classes of genes involved in inflammation, apoptosis, interleukin signaling, potassium balance, and WNT signaling were altered in the Tay Sachs cerebellum cells. To confirm and validate our observations, we used Quantitative Real Time PCR (qRT-PCR) and Western blot analysis. Our studies confirmed the up regulation of Caspase 1, Interleukin 6, Potassium Channel, Eotaxin, Phospholipase C, and Prostaglandin Synthase, and down regulation of Heme Oxygenease, Histone Deacetlyase, Sphingomyelin Phosphodiesterase, Calpain, and Inositol 1,4,5-Triphosphate Receptor. To establish a link between the altered genetic profile and the disease status of Tay Sach neuroglia cells, we treated cells with n-butyldeoxynojirimycin (NBDNJ) to prevent and alleviate GM2 storage. Using qRT-PCR to determine gene expression, we discovered that NDBNJ reversed to normal levels the expression of several of these genes. Genetic profiling of expression pointed to mediators of apoptosis and potassium-deprivation induced depolarization as the most responsive genes to GM2 accumulations. These mediators therefore may be the main players in the disease mechanisms associated with Tay Sachs phenotype.

Hypophosphatasia: c.1133A>T, p.D378V is the most common American *TNSALP* mutation. *S. Mumm[1,2], D. Wenkert[2], X. Zhang[1], M. Geimer[1], J. Zerega[2], M.P. Whyte[1,2]* 1) Div Bone & Min Dis, Wash Univ Sch Med, St Louis, MO; 2) Cntr Metab Bone Dis & Mol Res, Shriners Hosp, St Louis, MO.

Hypophosphatasia (HPP) is a rare, heritable, inborn-error-of-metabolism featuring defective skeletal mineralization caused by loss-of-function mutation in the tissue non-specific alkaline phosphatase gene (*TNSALP*). Severity ranges greatly from neonatal lethal disease to asymptomatic carrier. HPP can be inherited as an autosomal dominant or recessive trait. Generally, recessive disease is more severe. To better understand the molecular basis and inheritance of HPP, we performed mutation analysis of the *TNSALP* gene for approximately 155 HPP probands, primarily from North America. One particular missense mutation, the transversion c.1133A>T, p.D378V (previous nomenclature: D361V), was found in 22 probands, making this defect the most common *TNSALP* mutation in our cohort of patients. In all but one individual, a second *TNSALP* mutation was not detected, supporting autosomal dominant inheritance for the HPP in these kindreds. Genealogy has thus far not disclosed a common ancestor among these families, which all seem to have early American heritage. The clinical phenotype for these patients is generally mild (odonto-, childhood, or adult HPP), however, 3 probands had infantile HPP. An exon deletion of the other *TNSALP* allele has not been ruled out for these 3 cases. Thus, they could represent heterozygous, recessively inherited, HPP. Where investigated (20 of 22 kindreds), common *TNSALP* polymorphisms in exons 7, 8, and 9 were invariably found along with the D378V mutation. In cases where parental DNA was also studied, these polymorphisms cosegregated with the D378V mutation. We therefore propose that the D378V mutation in our North American cohort is due to a common founder, and when dominantly inherited usually causes a mild phenotype. Nevertheless, a surprisingly broad clinical expression of HPP manifests in these 22 families with this identical heterozygous *TNSALP* mutation. Hence, patients with c.1133A>T, p.D378V and relatively severe HPP bone disease likely have additional genetic or environmental factors that contribute to their HPP phenotype.

Myocilin and Optineurin Sequence Variants in Primary Open Angle Glaucoma in a Hispanic Population of Mexican Descent. *K. LaRocque-Abramson[1], X. Yin[1], C. Santiago-Turla[1], A. Ventura-Viray[1], L. Ramirez[2], M. Alvarez[2], A. Beltran[3], M. Pericak-Vance[1], M. Hauser[1], R.R. Allingham[1]* 1) Duke Univ Medical Ctr, Durham, NC; 2) Oftalmologos Associados, Nogales, Mexico; 3) Clinica Oftalmologica, Hermosillo, Mexico.

Glaucoma is a major cause of blindness and is characterized by progressive loss of retinal ganglion cells and optic neuropathy. Population-based studies demonstrate that the prevalence of primary open-angle glaucoma (POAG) is high in Hispanic persons of Mexican descent. Although POAG is often accompanied by elevated intraocular pressure (IOP) over 80% of patients in this population have a variant of POAG with IOP in the normal range. Variations in myocilin(MYOC) and optineurin(OPTN) have previously been reported to cause POAG. The goal of this study is to determine the contribution of disease-associated variants of MYOC and OPTN in POAG in Hispanic patients of Mexican descent. Patients were ascertained from 2 ophthalmology clinics in the Mexican state of Sonora. Criteria for inclusion included: glaucomatous optic nerve damage in both eyes, visual field defects in at least one eye, and age of diagnosis 40 years. Elevated intraocular pressure(IOP) was not used as a criterion for affected status. The MYOC and OPTN genes were PCR amplified from genomic DNA and sequenced. Sequences were assembled into contigs and analyzed using Sequencher. Three non-synonymous coding variants were found in MYOC. Arg7His and Lys398Arg were each found in 1% of cases. The non-pathogenic Arg76Lys variant was found in 19% of cases. Only the Lys98Met variant was found in OPTN, in 5% of cases. Both genes also show multiple synonymous coding and intronic variations. MYOC mutations account for 4% of POAG cases in other populations, so the 2% found here reflects a relatively low contribution of MYOC to disease in this population. The Lys98Met OPTN variant has been variably associated with glaucoma in some populations. Age- and ethnicity-matched controls are currently being analyzed to complete the assessment of POAG risk in this population. In conclusion, the role of MYOC and OPTN in POAG appears to be limited in Hispanics of Mexican descent.

Differential Alu retrotransposition in HeLa cells. *H.C. Kopera, J.L. Garcia-Perez, J.V. Moran* Human Genetics, University of Michigan, Ann Arbor, MI.

Long Interspersed Element-1 (LINE-1 or L1) is a non-long terminal repeat (non-LTR) retrotransposon that comprises ~17% of the human genome. A retrotransposition competent human L1 (RC-L1) is ~6 kb and contains a 5 UTR, two non-overlapping reading frames (ORF1 and ORF2), and a 3 UTR that ends in a poly (A) tail. Both the ORF1 and ORF2 encoded proteins (ORF1p and ORF2p) are required for retrotransposition, which likely occurs by a mechanism termed target site primed reverse transcription (TPRT). The proteins encoded by RC-L1s also are responsible for the mobilization of Alu elements and the formation of processed pseudogenes, which together comprise at least 10% of human DNA. Unlike L1 retrotransposition, Alu mobility only requires ORF2p, suggesting that other host factors functionally substitute for ORF1p. However, little is known about these host factors.

Here, we report two different HeLa cell lines that differ in their ability to support Alu retrotransposition in a cultured cell retrotransposition assay. Experiments conducted with engineered L1s demonstrate that the first cell line (HeLa-HA) supports L1 retrotransposition, ORF1p-dependent pseudogene formation, and Alu retrotransposition. By comparison, the second cell line (HeLa-JVM) supports L1 retrotransposition and ORF1p-dependent pseudogene formation at similar levels as HeLa-HA, but shows a 2-3 fold order of magnitude reduction in Alu retrotransposition. One possibility for this difference is that HeLa-JVM has lost a factor required for Alu retrotransposition. Here, we describe our efforts to functionally complement the defect in HeLa-JVM cells using a cDNA library constructed from HeLa-HA. These studies will further our understanding of Alu mobilization as well as the retrotransposon-host dynamic.

The Hunter Outcome Survey (HOS): A registry of mucopolysaccharidosis II (MPS II) patients. *R. Martin[1], M. Beck[2], E. Wraith[3], R. Giugliani[4], J. Clarke[5], J. Muenzer[6]* 1) St. Louis Children's Hospital, St Louis, MO; 2) Children's Hospital, University of Mainz, Mainz, Germany; 3) Willink Biochemical Genetics Unit, Manchester, UK; 4) Hospital de Clinicas de Porto Alegre, Porto Alegre, Brazil; 5) Hospital for Sick Children, Toronto, ON, Canada; 6) University of North Carolina at Chapel Hill, Chapel Hill, NC.

Mucopolysaccharidosis II (MPS II, Hunter syndrome) is a rare, progressive X-linked disorder of glycosaminoglycan (GAG) metabolism that results in progressive GAG accumulation within multiple tissues and organs, due to a deficiency of iduronate-2-sulfatase (I2S). The clinical features of MPS II include hepatosplenomegaly, central nervous system and skeletal involvement, upper airway and cardiac disease, decreased joint range of motion, and short stature. Currently there is no approved therapy for MPS II. Idursulfase, a recombinant human I2S, is being developed as a treatment for MPS II. The Hunter Outcome Survey (HOS) is a global patient registry of MPS II patients regardless of treatment status. The objectives of HOS are to enhance our understanding of the natural history of MPS II, to monitor the safety and efficacy of enzyme replacement therapy, and to provide the basis for the development of clinical management guidelines. The HOS database will include clinical information obtained during the routine follow-up of MPS II patients. All data will be captured and transmitted to the central database electronically. This registry is sponsored and supported by Shire Human Genetic Therapies, Inc., Cambridge, MA. HOS will benefit participating physicians by providing them with the opportunity to contribute to the study of the management of MPS II and by giving them the ability to review data on a larger population of MPS II patients. As of May 2006, fifty patients have been enrolled into HOS, from 15 sites across 7 countries. As enrollment increases, HOS will improve our understanding of MPS II, the long-term impact of enzyme replacement therapy and allow the development of evidence based clinical management guidelines.

Spastic paraplegia 5: locus refinement, candidate gene analysis and extension of the phenotype. *S. Klebe[1], A. Durr[1,2], N. Bouslam[1,3], D. Grid[4], C. Paternotte[5], A. Bouhouche[3], N. Elleuch[1], H. Azzedine[1], J. Hazan[5,6], A. Brice[1,2], G. Stevanin[1,2]* 1) IFR Neurosciences, INSERM U679-NEB, Paris, France; 2) APHP, Salpetriere hospital, Dept of Genetics, Paris, France; 3) Neurology B and Neurogenetics unit, Specialties hospital, Rabat, Morocco; 4) Généthon, Evry, France; 5) Genoscope, CNS, Evry, France; 6) Centre for Developmental Neurobiology, Kings College London, Guys Hospital Campus, UK.

Thirty-two different loci for hereditary spastic paraplegias (HSP) have been mapped, and 14 responsible genes have been identified. Autosomal recessive HSP (ARHSPs) usually have clinically complex phenotypes but the SPG5, SPG24 and SPG28 loci are considered to be associated with pure forms of the disease. Up to now, 10 families have been mapped to the SPG5 locus on chromosome 8q12. After exclusion of known ARHSP loci, a genome-wide screen performed in a French kindred provided evidence of linkage with a maximal multipoint lod score of 2.6 in the D8S1113-D8S1694 interval. This interval partially overlapped SPG5 and reduced it to a 5.9 megabase (Mb)-region between D8S1113 and D8S544. In a family of Algerian origin from a series of 17 other ARHSP kindreds, linkage to the SPG5 locus was supported by a multipoint lod score of 2.3 over the entire restricted interval. The direct sequencing of 5 candidate genes (RAB2, ASPH, TTPA, Q8NB32 and BHLHB5) did not detect mutations/polymorphisms in the index cases of both linked families. The phenotype of the two SPG5-linked families consisted of early onset spastic paraparesis associated with deep sensory loss. In several patients with long disease durations, there were also mild cerebellar signs suggesting that this genetic entity might also account for complex HSP. In conclusion, the frequency of SPG5 was ~10% (2/18) in our series of ARHSP families with pure or complex forms. In addition we have refined the SPG5 locus to a 3.8 cM interval on chromosome 8q12 and extended the phenotype of this form of ARHSP to include cerebellar signs.

Investigation of PARK10 gene for Parkinson disease. *Y.J. Li, J. Deng, G.M. Mayhew, X. Huo, J. Grimsley, J.M. Vance* Ctr Human Genetics, Duke Univ Medical Ctr.

Chromosome 1p was previously mapped to Parkinson disease (PD) by two independent studies including our linkage screen for age-at-onset (AAO) of PD (Li et al. 2000) and Hicks et al.s (2000) screen on Icelandic families, where they designated this locus as PARK10. Interestingly, these two linkage regions were almost identical regardless of different phenotypes used. We followed up this region by using a genomic convergence approach and an iterative association mapping across a 19.2 megabase region under the AAO linkage peak (Oliveria et al. 2005). The two most significant genes were: EIF2B3 for AAO (min P= 0.0004) and HIVEP3 for risk of developing PD (P0.006 for rs648178 and rs661225). Recently, Maragnore et al. (2005) conducted a whole genome association (WGA) study using 248,535 SNPs and reported LOC200008 as the potential PARK10 with two significant SNPs. Importantly, their study also found two moderate significant SNPs in HIVEP3 in the Tier 1 dataset (P0.008) and Tier 2 dataset (P=0.03). To further investigate the potential PARK10 gene, we revisited HIVEP3 and LOC200008 with an expanded PD family dataset (293 multiplex and 467 singleton families). Recently, other authors have not been able to replicate the LOC200008 findings, but none has tested it in a family dataset of equivalent size to ours. We have genotyped most available HapMap tagSNPs as well as additional SNPs, while maintaining an equal spacing within the gene (in total, six SNPs in LOC200008 and 36 in HIVEP3). We used the PDT and APL methods for testing association with PD risk, and the QTDT program for AAO. No significant results were found for any of the six SNPs in LOC200008. Significant association with PD was found in six SNPs in HIVEP3 including two our previously reported SNPs (min P=0.007). However, the two WGA SNPs in HIVEP3 were not significant. Our failure to replicate the LOC20008 findings supports the negative results of three recent replicate studies for this gene. It is encouraging that HIVEP3 was found to be significantly associated with PD in two independent studies, implying HIVEP3 as a strong candidate for PARK10. Further testing of HIVEP3 by other groups is encouraged.

HER-2/neu amplification, hyperdiploidy, and ERBB2 expression by IHC in breast cancers: A FISH analyses study of 165 cases. *T.J. Jodlowski, D.T. Walsh, L.A. Cannizzaro, K.H. Ramesh* Department of Pathology, Montefiore Medical Center, Bronx, NY. 10461.

Patients with a positive HER-2/neu gene amplification result by immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) may undergo further treatment with Herceptin (Trastuzumab) in combination with paclitaxel for a better prognosis. However, limitations in the FISH scoring criteria, including the relationship between hyperdiploidy and amplification, may rule out potential patients from receiving this or other beneficial treatments. By FISH, HER-2/neu amplification status is determined by the number of copies of the HER-2/neu gene localized to 17q11.2-q12, divided by the number of chromosome 17 centromeres present in infiltrating or invasive breast tumor cells. A ratio of 2.2 or greater for HER-2/neu is considered positive for amplification. In our study, cases with a ratio of centromere 17 signals to the number of cells analyzed, greater than 2.5 were considered hyperdiploid. By IHC, 1+ is considered negative for over expression of ERBB2, 2+ is weakly positive, and 3+ is positive. Of the 165 cases that we studied, a significant number were hyperdiploid for centromere 17 and negative for amplification (31%). Although multiple copies of HER-2/neu were indeed present in the hyperdiploid cases in our study, the scoring criteria for amplification dictates these cases to be reported as negative for HER-2/neu amplification. Of our 165 cases, 24% were 2+ by IHC, hyperdiploid and reported as negative for amplification by FISH. Additionally, 4% of our 165 cases were 3+ by IHC, hyperdiploid, and negative for amplification by FISH. Conversely, 5% of our 165 cases that were 3+ by IHC and hyperdiploid were amplified. Based on this study further evaluation of the FISH scoring criteria for HER-2/neu amplification and reporting of hyperdiploidy with or without HER-2/neu gene amplification in breast cancer is strongly suggested. Hyperdiploidy results, with or without HER-2/neu gene amplification obtained from studies on a larger cohort may provide valuable information that could be vital in the treatment and prognoses of patients with aggressive breast tumors.

DNA methylation analysis of the GABRB3 promoter in monozygotic twin pairs discordant for Non-syndromic Cleft lip and/or Palate. *J.W. Kimani, J.C. Murray* Department of Pediatrics, University of Iowa, Iowa City, IA.

Non-syndromic cleft lip with or without cleft palate (CL/P) and cleft palate only (CPO) are common birth defects of multifactorial etiology, involving both genetic and environmental factors. The gamma-aminobutyric acid (GABA-A) receptor, subunit beta 3 (GABRB3) gene is a clefting candidate gene: mouse knockouts of GABRB3 or its ligand result in a cleft palate, and one study has reported linkage disequilibrium between GABRB3 and CL/P. GABRB3 is located in the Prader-Willi and Angelman Syndromes 15q11-12 imprinted region, and is expressed specifically from the paternal allele, which is hypermethylated in the promoter region. As part of an ongoing twin study, we are testing the hypothesis that aberrant methylation in the GABRB3 promoter region may underlie discordance for clefting in a set of monozygotic (MZ) twins. Our samples include 3 pairs of control (unaffected) and 6 pairs of discordant twins. Using bisulfite sequencing, we have examined cytosine methylation at 114 CpGs in a 0.8kb region located within a CpG island immediately 5 of the GABRB3 gene. PCR products were gel extracted, cloned and sequenced. Analysis of at least 10 clones per individual showed varied methylation from clone to clone. The two chromosomes could not be distinguished due to lack of informative polymorphisms in the region, but some clones showing higher levels of methylation were suspected to be of paternal origin. Overall methylation profiles between pairs of twins (both control and discordant) were similar. Our current results do not provide support for the hypothesis that epigenetic differences between MZ twins who generally share an identical genotype may result in phenotypic discordance. Future studies include extension of the region studied, expansion of our sample size and examination of methylation differences at a global level.

Clinical Complications in Adults with Congenital Disorders of Glycosylation type Ia (CDG-Ia). *D. Krasnewich, K. O'Brien, S. Sparks* Medical Genetics Branch, NIH, Bethesda, MD.

Congenital Disorders of Glycosylation are a group of metabolic disorders resulting from defective synthesis of N-linked oligosaccharides. CDG-Ia is the most common of the sixteen known types defined by defects in different steps of the pathway. An increasing number of American adults with CDG-Ia are being recognized, but little is documented on the morbidity and mortality in this population. The clinical issues and management strategies for these affected adults will be discussed. We follow 4 adults with CDG-Ia, ages 19-36 years old. All are active, dysarthric conversant adults with moderate cognitive impairment. Although they are ataxic and wheelchair dependent, only the oldest man shows significant muscle atrophy. Three of four remain on anticonvulsants with only occasional seizures and none have had stroke-like episodes since their teen years. Their skeletal issues include severe kyphoscoliosis, joint contractures and osteopenia. Retinitis pigmentosa and myopia complicate their functional vision. The women do not menstruate and the men have small testes resulting from hypogonadotropic hypogonadism. A 33-year-old woman with CDG-Ia recently presented to NIH with a history of lower extremity deep vein thrombosis (DVT) and subsequent multiple pulmonary emboli. She was treated initially with heparin and Lovenox, required the placement of an inferior vena caval filter and is chronically anticoagulated on Coumadin. She developed immobilizing lower extremity pain and muscle contractions. Chronic muscle relaxant medication and physical therapy were necessary to regain her previous functional level. Hematologic evaluation revealed protein C, protein S, and antithrombin III deficiency, consistent with her diagnosis of CDG. Hematologic abnormalities have been described in CDG, including factor XI, antithrombin III, protein C and protein S deficiencies. DVTs have been published in three patients with CDG (ages 8, 17, and 18 years). Documentation of clinical complications and successful management strategies in adults with CDG will improve their quality of life and allow more informed prognostic discussions with families of younger affected individuals.

Prevalence of A69S in LOC38771, a gene associated with Age-Related Macular Degeneration, in a US population. *M. Mikula, A. Buller, W. Sun, C. Strom* Molecular Genetics, Quest Diagnostics Nichols Institute, San Juan Capist, CA.

Age-related macular degeneration (AMD) is a major cause of visual loss in developed countries with as much as 30 percent of the elderly population succumbing to some degree of impairment due to AMD. AMD is incurable although early treatment may prevent the more severe forms of the disease. Recent genome wide linkage studies have implicated two major AMD susceptibility loci, CFH on chromosome 1 and, more recently, LOC387715 on chromosome 10. Studies have determined the major AMD-susceptibility allele in LOC387715 to be a missense variant in exon 1, A69S. Data shows that this variant is responsible for a 2.85 increased risk of AMD in heterozygotes and an 8-fold increased risk in a German population where the frequency of the risk allele was 31.3% and 4.7% for heterozygotes and homozygotes respectively. (Hum Mol Gen 2005, 14(21)). There is a paucity of published frequency data for the A69S polymorphism in the general U.S. population. Based on the high prevalence of AMD and the A69S susceptibility allele determined thus far, additional frequency data in various susceptible populations is warranted. A single-nucleotide primer extension assay (SNaPshot) was used to screen anonymized samples previously characterized for Cystic Fibrosis and Factor V Leiden DNA analyses. The assay detects the A69S mutation by single-nucleotide extension of a primer next to the mutation, followed by capillary electrophoresis and fluorescent detection. 1219 samples were genotyped. 476 heterozygotes (39%) and 64 homozygotes (5.2%) for the risk allele were observed, similar to that of German population. The ability of the assay to detect the genotype at this locus has been confirmed by DNA sequencing.

TENR gene mutations in infertile males who display oligospermia or azoospermia. *T.D. Brown, L.P. Chorich, L.C. Layman* Dept of Ob/Gyn, Section of Reproductive Endocrinology, Infertility, & Genetics, Developmental Neurobiology Program, Institute of Molecular Medicine & Genetics, Medical College of Georgia, Augusta, GA.

The successful differentiation of male spermatids into mature spermatozoa is vital for normal fertility in the final stages of spermiogenesis. Tenr, an RNA binding protein, is exclusively expressed in the testis and appears to be involved in spermiogenesis. Disruption of this gene is predicted to interfere with male fertility. Homozygous male Tenr knockout mice exhibited infertility and no litters due to a 6-fold lower number of sperm in the epididymis, a 3-fold reduction in motility, and a 4.5-fold increase in abnormal sperm morphology. The purpose of the present study was to determine if TENR mutations occur in infertile human males who display oligospermia or azoospermia and to demonstrate TENR expression in human sperm. The TENR protein coding region (exons 3-13) and splice junctions from the genomic DNA of 66 infertile males (13 with azoospermia and 53 with oligospermia) and controls were subjected to PCR, agarose gel electrophoresis, and DNA sequencing. A silent mutation (Tyr338Tyr) c.270 C>T in exon 4 was identified; it was homozygous mutant in 2/64, heterozygous in 12/64, and homozygous wild type in 50 /64. To date, it has not been identified in controls. In addition, another potential mutation was identified in an intron (IVS12+20A>G); it was heterozygous mutant in 2/43 patients analyzed. We are currently determining if these mutations affect splicing of mRNA. Human TENR expression was demonstrated by RT-PCR in a control male with the presence of a predicted 2kb fragment using TENR-specific primers. The TENR gene represents a reasonable candidate gene for mutations in infertile males with abnormal semen analyses. However it appears that TENR gene mutations may not be a common cause of infertility in oligospermic or azoospermic males.

C677T and A1298C polymorphisms of the methylenetetrahydrofolate reductase gene and elevation of transaminases in Mexican patients with rheumatoid arthritis treated with methotrexate. *J. Mena[1], IP. Dávalos[1], L. González[2], J. Gamez-Nava[2], L. Sandoval[1], LE. Figuera[1], J. Sánchez[2], A. Celis-de la Rosa[1], M. Salazar-Páramo[2]* 1) División de Genética,CIBO, IMSS y CUCS, Universidad de Guadalajara; 2) División de Investigación, UMAE,HGZ 110, HE,CMNO, IMSS.

INTRODUCTION: The variants C677T and A1298C in the 5,10-methylenetetrahydrofolate reductase gene, has been involved in homocysteine metabolism and considered as a genetic risk factor in the increase of transaminases levels in Rheumatoid Arthritis patients treated with methotrexate (RA-MTX). **OBJETIVE:**To determine the genotype (GF) and allele frequencies (AF) of the C677T and A1298C variants of the MTHFR gene in mexican RA-MTX patients and the association with transaminasemia. **METHODS:**71 patients with RA (ACR 1987 criteria) and treated with MTX were included. Demographic, clinical laboratory and therapeutic data were obtained using a structured questionnaire. Molecular analysis was performed by PCR/RFLP method with the restriction enzymes *Hinf*I and *Mbo*II, for C677T and A1298C, respectively. For statistical analysis, chi square test with GDA and OR with de Finetti programs were used. **RESULTS:**From 71 patients, 13 (18%) had an increase in the serum transaminases levels. The 677T AF in RA-MTX patients with transaminasemia was slightly less (42.3% *vs* 44.7%)than in RA-MTX without transaminasemia. Otherwise the A1298C AF was greater in the RA-MTX with transaminasemia group (42.3%*vs* 21%), showing a p=0.02193 and OR=2.75. **CONCLUSIONS:** The A1298C polymorphism is associated with elevation of transaminases in RA-MTX patients. The identification of MTHFR A1298C in mexican patients with RA-MTX could be associated with elevation of transaminases. It allow to identify at risk patients and to give a treatment more individualized.

A novel autosomal dominant Hereditary Spastic Paraplegia with generalized dystonia: linkage to 2q34-2q31. *E. Leslie[1,2], D. Gilbert[1,2], M. Keddache[1,2], N. Leslie[1,2]* 1) Human Genetics, Cincinnati Children's Hospital Research Foundation, Cincinnati, OH; 2) University of Cincinnati.

The Hereditary Spastic Paraplegias (HSPs) are neurodegenerative disorders characterized by pyramidal tract disease and progressive spasticity and weakness of the legs and feet. In contrast, the dystonias are movement disorders characterized by extrapyramidal dysfunction and abnormal posturing. An extended family has been identified that spans 4 generations, with a novel combination of HSP, apparent anticipation, and generalized dystonia. Family members were considered affected if verified by neurological examination and unaffected if the exam was normal at age >40. All others, including those with increased reflexes, were considered uncertain. Initial whole genome linkage analysis of 42 pedigree members was performed using microsatellite markers from ABI linkage mapping set v2.5. Regions on chromosomes 2q and 4q showed maximum nonparametric mutlipoint lod scores above 4. To confirm and refine the region, SNP genotyping using the Illumina Linkage Array was performed on a limited set of 9 clearly affected individuals including those with uncomplicated HSP as well as more severely affected individuals with early onset and dystonic features. This approach identified a single critical region of 12 Mb on 2q (2q24-2q31) with a maximum nonparametric lod score of 17.4 (p value=0.0097). The multipoint parametric lod score with an AD model and 99% penetrance was 2.71 across a haplotype between rs2058996 and rs2007326. This region excludes the known HSP and Dystonia genes, including SPG 13, located telomeric to the identified region. A number of genes located in the region show expression in the brain and spinal cord and serve as attractive candidate genes for identification of the disease gene.

Towards Noninvasive Prenatal Diagnosis of Trisomy 21 Down Syndrome. *M. Hulten, F. Crea, W. Puszyk, R. Old*
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Currently many women delay childbearing until after the age of 35 years, leading to a dilemma caused by the combination of age-related reduced fertility and increased risk of fetal aneuploidy, in particular Trisomy 21 Down Syndrome. This dilemma is aggravated by the fact that traditional prenatal diagnosis, based on invasive procedures, such as chorionic villus sampling and amniocentesis, is associated with a procedure-related fetal loss rate in around 1-2 % of these (precious) pregnancies. It has been known for many decades that fetal cells enter the maternal circulation, and since 1997 that a relatively large proportion of free-floating DNA in maternal blood samples are of fetal origin, around 1-3 %. Thus it is now possible to use maternal blood samples for Noninvasive Prenatal Diagnosis (NIPD) based on the identification of fetal-specific, paternal DNA sequences, i.e. sex-linked and paternal dominant conditions. In this presentation we will review these advances and in particular highlight our own work on the development of epigenetic biomarkers for NIPD of common chromosome disorders, the majority of which are of maternal origin. We have developed a new assay for simple and rapid screening of epigenetic biomarkers in general and applied this method for identification of a number of chromosome 21-specific DNA sequences that show differential methylation patterns. These novel biomarkers are expected to allow specific identification and quantification of free-floating fetal DNA against a large background of maternal DNA- with the potential for NIPD of Trisomy 21 Down Syndrome in the first trimester of pregnancy. A large-scale clinical evaluation of this new NIPD assay is underway, using blood samples collected from pregnant women at high risk as ascertained by a combination of maternal serum screening and ultrasound (nuchal translucency) followed by QF-PCR aneuploidy diagnosis (and karyotyping) using chorionic villus samples. Any biomarker assessing the amount of free fetal DNA in maternal plasma may also be used for assessing risk of pregnancy-related disorders, such as pre-ecplampsia, premature labor and fetal intrauterine growth restriction.

Supernumerary marker chromosome 17: new case report, delineation of the phenotype, and comparison with other segmental 17p duplications. *R. McGoey[1], F. Tsien[2], M. Leake[2], M. Marble[3]* 1) Pathology, Louisiana State University, New Orleans, LA; 2) Genetics, Louisiana State University, New Orleans, LA; 3) Genetics, Children's Hospital of New Orleans, New Orleans, LA.

Trisomy 17p due to a supernumerary marker chromosome (SMC) is a rare and typically mosaic cytogenetic rearrangement. Ten patients with SMC(17) have been described but the molecular delineation is published in only four. A consistent genotype-phenotype correlation has not been established. We report a new patient with SMC(17) thus and extend the phenotypic spectrum associated with SMC(17).

Our patient is a 3 week old with cleft palate, hypotonia and a ventricular septal defect. She had hypertelorism, midface hypoplasia and micrognathia. Digits 2/4 were overriding the 3rd. Renal ultrasound and cranial CT were normal. Cytogenetics revealed a SMC which by FISH was from centromeric chromosome 17. This SMC(17) was identified in 60% of cells. Parental karyotypes were normal. At 2 months of age, she had reflux and was feeding via a gastrostomy tube. Her weight was 3.0kg (<3%), height 55cm (5-10%) and head circumference 36cm (2%).

To date, a consistent clinical pattern associated with SMC(17) has not been established. This may be due to the variable size and gene content of the SMC, the extent of mosaicism, and the fact that only four cases have been molecularly analyzed, all of which involved region 17p11.2. Among these cases, the more common clinical features included developmental delay, poor feeding, hypotonia and a triangular facies. Our patient shares these features but also has cleft palate and a heart defect. This combination of a facial cleft and heart defect has been reported in two SMC(17) patients, one with trisomy 17ptercen and another with trisomy 17pterp11. This may suggest potential clefting or cardiac genes in regions distal to 17p11.2. We will review the previously reported patients with SMC(17) emphasizing those cases with molecular refinement and those with clefting and heart defects. Follow-up molecular studies of our patient will also be presented.

Isolation of the t(1;19)(q10;p10) associated with human oligodendrogliomas using Conversion Technology. *R.B. Jenkins, H. Flynn, H. Blair, K. Meyer, P. Schneider, V. Marley, K. Schowalter, W.E. Highsmith* Div Laboratory Genetics, Mayo Clinic, Rochester, MN.

One of the goals of cancer cytogenetics is to isolate and clone chromosomal anomalies/breakpoints associated with malignancies. However, the isolation of these anomalies/breakpoints can be difficult because the amount of tissue may be limited and/or the tumor cells do not proliferate *in vitro*. Conversion Technology is a technique for the separation of human homologues in mouse-human somatic cell hybrids. For conversion, *msh2* deficient, embryonic mouse fibroblasts (E2) and human cells are fused and cultured with HAT selection. The hybrids are genotyped for the chromosome(s) of interest to determine which haplotype is present. Once hybrids containing the isolated homologues are identified, individual cell lines can be expanded. Here we report the use of Conversion Technology to isolate the t(1;19)(q10;p10) associated with human oligodendrogliomas that have combined 1p and 19q deletion. Cells from 3 different fresh glioma specimens were dispersed, washed and fused with mouse E2 cells. After 18 days of HAT selection, hybrid clones from each tumor were plucked into 96-well plates and cultured for an additional 10 days. DNA from replica plates was genotyped using 5 STR markers for both chromosomes 1 and 19. Based on the distribution of the STR markers, we were able to predict the genotypes of hybrids that contained the normal chromosome 1, the normal chromosome 19 and the derived t(1;19). We were able to isolate all three chromosomes from 2 gliomas with combined 1p and 19q deletion and all four chromosomes 1 and 19 from a glioma without combined deletion. FISH analysis of cells derived from passages 2 to 4 of the expanded hybrids confirmed the STR genotyping results. This study demonstrates that Conversion Technology can be used to isolate and clone normal and derived chromosomes 1 and 19 from fresh, uncultured, primary glioma specimens. Our results suggest that Conversion Technology can be applied to other primary malignancies for the isolation of normal and abnormal chromosomes. The isolation of such chromosomes may faciliate analyses of the human cancer genome.

***CCM2* deletions are a common cause of cerebral cavernous malformations.** *D.A. Marchuk[1], C.L. Liquori[1], M.J. Berg[2], F. Squitieri[3], T.P. Leedom[1], L. Ptacek[4], E.W. Johnson[5]* 1) Mol Genet & Microbiol, Duke Univ Medical Ctr, Durham, NC; 2) Strong Epilepsy Center, Dept of Neurology, Univ of Rochester Medical Ctr, Rochester, NY; 3) Neurogenetics Unit, Istituto di Ricovero e Cura a Carattere Scientifico, Neuromed, Pozzilli, Italy; 4) Dept of Neurology, Univ of California San Francisco, San Francisco, CA; 5) Molec Diagnostics and Biobanking, Prevention Genetics, Marchfield, WI.

Cerebral cavernous malformations (CCMs) are vascular abnormalities of the brain that can result in a variety of neurological disabilities, including stroke and seizures. Mutations in the gene *Krit1* are responsible for CCM1, mutations in the gene *MGC4607* are responsible for CCM2, and mutations in the gene *PDCD10* are responsible for CCM3. Over several years, we have collected 64 CCM probands with family history and/or multiple lesions, and sequence analysis showed that a high proportion (41%) of these probands lacked any identifiable mutation. We now report that large genomic CCM2 deletions represent a major component of this disease which are missed by routine mutation screens. We used multiplex ligation-dependent probe analysis (MLPA) to screen 26 non-CCM1, non-CCM2, and non-CCM3 probands for potential deletions or duplications within all three CCM genes. We identified a total of 14 deletions - 1 in the CCM1 gene, 13 in the CCM2 gene, and none in the CCM3 gene. Interestingly, 50% of the identified CCM2 deletions span exons 2-10. Sequence and deletion analysis of our 64 probands gave a frequency ratio of 39% (25/64) CCM1, 36% (23/64) CCM2, 6% (4/64) CCM3, and 19% (12/64) unknown. These data indicate that the prevalence of CCM2 is much higher than previously suspected and that the frequency of CCM1 mutations and CCM2 mutations are approximately equal. With respect to CCM2, we highly recommend that deletion screening should be performed to avoid missing over half of the responsible mutations. The fact that 19% of CCM mutations still remain unidentified suggests either that the remaining mutations cannot be identified by sequence or deletion analysis or that there may be an additional CCM gene(s).

A novel autosomal recessive degenerative phenotype of cerebellar atrophy : homozygosity mapping to a 1.5cM locus on chromosome 22. *Sh. Khateeb[1], O. Birk[1,2], R. Ofir[1], H. Flusser[3]* 1) The Morris Kahn Laboratory of Human Genetics Dept. of Development Genetics , Ben-Gurion University, Beer-Sheva Israel; 2) The Institute of Genetics,Beer-Sheva,Israel; 3) Soroka Medical Center,Beer-Sheva,Israel.

We describe a novel autosomal recessive syndrome of progressive cerebellar atrophy, affecting 6 members of 3 related families of a single Bedouin Israeli inbred kindred. Affected individuals, born to consanguineous marriages, were normal at birth. At age ~18 months began gradual psychomotor retardation with regression in cognitive and motor abilities, progressing to death of asphyxia at age 7-10 years. Clinical hallmarks were hypotonia of the trunk with enhanced tonus in the limbs, augmented tendon reflexes and pyramidal signs. Combined vertical and horizontal nystagmus and pallor of the optic discs were evident. There was no dysmorphism and no defects or phenotypic stigmata other than the neurological phenotype. Computerized tomography and MRI demonstrated cerebellar atrophy, with widened cerebellar folds and atrophy of the vermis. The fourth ventricle and the cysterna magna were enlarged, with normal lateral ventricles and no evidence of cortical brain atrophy. Genomic DNA samples of 22 family members including 6 affected individuals were tested: linkage analysis studies ruled out association with any of the known loci associated with phenotypes involving cerebellar atrophy. Genome-wide linkage analysis using 400 polymorphic markers failed to identify a locus associated with the disease. Genome-wide linkage analysis using Affymetrix 10K SNP microarrays identified a 1.5cM region of homozygosity on chromosome 22, with a maximum LOD score of [Zmax] = 4.7 at recombination fraction [q] = 0.0. Analysis of candidate genes within the defined interval is underway.

Investigations of the effect of CTCF on the promoters of the anti-apoptotic Bcl-2 gene. *A. Molouki*[1], *E. Klenova*[2], *H. Najmabadi*[1] 1) Genetics Department, University of Welfare Science & Rehabilitation, Tehran, Iran; 2) Department of Biological Sciences, University of Essex, Colchester, UK.

The highly conserved, ubiquitously expressed 11-Zn finger nuclear protein CTCF is involved in the mechanisms through which this transcription factor acts at various levels of gene regulation. This experiment is a follow-up to a series of recent investigations performed by Dr. Elena Klenova and colleagues on how Bcl-2 protein can overcome the effects of lowering CTCF levels and alternatively, how CTCF can overcome the effects of the pro-apoptotic protein, Bax in the cell. The main attention was focused on the role of CTCF in regulation of the promoters of the Bcl-2 gene in breast and non-breast cancer cell lines. Co-transfection assays using LB322 Bcl-2-Luc promoter-reporter constructs showed that CTCF protein strongly activates the expression of Bcl-2 in 293T and slightly in MCF-7 and CAMA-1 cancer cell lines. Further experiments revealed that CTCF can both repress and activate the promoter by binding to different regions of the promoter. Co-transfections of LB124, LB334 and LB556 with CTCF into the same cell lines showed to repress the expression while LB335 combined with CTCF resulted in activation of expression suggesting that CTCF can only activate the Bcl-2 expression and protect cells from apoptosis by binding to a sequence located between bases -170 and -1281 of the promoter. Furthermore these experiments demonstrate that CTCF anti-sense inhibits the CTCF transcription in MCF-7 cells resulting in a reduction of Bcl-2 expression. This result was not confirmed in 293T co-transfections.

KELVIN: A 2nd generation distributed multiprocessor linkage and linkage disequilibrium analysis program. *Y. Huang[1], A. Segre[2], J. OConnell[3], H. Wang[2], V. Vieland[1]* 1) Columbus Children's Research Institute , Columbus, OH; 2) University of Iowa, Iowa City, IA; 3) University of Maryland, Baltimore, MD.

Genetic analysis software has been widely used to map disease genes. This success is largely due to the development of statistical genetic analysis techniques and methodologies over the last few decades. Each method, and its software implementation, has specific strengths and weaknesses. LIPED, introduced in 1974, was the first model-based linkage analysis program. MLIP (Multiprocessor LIPed), developed at the University of Iowa, extends LIPED's core algorithm to calculate two-point LOD scores over a multi-dimensional trait parameter space using a cluster of computers. MLIP's parallel processing has greatly enhanced our ability to analyze complex traits. However, since LIPED is limited to two-point analyses, we are now implementing a new extended version of VITESSE into MLIP. VITESSE uses novel algorithms such as set-recoding and fuzzy inheritance algorithms to accelerate the likelihood calculation. Our new implementation, called KELVIN, uses data structures and software engineering techniques to allow for future expansion. The KELVIN design supports both two-point and multipoint linkage, dichotomous, quantitative, and mixed traits, linkage and linkage disequilibrium mapping, heterogeneity and other complications such as covariate dependent penetrances, and uses Bayesian sequential updating to accumulate evidence across multiple data sets. KELVIN will also, like MLIP but unlike VITESSE, employ large clusters of computers for distributed computing for improved speed of calculation. Moreover, we have also prototyped another innovative approach to exploit symbolic computation to reduce likelihood calculation costs significantly (see also abstract entitled "Fast Computation of Large Numbers of LOD Scores for Genetic Linkage Analysis"; by Wang et al.). The software firepower represented by KELVIN will support computationally-intensive calculations, like the PPL, over higher-dimensional spaces than are possible today.

The OpenArray Platform: Enabling high-throughput SNP genotyping applications in nanoliter volumes. *S. Liu-Cordero, K.D. Munnelly, J. Garcia, K. Yoder, J. Cho, A. Katz, T. Kanigan, T. Morrison, C. Brenan, D.G.W. Roberts*
BioTrove Inc, Woburn, MA.

Single nucleotide polymorphism (SNP) genotyping has become increasingly important in both pharmaceutical and agricultural biotechnology as a means of identifying biologically important genetic variations. Examples include understanding genetic variations associated with drug metabolism, association of polymorphisms and diseases, and marker assisted breeding. BioTrove has developed a SNP genotyping platform based on through-hole technology, a broadly applicable nanoliter fluidics technology for parallel and low-volume solution phase reactions. The OpenArray chip consists of 3072 through-holes that can be loaded with reagents to perform individual 33 nL reactions for use in endpoint genotyping applications. The unique configuration of the through-holes enables the researcher to interrogate a large number of nucleic acid samples against a large number of assays in a flexible, configurable format. By altering the number of assays or the number of samples the researcher can easily customize the OpenArray to meet their changing needs. Researchers using this technology benefit from the parallelism of microarrays and the data quality of solution phase reactions. Performance characteristics of the OpenArray platform will be presented and implications to genomic research will be discussed.

Validation of SNP Oligonucleotide Microarray Analysis (SOMA) for Clinical Cytogenetic Diagnosis. *V. Jobanputra[1], O. Nahum[2], K. Anyane-Yeboa[1], W. Chung[1], Y. Sun[2], E. Bottinger[2], W. Zhang[2], D. Warburton[1], B. Levy[1]*
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SNP arrays are high-density oligonucleotide-based arrays that can identify both loss of heterozygosity (LOH) at individual nucleotides and copy number alterations. In contrast to the CGH array approaches, in SNP arrays only one genomic sample is hybridized to the array, so copy number changes are identified by comparison with independent control hybridizations. In the current study, copy number gains and losses were determined by means of the Circular Binary Segmentation method. We are currently validating the Affymetrix 500K SNP Oligonucleotide microarray for clinical cytogenetic analysis (SOMA) and report on our first 18 cases. Cases were chosen to contain a variety of known cytogenetic aberrations, including whole chromosome aneuploidy, unbalanced rearrangements, marker chromosomes and microdeletions. All aberrations except one subtelomeric deletion were correctly identified by segmentation analysis of the SOMA data. With over half a million SNPs and a mean spacing of 5.8 kb, the 500K SNP array offers a comprehensive whole genome scan and has the potential to provide the highest resolution of copy number detection currently available. Since deletions and duplications can be precisely defined by using the SNP positions on the genome browser, SOMA will useful for the clinical interpretation of both visible and submicroscopic cytogenetic imbalances. Another advantage of using a SNP-based array lies in the concurrent availability of genotype information that would allow simultaneous DNA-based studies such as uniparental disomy (UPD), zygosity and maternal cell contamination. Further studies of normal populations are currently underway and these will be critical for delineating benign copy number variants. In addition, our ongoing study will also assess whether the coverage of the SNPs is adequate for most clinical cytogenetic diagnoses, including all known microdeletions and cryptic sub-telomeric gains and losses.

Evidence for FOXE1 Having an Etiologic Role in Nonsyndromic Cleft Lip With or Without Cleft Palate (CL/P).

L. M. Moreno[1], S. A. Bullard[1], J. Martin[1], M. A. Mansilla[1], M. Johnson[1], M. Arcos-Burgos[2], H. Mishima[1], B. S. Maher[3], T.H. Goldstein McHenry[3], M.E. Cooper[3], C. Valencia-Ramirez[4], A. Hing[5], E.J. Lammer[6], M. Jones[7], A. Jugessur[1], N. H. Robin[8], P. Chines[2], J. C. Murray[1], M. L. Marazita[3], A. C. Lidral[1] 1) Univ Iowa, Iowa City, IA; 2) NHGRI, NIH, Bethesda, MD; 3) Craniofacial and Dental Genetics, U. of Pittsburgh, Pittsburgh, PA; 4) Odontologia, U de Antioquia., Medellín, Colombia; 5) Univ Washington, Seattle, WA; 6) Children's Hospital Oakland Research Institute, Oakland, CA; 7) Children's Hospital, San Diego, CA; 8) Univ Alabama, Birmingham, AL.

Nonsyndromic (CL/P) is caused by failure of fusion between the nasal and maxillary prominences. Genes expressed in facial prominences during primary palatogenesis or in which mutations cause a clefting phenotype are good candidates for CL/P. FOXE1 is a transcription factor expressed in cranio-pharyngeal ectoderm and thyroid, yet Foxe1 expression has not been described during primary palatogenesis. Only two FOXE1 mutations are known, both causing Bamforth-Lazarous syndrome, which includes cleft palate and thyroid dysgenesis. FOXE1 maps within a major CL/P locus: human 9q22-q33 (Marazita et al., 2004). The goal of this study is to determine if it has a role in primary palate formation. Whole mount and section in situ hybridization were performed using a digoxygenin labeled Foxe1 RNA probe in C57BL/6J mouse embryos at E9.5, E10.5 and E11.5. Foxe1 expression was observed at E10.5 and E11.5 particularly in the epithelium involved in the fusion of the medial nasal and maxillary processes. These data are consistent with FOXE1 playing a role in lip development. Also, Foxe1 expression is similar to that of Shh which is known to regulate Foxe1 transcription (Brancaccio et al. 2004). Altering Shh function in chick embryos results in cleft lip (Hu & Helms 1999), suggesting that Foxe1 and Shh are in a pathway involved in lip formation. This is supported by human studies (Marazita et al. & Mansilla et al. this meeting) showing highly significant association between FOXE1 and CL/P. Grant Support: NIH RO1DE14677 (ACL, MAB, MLM), KO2DE015291 (ACL), NIH P60 DE13076 (JCM), MOD Grant #6-FY01-616 (ACL, MAB) and P50-DEO16215.

A principal components-derived psychosocial phenotype reflecting Type A Behavior and Ambition is linked to chromosome 11q in Mexican Americans. *J.W. Kent Jr.[1], S.P. Fowler[2], H.P. Hazuda[2], R. Arya[2], M.P. Stern[2], J. Blangero[1], R. Duggirala[1]* 1) Dept. of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX; 2) Div. of Clinical Epidemiology, Dept. of Medicine, University of Texas Health Science Center, San Antonio, TX.

Measures of mental state can be challenging phenotypes for quantitative genetic analysis, as they are not expected to map one-on-one with the underlying biochemical pathways regulated by genes. Such measures may be intercorrelated and represent effects of multiple underlying processes. The San Antonio Family Diabetes/Gallbladder Study (SAFDGS) is a large, extended family-based study of the genetics of diabetes and its comorbidities in Mexican Americans. We assessed by questionnaire 8 psychosocial measures (Ambition, Trait anger, Self esteem, Emotional lability, Mastery, Non-easygoingness, Perceived daily stress, and Type A behavior) in 450 participants (176 males and 274 females) in SAFDGS. Variance components-based quantitative genetic analyses were performed in SOLAR. All of the measures except Anger were heritable (range 0.23-0.41) and most exhibited significant genetic and/or environmental correlations in bivariate analyses. Genome-wide linkage scans of the individual traits yielded suggestive evidence of linkage but none at genome-wide significance (LOD ? 3). To maximize the information available from the correlations among traits, we performed a principal components analysis on the standardized traits. The first 3 principal components accounted for 69% of the total variance. Each of these components was heritable: PC1 (weighted on Mastery, Esteem, Anger, and Lability), 0.29; PC2 (Type A, Ambition), 0.38; PC3 (Non-easygoingness), 0.40 (all significant at $P<0.001$). PC1 showed suggestive linkage (LOD = 2.2) on Chr. 20. PC3 showed a suggestive LOD = 2.4 on Chr. 16. PC2 showed significant linkage (LOD = 3.2) on Chr. 11q. Several previous studies, including linkage analysis and analysis of chromosomal rearrangements, have associated this region with bipolar disorder. These results provide a preliminary glimpse at the genetic architecture of normal variation in personality and mood in this study population.

Chromosome 15q telomere imbalances: Association of *IGF1R* with growth abnormalities. *Z. Nawaz, D.H. Ledbetter, C.L. Martin* Department of Human Genetics, Emory University, Atlanta, GA.

Patients with cytogenetically visible deletions of the distal part of 15q have intrauterine growth retardation (IUGR), postnatal growth delay and variable mental retardation. In contrast, patients with duplication of distal 15q have macrosomia at birth, overgrowth, macrocephaly and mild developmental delay (DD). It has previously been shown that deletions, duplications or mutations of the insulin-like growth factor 1 receptor gene (*IGF1R*), which maps to 15q26.3 and codes for a kinase receptor-containing transmembrane protein, disrupts normal growth. Using genome-wide telomere FISH analysis, we identified three patients with *de novo* deletions of the 15q telomere region: one patient had a terminal deletion while the other two had unbalanced translocations with X/Yq and 3q, respectively. All three patients had IUGR, postnatal growth retardation and significant DD. To delineate the size of the deleted material on chromosome 15q and determine if the *IGF1R* gene was included in these deletions, a combination of array comparative genomic hybridization (aCGH) with a homebrew chromosome 15 BAC array and FISH analysis were utilized. The 15q deletion in the patient with an unbalanced translocation between 15q and X/Yq was determined to be ~4.6-5.5 Mb while the 15q deletion in the patient with the unbalanced translocation with 3q was ~4.3-4.6 Mb. The patient with the pure terminal deletion had a deletion ~7.5-8.0 Mb in size. The deleted region in all three patients includes *IGF1R*, indicating that they are hemizygous for this gene, which is believed to be responsible for their growth delays. We also identified a patient with overgrowth and a cytogenetically visible duplication of the 15q telomere region resulting from an unbalanced translocation with 14p. FISH analysis revealed that the duplicated portion of 15q includes *IGF1R*. Our data and the published data on 15q imbalances suggest that this cytogenetic rearrangement should be considered in patients with growth abnormalities. In addition, since three of our cases had normal G-banded chromosome analysis, targeted molecular cytogenetic analysis for *IGF1R* is warranted if routine G-banding is normal.

The role of Wnt9b during primary palatogenesis. *H. Mishima[1], S.A. Bullard[1], L.M. Moreno[1], T. Busch[1], M. Arcos-Burgos[8], C. Valencia[3], A. Hing[4], E.J. Lammer[5], M. Jones[6], N. Robin[7], B.S. Maher[2], M.E. Cooper[2], T. McHenry[2], M.L. Marazita[2], A.C. Lidral[1]* 1) Univ Iowa, IA; 2) Univ Pittsburgh, PA; 3) Univ Antioquia, Colombia; 4) Children's Hosp., Seattle, WA; 5) Children's Hosp., Oakland, CA; 6) Children's Hosp., San Diego, CA; 7) Univ Alabama, AL; 8) NIH, MD.

Cleft lip with or without cleft palate (CL/P) is a common human birth defect. Studies using a mouse strain with spontaneous CL/P have identified 2 epistatically interacting loci, including the *clf1* locus containing the Wnt9b and Wnt3 genes. A retrotransposon insertion has been discovered downstream of Wnt9b (Juriloff et al. 2005), suggesting Wnt9b is involved in primary palate development, which is corroborated by incompletely penetrant CL/P occurring in Wnt9b null mice (Carroll et al. 2005). This study aims to characterize the role of Wnt9b during primary palatogenesis. Mouse embryos from heterozygous Wnt9b null matings were observed at E16.5 to determine frequency of CL/P. Eleven of 22 Wnt9b null embryos had clefts, whereas no clefts occurred in 25 wildtype embryos, and 1 of 35 Wnt9b null heterozygous embryos had cleft palate. In situ hybridization showed both Wnt9b and Wnt3 are expressed in the facial ectoderm during primary palatogenesis. However, Wnt9b is expressed in the ectoderm between the fusing medial and lateral nasal process whereas Wnt3 is not. Expression of potential downstream genes, Bmp4, Cux1, Cux2, Msx1 and Fgf8 revealed incomplete penetrance with reduced Fgf8 expression in the lateral nasal processes. Crabp1, a neural crest cell marker, showed reduced expression at E11.5 in Wnt9b null embryos. No reduction in Axin2, Dkk1, and Fath, which are Wnt canonical pathway markers, was observed. The Crabp1 data suggests normal NCC migration at earlier stages, but loss of Crabp1 induction or decreased NCC survival at E11.5. Persistent Wnt canonical signaling suggests functional redundancy or Wnt9b acts through a non-canonical pathway in the face. Reduced Fgf8 expression connects the Wnt and Fgf signaling pathways during facial development. The syntenic region for *clf1* is on human 17q, and has been implicated in human multifactorial CL/P. We hope to report results evaluating this region in humans.

Candidate gene analysis at 16q12-13 for SLE susceptibility. *S.K. Nath[1], X. Kim-Howard[1], F. Ota[1], M. Gains[1], P. Viswanathan[1], R. Vijayaraghavan[1], D. Mandhyan[1], J. Kelly[1], K. Kaufman[1], J.B. Harley[1,2]* 1) Arthritis & Immunology, Oklahoma Medical Research Foundation, Oklahoma City, OK; 2) US Department of Veterans Affairs Medical Center, Oklahoma City, OK, USA.

Systemic lupus erythematosus (SLE) is a multi-system autoimmune disease with high morbidity and mortality morbidity and mortality with an etiology and pathogenesis that are largely unknown. Genetic susceptibility plays an important role in the development of SLE. SLE susceptibility at 16q12-13 was demonstrated previously by multiple independent research groups. We confirmed this linkage in two independent data sets. Additionally, our recent meta-analysis based on 9 published whole-genome scans has further identified this genomic region as one of the most promising regions that may harbor a SLE susceptibility gene. Therefore, repeated evidence for linkage in several independent reports, together with our two individual reports and a meta-analysis, provide strong evidence for a SLE susceptibility gene at 16q12-13. In this study we examined the role of 30 SNPs from 4 plausible candidate genes using the LD based association mapping approach. We have assessed the genotypic as well as allelic association on 1490 cases and 1965 controls, which consisted of 4 separate groups. These are European-American (n=2043), African-American (n=1144), Hispanic (n = 418) and Gullah (n= 240). The Gullah are an African-American population living in the Sea Islands and the nearby coastal low country region of South Carolina. Most of the SNPs were in Hardy-Weinberg Equilibrium for the controls; only 2 SNPs from African-American controls were not in HWE. Using single SNP at a time as well as moving-window haplotype-based analyses, we have found significant evidence of statistical association (p-value ranges from 0.01 to 0.001) between multiple SNPs and SLE phenotype in multiple ethnic groups. The estimated odds ratio per SNP ranges from 1.2 to 2.4. We also tested the association between a subset (lupus nephritis) from the SLE cases and normal controls. We discuss the implication of these associations and the involvement of the candidate genes in the pathogenesis of SLE.

Exploiting genetic model information to identify homogenous pedigrees. *M.W. Logue[1], J.W. Park[2], J.F. Cremer[2], A.M. Segre[1,2], V.J. Vieland[1,3]* 1) Center for Statistical Genetics Research, University of Iowa, Iowa City, IA; 2) Department of Computer Science, University of Iowa, Iowa City, IA; 3) Department of Psychiatry, University of Iowa, Iowa City, IA.

After a linkage peak has been identified based on a set of pedigrees it is often necessary to identify a homogeneous subset of those pedigrees for use in follow-up molecular work with the goal of localizing the putative susceptibility gene. In the absence of relevant covariate information (e.g., age of onset), this is usually done by selecting the pedigrees that individually yield a suitably positive LOD score in the region of the peak. Here we propose selecting a subset with a clustering algorithm that includes pedigrees based on their ability to contribute to the total integrated linkage likelihood (TILL), defined as an expected LOD score, with the expectation taken with respect to the genetic model and . Pedigrees are greedily added to the cluster in descending order of contribution until the percentage TILL increase associated with a newly added pedigree falls below a certain threshold. This method exploits additional information implicit in the shape of the likelihood surface, and only adds pedigrees to the cluster which are compatible in terms of inheritance models. In a series of preliminary simulations, we show that using the genetic model information to identify pedigrees linked to a particular locus has the potential to greatly increase accuracy and reliability when compared to a simple maximum LOD score selection criteria.

Construction and analysis of a novel database of human neutral polymorphisms. *M.F. Hammer[1], M. P. Cox[1], D.W. Garrigan[1], A. Woerner[1], T. Severson[1], J.D. Wall[2]* 1) ARL Division of Biotechnology, University of Arizona, Tucson, AZ; 2) University of Southern California, Los Angeles, CA.

The accurate characterization of human demographic history requires the analysis of neutral polymorphisms from a large number of independently evolving loci. Towards this goal we are re-sequencing 15-20 kb segments from 90 regions of the genome in 90 humans from six geographically diverse human populations (e.g., three from sub-Saharan Africa and three from Eurasia) and 4 great apes. While several larger-scale publicly available re-sequencing databases exist, none of these focuses entirely on re-sequencing non-coding DNA from genomic regions with moderately high recombination and low gene density. Hence, we are in a position to make inferences about human demographic history that are minimally confounded by the effects of natural selection. Statistical approaches are being developed for explicitly testing a number of hypotheses pertaining to the structure of the ancestral population giving rise to *H. sapiens* and the extent of admixture with archaic humans. We have also been working on new quantitative methods for estimating population parameters for a basic model of recent human population history. This model includes several factors known to be important in recent human demography: population divergence between different continental groups, migration between different continental groups, recent population growth and a bottleneck in non-African populations.

Comparison Gene Expression Micro-Array Analysis Between a Term Pregnant Mother and Her Infant Identifies Unique Fetal Biomarkers in Maternal Whole Blood. *J.L. Maron[1], K. Johnson[1], C. Lai[2], M. Ramoni[3], D. Slonim[4], Z. Jarrah[1], D.W. Bianchi[1]* 1) Division of Genetics, Tufts-NEMC, Boston, MA; 2) Nutrition and Genomics Lab, JM-USDA HNRC, Boston, MA; 3) Partners Center for Genomics, Boston, MA; 4) Tufts University, Medford, MA.

Background: The discovery of fetal mRNA transcripts in the maternal circulation holds great promise for noninvasive prenatal diagnosis. Previous studies have been limited in their scope by identifying only placental-derived or Y chromosome gene transcripts. Here, we suggest that mRNA extracted from maternal and umbilical cord whole blood samples, and analyzed with the use of comparative oligonucleotide microarrays, identifies unique fetal transcripts representing broad biological functions. Methods: Comparison HGU133A microarray analyses were performed using extracted and amplified mRNA from whole blood samples. Gene transcripts present in both the womans blood prior to delivery and her infants umbilical cord blood, but absent from the woman within 1 day of delivery were identified as potential fetal biomarkers. Results: Of 14,500 genes on the microarray, 253 genes were identified. Of these, 60 appear to be fetal specific. These include 34 genes involved in embryonic or fetal development including a predominance of neural developmental genes, and embryonic regulatory genes such as *HOXA7*, *SHOX*, and *AMN*. Interestingly, 10 sensory genes involved in auditory, visual and olfactory perception are upregulated in the fetus and detected in the antepartum mother. 16 candidate genes of physiological interest were identified including *KLF1*, which facilitates transition of fetal to adult hemoglobin, and atrionatriuretic peptide receptors, *NPR1* and *NPR2*. Conclusions: Microarray analysis of mRNA gene transcripts in maternal whole blood is feasible, and can identify novel fetal genes in the maternal circulation. The use of whole blood, as compared to prior reports using plasma, identifies a unique subset of fetal genes not previously recognized, which represent a diverse group of biological functions and may allow noninvasive monitoring of fetal development.

Lack of association of two SNPs on chromosome 10 (*rs498055* and *rs4417206*) with Alzheimers Disease. *R.L. Minster*[1], *S.T. DeKosky*[2], *M.I. Kamboh*[1] 1) Department of Human Genetics; 2) Department of Neurology, University of Pittsburgh, Pittsburgh, PA.

Only association between sequence variation in *APOE* and late-onset Alzheimers disease (LOAD) has been consistently replicated, but that variation is estimated to explain only part of the genetic variation in the risk of developing LOAD. Several groups have reported evidence of linkage on chromosome 10 to LOAD. A recent scan of single nucleotide polymorphisms (SNPs) on the long arm of chromosome 10 reported significant associations between *rs498055* and *rs4417206* and risk of LOAD (Grupe et al. *Am J Hum Genet* 78). We examined the two SNPs in up to 1,060 Caucasian Americans with LOAD and up to 810 age-matched healthy Caucasian Americans. Both SNPs were genotyped by template-directed dye terminator incorporation assays detected with fluorescence polarization. Measures of linkage disequilibrium between the two SNPs (D' = 0.881, r^2 = 0.445) in our sample were similar to those calculated in Grupe and colleagues. Genotype frequencies in both SNPs did not deviate significantly from those expected under Hardy-Weinberg equilibrium. We observed no statistically significant differences in allele frequencies between the cases and controls at either locus. The frequency of the minor allele of the *rs498055* SNP was 0.462 in cases and 0.464 in controls (p = 0.884), and for the *rs4417206* SNP it was 0.343 and 0.314 (p = 0.139). We also examined the impact of genotypes at these loci on age-at-onset and found no significant association. As has too often been the case with initial association studies, we were unable to replicate previous results in our relatively large case-control cohort, and a definitive source for the chromosome 10 linkage peaks remains elusive.

Clinical Presentation and Diagnosis of Patients with Hutchinson-Gilford Progeria Syndrome. *M.A. Merideth[1], W.J. Introne[1], L.B. Gordon[2], A.C.M. Smith[1], W.A. Gahl[1]* 1) SHBG, MGB, ORD, NHGRI/NIH, Bethesda, MD; 2) Brown University, Providence, RI.

Hutchinson-Gilford Progeria Syndrome (HGPS) is a sporadic autosomal dominant segmental premature aging syndrome with an incidence of 1 in 4-8 million. The basic defect involves an abnormal lamin A that disrupts the inner nuclear membrane, with protean, multisystemic manifestations. Since February of 2005 we have studied 15 patients with HGPS at the NIH under an IRB-approved natural history protocol. The clinical presentations of children with HGPS at the time of diagnosis have been remarkably consistent, confirming DeBusks description of the HGPS phenotype as so constant that patients with the condition bear an uncanny resemblance to one another (DeBusk, FL. The Hutchinson-Gilford progeria syndrome, J. Pediatr 80:697, 1972). The 15 patients seen at the NIH were diagnosed at a median age of 19 months (range 3.5 months to 4 years). The diagnosis was made most frequently by geneticists (7 of 15 patients), followed by dermatologists (5 of 15 patients), and a gastroenterologist in 1 case. The diagnosing physicians specialty was unclear in 2 patients. At the time of diagnosis, all of the children had skin findings, generally described as sclerosis, and all had been diagnosed with failure to thrive. Alopecia was present in 87% (13 of 15 patients) at the time of diagnosis, and 73% (11 of 15 patients) were described by their parents as sleeping with their eyes partially open. Circumoral cyanosis was observed in 60% (9 of 15 patients) and prominent scalp veins were observed in 53% (8 of 15 patients). Joint contractures were present at the time of diagnosis in 33% (5 of 15 patients), with decreased joint range of motion observed in an additional 2 patients. Our clinical natural history protocol will provide detail to the phenotypic description of patients with HGPS. In addition, therapies addressing the basic defect in HGPS are now being considered. Recognition of the earliest features of HGPS will prompt more rapid diagnosis and may hasten the implementation of salutary interventions.

A novel program for allele- and genotype-based association tests on quantitative traits. *Y.W. Li*[1], *L. Zhang*[2], *E.R. Martin*[2], *Y.J. Li*[2] 1) Stat Dept,NC State Univ; 2) Ctr for Human Genetics, Duke Univ Med Ctr.

The QTDT program (Abecasis et al. 2000) is a frequent used tool for association study for quantitative traits. QTDT includes five association methods, which are all allelic-based tests and applicable to nuclear families. Since genotype of a marker is a direct observation for an individual, it is desirable to assess association at the genotypic level. In some cases, family data includes extended pedigrees, but the current methods, such as the method proposed by Monks and Kaplan (2000) (MK method), use only partial information from the general pedigree, which may result in the loss of statistical power. In this study, we extended the MK method and developed a novel program that could perform both allele- and genotype- based association tests in any pedigree structures. The new genotype-based MK method provides test statistic for each genotype and a global test for combined genotypes. We evaluated the power and type I error rates of our genotype-based MK method through a series of simulation studies. Nuclear families with sibship sizes of one to six were simulated under various genetic parameters including different QTL heritability, allele frequencies of marker and QTL, degree of linkage disequilibrium between marker and QTL, and different genetic models. To evaluate the performance of the new program, we utilized simulated two generations pedigree data and real data. The simulation results demonstrate that our proposed genotype-based MK method has correct type I error and comparable statistical power with the allele-based MK method. Interestingly, genotype-based MK method shows higher power than the allele-based MK method under the overdominate model where the dominant effect exceeds additive effect at the QTL (max difference: 83.1%). The modified version of allele-based MK method in program showed correct type I error for general pedigree. We also demonstrate the utility of the program applied to real data to test association between GSTO1 and age-at-onset data of Alzheimer disease (P=0.025 for the CC genotype of rs4925). From the overall testing, we proved that the program is a robust tool to analyze the quantitative trait data in general pedigrees.

Identifying Genetic variants in Urea Cycle Enzymes. *S. Mitchell, M. Neill, L. Hall, M. Summar* Human Genetics, Vanderbilt Univ, Nashville, TN.

Our lab is carrying out a comprehensive screen of the genes encoding urea cycle enzymes to identify genetic variants. The urea cycle functions to remove nitrogen waste from the body and to generate intermediate products necessary for other metabolic functions. A number of inherited metabolic disorders are associated with the urea cycle.

There are five key enzymes within the urea cycle: carbamoyl phosphate synthetase I(CPSI), ornithine carbamoyltransferase(OTC), argininosuccinate synthase(ASS), argininosuccinate lyase(ASL), and arginase 1(ARG1). Additionally, a sixth enzyme, N-acetyl glutamate synthase (NAGS) is necessary for formation of N-acetyl glutamate which is an allosteric activator of CPSI, the first enzyme in the cycle. Deficiencies in any of these enzymes result in hyperammonemia; which is characterized by high levels of blood ammonia. Ammonia is a toxic compound and has detrimental effects when its levels are elevated, including central nervous system dysfunction, brain damage, coma and death. We hypothesize that functional polymorphisms exist within these enzymes which may confer susceptibility for disease or dysfunction particularly when this system is stressed.

Although some polymorphisms have been described, a comprehensive screen of all the urea cycle enzymes has not been performed. Variants are identified via single stranded conformational polymorphism (SSCP) analysis and sequencing. We have identified a number of polymorphisms in the CPSI gene, and functional characterization of the T1405N substitution is underway. Kinetic enzyme assays are being performed for functional characterization of the T1405N CPSI polymorphism. Preliminary results obtained from enzyme assays reveal differential activity of CPSI. Screening of NAGS, OTC and ARG1 is ongoing. In a preliminary screen of NAGS, polymorphisms were observed in exon 4 and intron 6. Once these screens are complete we will focus on the final two enzymes, ASS and ASL. Through these studies we will gain a better understanding of how genetic variants contribute to urea cycle deficiencies and influence other metabolic processes.

Cell-Free Fetal DNA in Amniotic Fluid: Unique Fragmentation Signatures in Euploid and Aneuploid Fetuses. *O. Lapaire[1], D.W. Bianchi[2], I. Peter[3], B. O'Brien[2], H. Stroh[2], J.M. Cowan[2], U. Tantravahi[4], K.L. Johnson[2]* 1) Univ Womens Hospital, Univ of Basel, Switzerland; 2) Div Genetics, Dept Pediatrics, Tufts-New England Med Ctr, Boston, MA; 3) Inst for Clinical Research and Health Policy Studies, Tufts-New England Med Ctr, Boston, MA; 4) Dept of Pathology, Women and Infants Hospital, Providence, RI.

Background. Circulating cell-free fetal deoxyribonucleic acids (cffDNA) are novel biomarkers with many clinical applications. Amniotic fluid (AF) is a rich source of cffDNA. Our objective was to investigate the biophysical characteristics of cffDNA in AF. **Methods.** Ten mL of fresh AF supernatant, taken for clinical indications, were obtained from women carrying euploid fetuses (n=39) and aneuploid fetuses (n=4). To test the effects of storage and karyotype, samples frozen at -80C were also obtained from euploid fetuses (n=19) and aneuploid fetuses with trisomies 21 (n=16), 18 (n=9), 13 (n=3), triploidy (n=4), and monosomy X (n=2). AF cffDNA was characterized by real-time PCR amplification of *GAPDH*, gel electrophoresis, and analysis of the DNA fragmentation signature. **Results.** The amount of fresh AF cffDNA from euploid fetuses correlates significantly with gestational age ($R2=0.77$, $p<0.0001$), but frozen cffDNA does not ($p=0.63$). The median amount of cffDNA in frozen euploid samples was significantly lower than in fresh samples ($p<0.0001$). Compared to frozen euploid samples, a statistically significant decrease in the median amount of cffDNA was observed in frozen aneuploid samples, when adjusted for gestational age ($p=0.0005$). Analysis of the cffDNA size distribution showed statistically significantly different and qualitatively unique patterns for each karyotype. **Conclusions.** Our data suggest that gestational age, karyotype, and sample storage time affect quantitative levels and fragment size in AF cff DNA. This may be due to fundamental differences in tissue sources, excretion modes, or kinetic pathways. Characteristic fragmentation signature patterns, unique for each common aneuploidy, may offer the possibility of using DNA fragmentation analysis as a rapid and cost-effective means of triaging amniotic fluid samples.

Hierarchical Bayes Prioritization of Marker Associations from a Genome-Wide Association Scan for Further Investigation. *J.P. Lewinger, D.C. Thomas, D.V. Conti, J. Baurley, T. Triche* University of Southern California, Los Angeles, CA.

We describe a hierarchical regression modeling approach to selection of a subset of markers from the first stage of a genomewide association scan to carry forward to subsequent stages for testing on an independent set of subjects. Rather than simply selecting a subset of most significant marker-disease associations at some cutoff chosen to maximize the cost-efficiency of a multistage design, we propose a prior model for the true noncentrality parameters of these associations comprised of a large mass at zero and a continuous distribution of nonzero values. The prior probability of nonzero values and their prior means can be functions of various covariates characterizing each marker, such as their location relative to genes or evolutionary conserved regions, or prior linkage or association data. We propose to take the top ranked SNPs based on either the posterior probability that the noncentrality parameter is not zero or the posterior expectation of the noncentrality parameter for confirmation in later stages of a genomewide scan. The statistical performance of this approach is compared with the traditional p-value ranking by simulation studies. The method is applied to data from a recent genomewide scan for age-related macular degeneration.

The effect of heterogeneity on the chromosome 10 risk in late-onset Alzheimer Disease. *X. Liang[1], E.R. Martin[2], N. Schnetz-Boutaud[1], J. Bartlett[1], B.M. Anderson[1], S. Zuchner[2], H. Gwirtsman[1], D. Schmechel[2], R. Carney[2], J. Gilbert[2], M.A. Pericak-Vance[2], J.L. Haines[1]* 1) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 2) Center for Human Genetics, Duke University, Durham, NC.

Late-onset Alzheimer disease (LOAD) is a complex neurodegenerative disorder with a strong genetic component. However, with the exception of APOE, no universally accepted genetic association has been identified. A broad region of chromosome 10 has engendered continued interest generated from both preliminary genetic linkage and candidate gene studies. To better examine this region, we combined unbiased genetic linkage with candidate gene association studies. We examined five functional candidate genes (VR22, LRRTM3, PLAU, TNFRSF6, IDE) that also fell within the broad area of linkage. 50 SNPs were genotyped across the genes in a case-control dataset containing 745 cases and 998 controls and an independent family-based dataset containing 1337 discordant sib pairs in 567 multiplex families. Simultaneously we attempted to narrow the peak region of linkage by analyzing the family-based dataset using both covariate and subset analyses. We genotyped an additional 36 SNPs evenly spaced across 80.2 Mb. 24/50 SNPs in the five candidate genes gave nominally positive association results in at least one dataset, with at least one positive SNP in each gene. SNPs rs2441718 and rs2456737 in VR22 (67.8 Mb) and SNP rs203162 in TNFRSF6 (90.7 Mb) showed association in both family-based and case-control datasets, with odds ratios (ORs) of 1.29 (95% CI=1.02-1.62, p=0.03), 0.75 (95% CI=0.59-0.94, p=0.01) and 1.23 (95% CI=1.04-1.46, p=0.02), respectively. A two point LOD score of 2.69 was obtained at SNP rs1890739 (45.1 Mb, p=0.03 in 21% families) when the families were ordered from low to high by ApoE LOD score using Ordered Subset Analysis (OSA). These data continue to support a role for chr 10 loci in AD. However, the candidate gene and linkage analysis results did not converge, suggesting that there is more extensive heterogeneity on chromosome 10 than previously appreciated.

A Preliminary Study on X-ray Repair Cross Complementing (XRCC) Gene Polymorphisms as Possible Biomarkers of Breast Cancer Susceptibility among Cypriot Women. *A. Hadjisavvas[1], M. Loizidou[1], M. Daniel[2], E. Kakouri[2], S. Malas[3], Y. Marcou[2], K. Kyriacou[1]* 1) EM and Molecular pathology, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus; 2) Bank of Cyprus Oncology Centre, Nicosia, Cyprus; 3) Department of Oncology, Limassol General Hospital, Limassol, Cyprus.

Population-based molecular epidemiology studies on genetic variations of genes have shown associations between specific genetic polymorphisms and breast cancer susceptibility. A number of studies have demonstrated that common variants of genes involved in the DNA Double-Strand Break pathway can act as low penetrance breast cancer susceptibility alleles. This preliminary molecular epidemiology study which is carried out for the first time on a Cypriot cohort of patients/controls, aims to investigate the role of polymorphisms in DNA repair genes XRCC1, XRCC2 and XRCC3 in breast cancer. We analysed the XRCC1 Arg194Trp, Arg280His and Arg399Gln variants as well as the XRCC2 Arg188His and XRCC3 Thr241Met gene polymorphisms. To identify these polymorphisms PCR and restriction enzyme digest were performed on DNA obtained from 500 Cypriot breast cancer patients age between 40-70 and 600 age-matched normal controls. Extensive demographic, epidemiological and pathological data were also recorded. Using the Chi-square analysis, we identified a statistically significant ($p = 0.05$) difference between the case group and the control group for the XRCC1 Arg280His polymorphism. Evaluation of potential underlying gene-gene or gene-environment interactions, which might magnify the effect of the variants under study, will require even larger sample sizes. We are currently expanding our analysis to include a greater number of subjects to determine the importance of these initial findings and improve our knowledge on the effect of XRCC gene polymorphisms, on breast cancer susceptibility in Cypriot women.

Determination of mitochondrial sequence variants in familial heart disease using resequencing arrays. *M.K. Halushka[1,4], J. Albertus[2,4], B.R. Bishe[2,4], A. Chakravarti[2,4], R.D. Cohn[2,4], D.J. Cutler[2,4], N. Johnson[3,4], D.P. Judge[3,4], J. Lu[1,4], D.E. Arking[2,4]* 1) Department of Pathology; 2) McKusick-Nathans Institute of Genetic Medicine; 3) Division of Cardiology; 4) Johns Hopkins Medical Institutions, Baltimore, MD.

Despite recent gains in identifying genes responsible for familial forms of heart disease, there is still a significant group of individuals whose underlying genetic mutations remain unknown. This study investigated sequence variation in mitochondrial DNA (mtDNA) as a source of unexplained familial cardiomyopathies. Eight patients from 6 families with familial heart disease (hypertrophic cardiomyopathy, dilated cardiomyopathy (DCM), restrictive cardiomyopathy or exercise intolerance (EI)) were selected based on both the inability to exclude matrilinear inheritance and other phenotypes suggestive of mitochondrial disease. A subset of individuals had prior limited genomic sequencing which failed to identify a cause of disease. mtDNA analysis was performed using the Affymetrix GeneChip Human Mitochondrial Resequencing Array 2.0 on lymphocyte DNA. The ABACUS algorithm assuming homozygosity at each nucleotide was used to scan the chips for variation. The overall nucleotide call rate on the chips was 97% (range 95-98%). Ninety-five variant sites were identified, of which 75 were common variants and 18 were rare variants. Two novel sequence variants, not described in over 2,400 previous human mtDNA sequences were detected. One novel variant was located in the D-loop. The second, in a patient with EI, caused a mutation in a highly conserved threonine in the NADH dehydrogenase 2 (ND2) gene. Three family members with DCM shared a rare variant (frequency ~ 1/800 individuals) located in the 16s rRNA. Mutations in 16s rRNA and ND2 have been shown to cause DCM and a syndrome including EI respectively. Functional testing remains to be performed to confer function on these variants. We have identified two entirely novel sequence variants and one known rare variant which may contribute to the familial cardiomyopathies observed. We found the Mitochip to be a robust, inexpensive and high throughput mechanism to investigate mtDNA.

Reduced MeCP2 expression is frequent in autism frontal cortex and correlates with aberrant *MECP2* promoter methylation. *R.P. Nagarajan[1,2,3,4], A.R. Hogart[1,2,3,4], M.R. Martin[1,3,4], Y. Gwye[1,3,4], J.M. LaSalle[1,2,3,4]* 1) Dept of Med Microbiology and Immunology; 2) Genetics Graduate Group; 3) Univ of California Davis; 4) Rowe Program in Human Genetics.

Mutations in *MECP2*, encoding methyl CpG binding protein 2, cause Rett syndrome, an X-linked neurodevelopmental disorder. Both Rett and autism are pervasive developmental disorders and share many clinical features. Although *MECP2* coding mutations are a rare cause of autism, MeCP2 expression defects were frequently found in brain samples from autism and autism spectrum disorders. MeCP2 immunofluorescence was quantified by laser scanning cytometry on a large tissue microarray containing autism, autism-spectrum, and control postmortem cerebral cortex samples. A significant reduction in MeCP2 expression compared to age-matched controls was found in 11/14 autism, 10/10 Rett, 4/4 Angelman, 3/4 Prader-Willi, 3/5 Down, and 2/2 attention deficit hyperactivity disorder frontal cortex samples. We then tested the hypotheses that *MECP2* promoter mutations or aberrant promoter methylation cause reduced expression in cases of idiopathic autism. One autism female was heterozygous for a rare *MECP2* promoter variant that correlated with reduced MeCP2 expression. A more frequent occurrence was significantly increased *MECP2* promoter methylation in autism male frontal cortex compared to controls. Furthermore, percent methylation of *MECP2* significantly correlated with reduced MeCP2 protein expression. These results demonstrate that both genetic and epigenetic defects lead to reduced MeCP2 expression and may be important in the complex etiology of autism.

Altered expression of lipid-metabolism genes in the frontal cortex of suicide completers: validation of microarray data using real-time PCR. *A. Lalovic[1], A. Sequeira[1], L. Canetti[1], J. ffrench-Mullen[2], T. Klempan[1], G. Turecki[1]* 1) McGill Group for Suicide Studies, Douglas Hosp Research Centre, Verdun, QC, Canada; 2) Gene Logic Inc., Gaithersburg, MD, USA.

An association between low levels of serum cholesterol and suicidal behavior has frequently been reported. Studies investigating this association have generated data to suggest that lipid metabolism and cytokines may play interacting roles in suicidal behavior. We performed large-scale microarray gene expression analysis using the Affymetrix HG-U133 chipset and investigated the expression profile of genes related to lipid metabolism in post-mortem brains from suicide completers and controls. We used tissue from three regions of the frontal cortex (BA 8/9, 11, and 47) from 13 psychiatrically normal male controls and 22 male suicide completers, 15 of whom were diagnosed with major depressive disorder. Of the 196 lipid metabolism genes differentially expressed (fold-change (FC) 1.3 and p 0.05) in the three regions in total, a number of genes were selected for validation using real-time PCR, given their consistency across all three brain regions examined. Stearoyl-CoA desaturase (SCD) was found to be down-regulated in suicides with major depression in all regions (BA 8/9: FC=-1.47, p=0.002; BA 11: FC=-1.74, p=0.009; BA 47: FC=-1.67, p=0.01). Leptin receptor (LEPR) was also down-regulated in suicides in all three brain areas (BA 8/9: FC=-2.26, p=0.002; BA 11: FC=-2.02, p=0.01; BA 47: FC=-1.4, p=0.05). Good correlations were found between our microarray and real-time PCR data for these genes (LEPR: BA 8/9: r=0.49, p=0.02; BA 11: r=0.53, p=0.02, BA 47: r=0.65, p=0.0001). Western blotting experiments are underway to investigate alterations at the protein level. The possible functional roles of these proteins in the context of suicidal behavior will be considered, taken together with previous findings suggesting that leptin may play a role in regulating SCD expression.

Identification of genes involved in tooth development by differential microarray gene expression. *F. Li[1], T.J. Pemberton[1], G.A. Mendoza[1], Y. Hsu[1], M.L. Snead[2], R. Mehrian-Shai[1], P.I. Patel[1,2]* 1) Institute for Genetic Medicine, University of Southern California, Los Angeles, CA; 2) Center for Craniofacial Molecular Biology, University of Southern California, Los Angeles, CA.

Anomalies involving tooth number, such as hypodontia or supernumerary teeth, anomalies of tooth shape and size, and defects involving mineralization are common developmental dental abnormalities. Positional cloning approaches and knock-out mice have identified a number of genes critical in tooth development. As an alternative approach to identifying novel genes in the process, we have used the Affymetrix MGE 430 2.0 microarray to examine gene expression within developing mouse molar teeth between post-natal days 1 to 10. Analysis of the top-100 genes identified as up-regulated in the tooth RNA when compared with 16 control tissues found that they were enriched for membrane-associated, structural, and nuclear genes, and that the protein product of 16% is present in the extracellular matrix. Their functions were greatly enriched for integrin binding, extracellular matrix structural components conferring compression resistance and tensile strength, and for genes involved in biomineral formation, tissue remodelling, skeletal development, osteoblast differentiation, organ morphogenesis, and signal transduction regulation. Twenty-four of these genes have been previously reported to be involved in tooth development, mutations within which are known to cause the tooth developmental anomalies amelogenesis imperfecta, dentinogenesis imperfecta, and trichodentoosseous syndrome. Sixteen genes of unknown function were also identified. RT-QPCR has confirmed the up-regulation of 25 of these genes within the tooth RNA. However, further work is required to confirm the localization of expression within the developing tooth and to determine their role in tooth development. We conclude that microarray and RT-QPCR expression analyses of the developing tooth can facilitate the identification of novel genes involved in the process, which can aid future candidate gene selection during the investigation of the genetic aetiology of dental anomalies.

Parallel Selection on TRPV6 in Human Populations via Soft Sweeps. *D.A. Hughes[1], K. Tang[1], R. Strotmann[2], T. Schöneberg[2], B. Nilius[3], M.A. Stoneking[1]* 1) Dept. of Evolutionary Genetics, Max-Plank Institute for Evolutionary Anthropology, Leipzig, Germany; 2) Institute of Biochemistry, Molecular Biochemistry, University of Leipzig, Leipzig, Germany; 3) Laboratory of Physiology, Catholic University of Leuven, Leuven, Belgium.

To identify and characterize genes under local selection we screened the SeattleSNPs data set using two summary statistics molecular Fst and lnRHap. This screen identified TRPV6, as others have, as a candidate gene for local directional selection in Europeans. We further characterized selection at this locus with a modification of the extended haplotype homozygosity test in order to test the EHH for the same allele between two different populations. With this test TRPV6 in Europeans violates neutral expectations determined from both empirical and simulated distributions. Three tagging SNPs, which are also non-synonymous mutations, were then genotyped in the CEPH diversity panel to determine if this pattern may be limited to Europeans. In contrast to Africans, we discovered that all non-African populations were fixed, or nearly so, for the derived haplotype. Approximately 4kb of non-consecutive sequence in the TRPV6 region was then re-sequenced in 92 individuals from four global populations. We again performed the REHHB test and found that all of these populations have EHH values equal to or greater than that found in Europeans, suggesting that all non-African populations have experienced selection at this locus. We then dated the time since fixation of the European allele to ~12,000ybp, a date too young for there to have been a single selection event for all populations outside of Africa. Moreover, the signature of selection is not limited to non-Africans, for we also find a signature of selection in African-Americans on the same haplotype, suggesting multiple independent selective events for the same haplotype. To determine if there is a functional difference between the two protein alleles we performed multiple functional assays including patch-clamp analyses. These assays show no measurable functional differences between the alleles, suggesting that selection may be on expression differences or alternative isoforms.

Analysis of the C-reactive protein gene in sibling pairs extremely discordant for neovascular age-related macular degeneration. *I.K. Kim[1], F. Ji[2], S. Adams[1], A. Capone[3], J.W. Miller[1], J. Ott[2], T.P. Dryja[1], M.M. DeAngelis[1]* 1) Dept of Ophthalmology, Harvard Medical School, Mass Eye & Ear Infirmary, Boston, MA; 2) Laboratory of Statistical Genetics, Rockefeller University, New York, NY; 3) Associated Retinal Consultants, PC, William Beaumont Hospital, Royal Oak, MI.

Purpose: Inflammation appears to play a role in the pathogenesis of age-related macular degeneration (AMD). Some studies have suggested a correlation between circulating C-reactive protein (CRP) levels and AMD. Genetic variations in CRP partially influence plasma CRP levels. We sought to examine whether genetic variants in the CRP gene were associated with risk of neovascular AMD. **Methods:** We ascertained 144 extremely discordant sibpairs where at least one member had neovascular AMD and another had normal maculae and was past the age at which the affected sibling was diagnosed with neovascular AMD. Disease status for each participant was confirmed by grading of fundus photos by at least two of the investigators, or a home visit. Genotypes were determined by sequencing 9 single nucleotide polymorphisms (SNPs) in the CRP gene. Statistical analyses were performed by McNemars test or conditional logistic regression. **Results:** No significant association between any of the CRP SNPs and risk of neovascular AMD was detected. Stratification of the sibpairs according to complement factor H (CFH) genotypes (Y402H) demonstrated no association between any of the CRP SNPs and risk of neovascular AMD. However, with stratification by smoking status (index patient 10 pack-years vs. normal sibling <10 pack-years), the presence of one or two minor alleles of SNP rs3091244 (-286C > T/A) was found to be negatively associated with neovascular AMD (p = .04; OR: 0.125; CI: .003 - .932). **Conclusions:** No statistically significant association was detected between any of the 9 SNPs in the CRP gene and neovascular AMD when considering disease status alone or with stratification by CFH genotype. Among sibling pairs extremely discordant for smoking history as well as AMD, the minor alleles at SNP rs3091244 may be protective for neovascular AMD. However, this finding is of modest significance and may be due to the effect of smoking alone.

Macrocephaly on Fetal Ultrasound as a sign for Congenital Myotonic Dystrophy. *N. Martin[1], M. Thomas[1], D. Chitayat[1,2]* 1) Dept. of Obstetrics and Gynecology, The Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital; 2) Division of Clinical and Metabolic genetics, Dept. of Pediatrics, Hospital for Sick Children; University of Toronto, Toronto, Ontario, Canada.

Myotonic Dystrophy Type 1 (DM1) is an autsomal dominant condition associated with expansion of a CTG trinucleotide repeat and has variable clinical manifestations ranging from mild (late onset) to severe (congenital onset). Congenital DM1 has a high rate of morbidity and mortality and is characterized by severe hypotonia, respiratory insufficiency and mental retardation. We report two cases that presented with a fetal ultrasound finding of macrocephaly among other findings and were later found to have congenital DM1. **Case 1:** A 21- year-old woman presented at 35 weeks gestation with polyhydramnios, mild unilateral cerebral ventriculomegaly, prominent 3rd ventricle and macrocephaly. The biparietal diameter measured 100 mm, which is equivalent to 42 weeks gestation. Delivery was by caesarean section at 38 weeks gestation. The newborn was severely hypotonic, macrocephalic and DNA analysis of the *DMPK* gene showed 1500 CTG repeats, which is consistent with congenital DM1. Subsequently, the mother was found to carry a CTG repeat expansion of 700. **Case 2:** Fetal ultrasound done at 20 and 28 weeks gestation showed mild bilateral ventriculomegaly and mild polyhydramnios and at 30 weeks gestation a distended abdomen, polyhydramnios and macrocephaly were noted. Delivery was at 33 weeks gestation and the baby died 10 minutes after birth. On autopsy, no significant abnormalities were identified apart from megalencephaly (OFC of 38 GA). The mother had features of DMI not noted before and on DNA analysis of the *DMPK* gene had 100 CTG repeats. The baby had 3800 CTG repeats. Macrocephaly has only been reported in one other case series in association with congenital DM1. We would suggest that congenital myotonic dystrophy should be considered when fetal ultrasound shows macrocephaly. Further investigations may be required such as physical examination of the mother and possible DNA analysis for DM1.

New CFTR gene variants in Cystic Fibrosis and Chronic Rhinosinusitis Chilean patients. *G. Molina[1], C. Musri[1], M. González[1], A. Vera[1], F. Vásquez[1], F. Krause[1], A. Milinarsky[2], V. Lezana[2]* 1) Laboratorio de Biología y Genética Molecular. Escuela de Medicina. Universidad de Valparaíso, Valparaíso. Chile; 2) Departamento de Pediatría. Escuela de Medicina. Universidad de Valparaíso,Valparaíso. Chile.

Cystic Fibrosis (CF) is the most prevalent autosomal recessive disease in the Caucasian population (1/2000 newborns). Its prevalence in Chile could vary between 1/4000 to 1/2000 newborns in different regions. CF is caused by mutations in the cystic fibrosis transmembrane regulator (CFTR) gene, which encodes a protein that functions as a chloride channel. This protein regulates electrolytes and water transport through secretory cells. The failure of this protein produces a greater viscosity of mucus and other secretions. Chronic Rhinosinositis (RC) is a CFTR related disease, since greater viscosity of mucus is one of its causes. More than 1000 CFTR gene mutations have been described. We have searched for new mutations in 20 CF patients, using SSCA and sequencing of exon4, exon7, exon10, exon 11, exon12, and exon17b. We have already found two variants (p.T351S, p.G528G). We have not detected the p.T351S variant in 100 chromosomes of individuals from Valparaisos general population, thus it could be a CF causative mutation. Conversely, p.G528G is a common polymorphism. Furthermore, we have determined that homozygous individuals for this variant have an exon 10 normal splicing, despite the fact that it modifies the last base of exon 10. In RC patients we have performed a complete screening of CFTR coding sequencing using RT-PCR of peripheral lymphocytes RNA. We have found splicing variants causing exon 5, 14a, 17a skipping. This approach could lead us to discover new mutations responsible for the unknown 50% of CF causative mutations in our CF patients and to clarify the relationship between CF and RC. DIPUV 39/2004.

Assessing copy number variation in individuals with autism spectrum disorder using Affymetrix 500K SNP arrays. *C.R. Marshall[1], L. Feuk[1], L. He[1], T. Miller[1], J. Skaug[1], P. Szatmari[2], L. Zwaigenbaum[2], B. Fernandez[3], W. Roberts[1], S.W. Scherer[1]* 1) The Hospital for Sick Children, Toronto, Canada; 2) McMaster University, Hamilton, Canada; 3) Memorial University, St. John's, Canada.

Autism is a neurodevelopmental disorder characterized by impairments in social-communication and by a preference for repetitive activities. Twin studies suggest autism is mainly a genetic condition of complex etiology, but the major susceptibility genes are not known. Our studies have shown that 7% of autistic patients have chromosomal anomalies detectable upon karyotyping. We propose that sub-microscopic copy number variation (CNV) in the human genome may also contribute to autism. For detection of sub-microscopic unbalanced rearrangements in autism we are now using the Affymetrix 500K SNP array to (1) characterize all samples having cytogenetically detectable rearrangements and (2) assess the genome in 500 probands with normal karyotypes. We have already mapped 67 cytogenetically-visible breakpoints in 36 patients and will use this for comparison to CNV data. For aim 2, we have inferred copy number changes in 200 samples using a highly stringent Markov Model built into DNA-Chip Analyzer (dChip) software. On average, we detect ~5 CNVs/sample and so far our data does not indicate prevalence for sub-microscopic changes in autism compared to controls. The majority of the variants found in multiple patients are found at similar frequency in controls, indicating that these are common copy-number polymorphisms. For those CNVs that are not found in controls we are prioritizing characterization of regions based on: (1) Recurrent intervals in unrelated samples that contain genes and (2) Large single CNVs that contain candidate gene(s). We then assess these intervals in the larger autism collection and controls. Our combined cytogenetic and microarray data identifies (microscopic and sub-microscopic) potentially phenotype-associated loci on chromosome 1p21.3, 3p14, 7q31.2, 7q31.3, 7q34, 9p22, 15q11.2-15q13.3, 17q12, 22q11.21 and at other regions. Approximately 10% of cases have at least one de novo large CNV that may be associated with the disease providing critical information for clinical diagnostics.

Molecular analysis of *MCCC1* and *MCCC2* genes of infants whose newborn screening results are suggestive of 3MCCD. *P.B. McWhorter, J.D. Cogan, R. Hamid, M.T. Weingartner, J.A. Phillips III* Pediatrics, Vanderbilt University School of Medicine, Nashville, TN.

Introduction: MS/MS newborn screening (NBS) detects elevated 3-hydroxyisovaleryl carnitine (C5OH) which is associated with 3-Methylcrotonyl CoA Carboxylase Deficiency (3MCCD). While often asymptomatic, some infants with 3MCCD present with hypotonia or a Reye-like illness. Reported incidence is about 1:50,000 with no known high risk populations. Products of the *MCCC1* and *2* genes comprise the subunits of 3MCC. **Hypothesis:** Variations in the *MCCC1* and/or *2* genes are associated with elevated C5OHs on NBS in middle TN, especially among Hispanics and Kurds. **Methods:** *MCCC1* and *2* transcripts were sequenced for those with repeated C5OH elevations on NBS and frequencies of variations were determined in controls. **Results:** From July 2004 to present 107 neonates had C5OH elevations on their NBS. Of these 16% were evaluated because they also had 3-OH-isovaleric acid (3OHIA) elevations suggestive of 3MCCD. Ethnicity was 53% European, 18% Hispanic, 18% African American, and 12% Kurdish (all consanguineous). Sequencing *MCCC1* and *2* RT-PCR products from lymphoblastoids treated with puromycin to prevent NMD identified novel mutations in each. Case 1 was heterozygous for a single G deletion (1073delG) that is predicted to cause a frameshift starting with codon 358 of *MCCC1*. Case 2 was heterozygous for a 2bp deletion (640-641delGG) that also causes a frameshift and results in NMD of *MCCC1* transcripts. Case 3 was homozygous for a G to A transition (G1015A) encoding a Val339Met substitution in the *MCCC2* gene. Val339 is highly conserved in all mammalian species. No *MCCC1* 1073delG or *MCCC2* Met339 alleles were detected in 200 control alleles. **Conclusions:** 1) Elevated C5OH and 3OHIA were seen in ~1:3,800 births, 2) Hispanics and Kurds comprise 30% of our cases but less than 3.1% of our population, 3) all of our first three samples analyzed showed rare allelic variants in *MCCC1* or *2* that are plausible causes of perturbed 3MCC function, and 4) determining the mechanisms by which these variations perturb 3MCC expression or function will require further studies.

SNP microarray analysis in an apparently balanced complex chromosome rearrangement. *B. Hay[1], P. Minehart Miron[2,3], P. Crowley-Larsen[2], M. Ito[4], S. Ramakrishnan[2], X-L. Huang[4], J.M. Milunsky[4,5,6]* 1) Dept Pediatrics, Univ Massachusetts Med Ctr, Worcester, MA; 2) Dept Hospital Labs, UMass Med Ctr, Worcester, MA; 3) Dept Pathology, UMass Med Ctr, Worcester, MA; 4) Center for Human Genetics, BU School of Medicine, Boston, MA; 5) Dept Pediatrics, BU School of Medicine, Boston, MA; 6) Dept Genetics & Genomics, BU School of Medicine, Boston, MA.

Complex chromosomal rearrangements are rare events, typically reported in association with abnormalities in a proband. Parental concern regarding prenatal ventriculomegaly and pyelectasis led to chromosome analysis in a phenotypically normal infant that revealed a de novo structural rearrangement involving chromosomes 2, 3, 4 and 9. These rearrangements appeared balanced by initial cytogenetic studies, including analysis of GTG-banded metaphase chromosomes at the 500 band level and metaphase FISH with whole chromosome paints for chromosomes 2, 3 and 4 plus centromere probes for chromosomes 2 and 9. This yielded the following karyotype: 46,XY,der(2)del(2)(p16)ins(2;3)(p16;p13p24.2),del(3)(p13p24.2),der(4)t(2;4)(p16;q28),ins(9;4)(q31;q35q28). Subtelomere probes for 2p, 3p, 4q and 9q revealed disomy for each and localization consistent with the described karyotype. High resolution metaphase comparative genomic hybridization (resolution ~2.5Mb) was performed and was normal. Further investigation using the Affymetrix 250K SNP microarray revealed deletions at 3p24.3 (~2Mb with at least 7 genes) and 4q26 (~200kb with at least 1 gene). To our knowledge, a rearrangement involving these four chromosomes has not been reported. At 1 year of age, this child is exhibiting mild developmental delays and a few minor anomalies. Utilization of the SNP microarray provides more precise data regarding areas of cryptic unbalanced rearrangements, which may be beneficial for clinical management and prognosis.

Cornelia de Lange Syndrome and the Cohesinopathies. *I. Krantz[1], D. Yaeger[1], M. Kaur[1], L. Jackson[2], M. Deardorff[1]* 1) Division of Human Genetics and Molecular Biology, The Childrens Hospital of Philadelphia, Philadelphia, PA; 2) Division of Obstetrics and Gynecology, Drexel University School of Medicine, Philadelphia, PA.

Disorders affecting the sister chromatic cohesion complex likely represent an underappreciated class of clinical disorders. A major component of this complex, Cohesin, consists of the subunits Smc1, Smc3, Stromalin and Rad21. The former two comprise a ring that encircles sister chromatids and the latter two fasten the structure. NIPBL is believed to play a major role in loading Cohesin onto chromosomes. A number of other proteins have also been implicated in the proper regulation of Cohesin. Of these, Eco1 regulates maintenance of Cohesin on chromosomes. The essential function of the Cohesin complex suggests that its disruption would be lethal, however several alterations lead to specific patterns of developmental defects.

The Cornelia de Lange syndrome (CdLS) is a multisystem developmental disorder characterized by facial dysmorphia, hirsutism, growth and cognitive retardation, gastrointestinal abnormalities and limb deficiencies. We have demonstrated that mutations in NIPBL cause CdLS, associating disruption of the Cohesin complex with a developmental disorder. Despite extensive screening, we have found NIPBL mutations in only 50% of patients with CdLS. In the past year, several other members of the sister chromatid cohesion complex have been implicated in human congenital disease. The human homolog of Eco1 (ESCO2), has been found to be mutated in patients with Roberts syndrome/SC phocomelia, which have phenotypic overlap with CdLS. Most recently, mutations in a human Smc1 homolog (SMC1L1) have been found in patients with an X-linked variant of CdLS. We have screened a cohort of 95 NIPBL mutation-negative patients with CdLS and have identified SMC1L1 mutations in 8. These individuals represent mild variants of CdLS. This work confirms that mutations in the Cohesin complex result in CdLS and suggests that mental retardation-associated phenotypes due disruption of this complex may be more common than appreciated.

Large scale case-control, family-based and cohort studies of diabetes-susceptibility variants in the *TCF7L2* gene.

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Evidence is accumulating that common variants in *TCF7L2* represent the most important genetic determinants of type 2 diabetes (T2D) susceptibility yet uncovered. To explore the physiological mechanisms involved, we genotyped four *TCF7L2* SNPs (the two most-associated in Icelandic subjects [rs12255372, rs7903146] plus two well-correlated Hapmap proxies [rs4506565, rs12243326]), in diverse European samples. A case-control analysis in 2158 UK T2D subjects and 2574 controls demonstrated powerful T2D associations at all SNPs, with the strongest effect at rs7903146 (allele-wise RR 1.36 95% CI 1.24-1.48) ($P=1.3x10^{-11}$). Data suggested a multiplicative model, and a population attributable risk between 15-20%. Independent replication was seen in 388 parent-offspring trios (eg rs4506565: 62% transmission, $P=8x10^{-5}$) generating a combined association signal of $P=4.4x10^{-14}$. Overall, T2D-associated *TCF7L2* genotypes were more frequent in cases selected for family history and early onset (rs4506565, P=0.02). Consistent with effects on insulin secretion, diabetes-associated genotypes were, in cases, associated with lower BMI (rs7903146, P=0.0002). In the population-based N Finland Birth Cohort of 1966 (n=5646), we found no association with BMI at age 31, but *TCF7L2* variants were associated with increased fasting glucose (rs12255372 homozygotes differ by 0.06mmol/l, p~0.01) and reduced insulin secretion (HOMA%B, p=0.015). There was a significant but weak effect of the T2D-risk allele at rs12255372 to increase birth weight (62g difference between homozygotes, P=0.028). Our data provide powerful replication of the association between *TCF7L2* and T2D and support an effect mediated through reduced beta-cell function. However, the birth weight association suggests that, in those with the risk variant, insulin secretion may be increased during the prenatal period.

Redundant function of Smad1 and Smad5 during chondrogenesis. *B. Keller[1], P. Hermanns[2], B. Zabel[2], B. Lee[1]* 1) Dept. of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Center of Pediatrics & Adolescent Medicine, University of Freiburg, Germany.

Bone morphogenetic proteins (BMPs), members of the TGF- superfamily, play a pivotal role in the development of the vertebrate skeleton. The BMP-Receptor BMPR-1B (ALK-6) is expressed in all types of cartilage and expression of a dominant-negative form of BMPR-1B is able to block chondrogenesis and osteogenic differentiation. Smad1, Smad5 and Smad8 are the mediators of the BMP signaling pathway. Binding of BMP to the receptor leads to the phosphorylation of the Smads. They undergo a conformational change and are able to bind the common-partner Smad (co-Smad) Smad4. This complex translocates into the nucleus and activates or represses the transcription of target genes. *Smad1* and *Smad5* are expressed in proliferating and maturating chondrocytes, whereas *Smad1* and *Smad8* are shown to be expressed in mesenchymal cells. We examined the role of Smad1 and Smad5 in chondrogenesis by knocking out *Smad1* in proliferating chondrocytes using the cre-loxP-system. P1 mutant mice skeletons stained with alcian blue and alizarin red appeared to be normal and no obvious patterning defect has been observed. It has been discussed that *Smad5* might compensate for *Smad1* function. To address this issue we crossed mice with a *Smad1* loss of function in proliferating chondrocytes onto a *Smad5*+/- background. While there is no obvious difference in P1 mice, we could observe a weight difference in 8 week old mutants compared to their wt-littermates as well as a subtle disorganization of the growth plate.

Effects of microsatellite marker choice on measures of human interpopulation genetic relatedness and inferred phylogenies. *J. Listman[1], R.T. Malison[2,3], B.Z. Yang[2], A. Sughondhabirom[4], N. Thavichachart[4], H.R. Kranzler[5], S. Tangwonchai[4], A. Mutirangura[4], T. Disotell[1], J. Gelernter[2,3]* 1) Dept Anthropology, New York Univ, New York, NY; 2) Dept Psychiatry, Yale Univ Sch of Med, New Haven, CT; 3) VA CT, West Haven, CT; 4) Chulalongkorn Faculty of Med, Bangkok, Thailand; 5) Dept Psychiatry, Univ CT Sch of Med, Farmington, CT.

Short Tandem Repeats (STRs) are highly polymorphic in contrast to diallelic markers. This is thought to make them useful for human population genetic studies. STRs are used to measure interpopulation genetic distances and genetic diversity, identify first-degree relatives, infer population phylogenies, and assign individuals to a population. The usefulness of STR markers for interpopulation comparisons has been questioned, due to the supposed confounding effects of homoplasy. We used a panel of 35 unlinked autosomal STRs (15 tetranucleotide and 20 dinucleotide repeats) to examine the effects of marker choice and possible homoplasy on inferences of interpopulation genetic distances and phylogenetic relationships for 5 populations. Comparisons were made between 6 measures of genetic distance (D, R_{ST}, D_{SW}, D_a, D_m, D_S, and delta ()). Self-identified Thai (N=45), Thai-Chinese (N=28), Hmong (N=44), European Americans (N=91), and African Americans (N=54) were genotyped for all 35 STRs (4.8% missing data). Marker panels were created based on alleles per marker, repeat size, or semi-random selection to calculate distance measures which were then used to infer neighbor-joining trees. For all distance measures and resultant NJ trees, correlations were lowest between tetranucleotide and dinucleotide marker panels (.49 to .84). For each marker panel, correlations were lowest between R_{ST} or D and other measures. This indicates that design of STR panels can affect relative measures of genetic distance between populations, requiring interpretation of phylogenies within the context of marker properties and chosen distance measure. STRs may be more reliable for population differentiation, identification of close relatives, and population assignment, than for inferring population phylogenies, particularly when only a small number of markers is available.

Losartan modifies the predisposition for dissection in a mouse model of Marfan syndrome. *J.P. Habashi[1], M. Gamradt[1], M. Awad[1], E. Klein[1], D. Bedja[1], H.C. Dietz[1,2]* 1) Johns Hopkins University, Baltimore, MD; 2) HHMI, Baltimore, MD.

Marfan syndrome(MFS)is an autosomal dominant connective tissue disorder with a predisposition for aortic dilation and rupture. blocker therapy has been used to decrease the rate of aortic dilation but has not been shown to prevent the need for aortic root replacement or the risk of sudden death. Many manifestations of MFS, including aortic enlargement, have been shown to be mediated by excess activation of and signaling by the TGF family of cytokines. We demonstrated that losartan, an angiotensin II type 1(AT1)receptor blocker known to antagonize TGF, decreases the rate of aortic root enlargement and improves the architecture of the aortic media in a mouse model of MFS. This model(C1039G/+) shows histologic manifestations of failed aortic wall homeostasis, but fails to progress to dissection or death. While aortic size remains the best predictor of risk of dissection in people, the utility of this parameter has not been tested in mouse models. To determine if losartan therapy modifies the pathogenetic events culminating in dissection, we performed a clinical trial of losartan in mice homozygous for a hypomorphic Fbn1 mutation, (mgR/mgR)that show early aortic dissection. We randomized mgR/mgR mice to receive placebo(n=8),propranolol(50 mg/kg,n=8)or losartan(30 mg/kg, n=19)starting at 7 weeks, with propranolol and losartan doses titrated to achieve comparable hemodynamic effects. Echocardiograms were performed at baseline and monthly until death. While ongoing, the mice have been followed for 7 months and are necropsied on the day of death. At baseline, there was no significant difference in aortic root or ascending aortic size between the placebo, propranolol and losartan groups. Losartan significantly increased survival to 7 months of age(13/19 mice) as compared to placebo(1/7 mice; $p<0.02$) and propranolol(1/8 mice; $p<0.01$). Of the long-term survivors in the losartan treatment group, 3/13(23%) showed stable aortic dimensions while 7/13(54%) showed regression in aneurysm size. These data document that losartan has the capacity to modify the predisposition for aortic dissection in MFS, even in a mouse model with an artificially severe genotype.

Refinement of the FEVE locus and candidates. *B.A. Johannes[1], B.G. Elyas[1], M. Hicks[1], S.M. Haase[1], J.S. Bamforth[1,2], H.F. Pabst[2], M.A. Walter[1], K.A. Sprysak[1], L.M. Vicen-Wyhony[1], M.J. Somerville[1,2]* 1) Dept Medical Genetics, University of Alberta, Edmonton, AB, Canada; 2) Dept Pediatrics, University of Alberta, Edmonton, AB, Canada.

Familial Enteropathy with Villous Edema (FEVE; OMIM 600351) is an autosomal dominant disorder with variable penetrance that typically manifests in childhood as a recurrent acute, life-threatening secretory diarrhea associated with distinctive jejunal histologic changes and IgG2 subclass deficiency. We previously used a genome-wide microsatellite screen on a Mennonite kindred to define linkage of FEVE to D11S908 on chromosome 11q23 with a LOD score of 6.2 at theta = 0. Subsequent work using a higher density microsatellite marker array has refined the critical interval to a 2 Mb interval between D11S4142 and D11S1364.

Six genes from the candidate region have been sequenced: Interleukin 10 Receptor Alpha (IL10RA), Platelet-Activating Factor Acetylhydrolase Beta (PAFAH1B2), Sodium Channel Beta 2 subunit (SCN2B), Epithelial V-like Antigen 1 (EVA1), the delta subunit of CD3 antigen (CD3D), and Adhesion Molecule Interacting with CXADR Antigen 1 (AMICA1). IL10 down-regulates the inflammatory response, and protects against the lethality of intra-abdominal infection and sepsis. PAFAH1B2 is a catalytic subunit that inactivates the Platelet Activating Factor (PAF), where PAF is responsible for reducing inflammation. SCN2B modifies voltage-gated sodium ion channel function. EVA1 is involved mediating homophilic cell-cell adhesion. CD3D antigen has been associated with intestinal malabsorption. AMICA1 is an adhesion molecule in the immunoglobulin super-family. SNPs were found in several of these genes, but no causative mutations have been identified.

Serine proteases are involved in a wide variety of biological processes, including immune response and inflammation. Transmembrane Protease Serine 4 (TMPRSS4) maps to the FEVE critical region, is expressed in the gastrointestinal tract, and is currently being sequenced. Identification of the gene responsible for FEVE is expected to have relevance to other gastrointestinal and/or inflammatory disorders.

Allelic Variability and Tests for Natural Selection at the Human *ALDH2* Locus. *J. Long*[1], *C. Lewis*[1], *J. Li*[1], *R. Malhi*[2], *K. Hunley*[3] 1) Dept Human Genetics, Univ Michigan Medical Sch, Ann Arbor, MI; 2) Dept Anthropology, University of Illinois, Urbana, IL; 3) Dept Anthropology, University of New Mexico, Albuquerque, NM.

This research tests the hypothesis that natural selection has influenced variation at the human *ALDH2* gene locus. The enzyme product of this locus catalyzes the oxidation of aldehydes and is important in alcohol metabolism. *ALDH2* shows unusually high allelic differentiation among human populations. There is a deficiency variant, *ALDH2-2*, that is common in East Asians but absent in other places. This observation implicates the action of natural selection, but other processes can produce similar results. In this study, the null hypothesis of selective neutrality is tested by comparing the *ALDH2-2* allele frequency to the extent of intraallelic variability. In our tests of neutrality, we allow for a structured population. We have sequenced 5 kb of DNA surrounding the functional substitution at the *ALDH2* locus in a total of 134 people. These people represent sixteen populations, including four indigenous populations from each of four continental regions: Africa, Europe, East Asia, and the Americas. As expected, the *ALDH2-2* allele is present in all four East Asian populations, but not in populations from any other continental region. Also as expected, nucleotide diversity is highest in the four African populations and lowest in the four American populations. An old age for the *ALDH2-2* allele is suggested by background substitutions (i.e., intraallelic variability). This is consistent with its high frequency in Asians. However, it is important to examine variability at the locus in the context of the geographic structure of our species. To do this, we established neutral baselines for *ALDH2* sequence diversity in the geographically structured world-wide sample by fitting nested population structure models to the CEPH microsatellite diversity data. When the world-wide population structure was considered, the presence of intraallelic variability and a high allele frequency was inconsistent with the restriction of the *ALDH2-2* allele to the Asian continental region.

Correction of Fasting Hypoglycemia in a Rodent Model of Very Long-Chain Acyl-CoA Dehydrogenase Deficiency (VLCADD) by AAV8 Mediated Gene Transfer. *J.L. Merritt[1,2], T.V. Nguyen[1], D. Matern[1,2], J. Daniels[1], D.B. Schowalter[1]* 1) Dept. of Medical Genetics, Mayo Clinic, Rochester, MN; 2) Dept. of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.

VLCADD is a disorder of fatty acid beta-oxidation with clinical presentations ranging from severe neonatal-onset with cardiomyopathy, recurrent episodes of hypoketotic hypoglycemia, and adult-onset skeletal myopathy and rhadbomyolysis. Treatment aims to prevent metabolic decompensation by avoidance of fasting, a diet low in long-chain fatty acids, and vitamin supplementation. The variable phenotype and intracellular expression of the VLCAD protein within multiple tissues makes VLCADD a challenging disorder to treat by gene therapy. We prepared an AAV2/8 based gene delivery system able to correct the metabolic block seen in VLCADD. Six to eight week old VLCAD deficient mice were injected with $1x10^{12}$ genome particles of AAV8-CMV-hVLCAD. Ten days post-injection acylcarnitine analysis in blood demonstrated a significant improvement of C14 and C16 saturated and unsaturated acylcarnitine species of treated mice to levels comparable to wild-type levels. Consistent with these findings, immunohistochemical hepatocyte staining revealed VLCAD expression and Western blot demonstrated the 70 kDa hVLCAD band. Western analysis of liver, muscle and heart tissue demonstrates the majority of gene expression occurs in the liver. Measurement of fasting serum glucose levels pre-treatment (average 43.3 mg/dL +/- 3.3) revealed glucose levels at more physiological levels by day eleven (average 100.3 mg/dL +/- 6.1). Ten weeks post-injection acylcarnitine analysis demonstrated an elevation of acylcarnitine species to near normal pre-treatment levels. These findings demonstrate the short term correction of the biochemical phenotype of VLCADD in mice and provide the basis for further development of a human gene replacement strategy for VLCADD. The short duration of expression most likely is related to CMV promoter down regulation in the liver which has previously been seen with this promoter in other gene delivery systems. Further studies looking at tissue distribution of protein expression over time are in progress.

Prenatal cytogenetic study and molecular genetic characterization of a *de novo* monosomy 1q42-qter. *K. Krabchi[1], M. Ferland[1], A. Demers[2], A.L. Rougemont[3], H. Sartelet[3], R. Drouin[1]* 1) Dept Pediatrics & Genetics, Sherbrooke Univ, Sherbrooke, Qc; 2) Dept of Obstetrics & Gynecology, CHUS, Sherbrooke, Qc; 3) Dept of Pathology, Ste justine hospital, Montreal, Qc, Canada.

High levels of integrated serum markers suggested an amniocentesis be performed in a 33 year old woman. The standard cytogenetic analysis of amniocytes showed a terminal long-arm deletion of chromosome 1. FISH performed on amniocytes, using chromosome 1 specific subtelomeric probes confirmed the distal deletion. The G-banding karyotypes of parents performed on lymphocytes were normal suggesting that the fetal monosomy 1q42-qter had occurred de novo. Hybridization of the same subtelomeric probes on interphasic nuclei from 1 milliliter of maternal peripheral blood enabled us to quantify the total number of fetal nucleated cells (FNCs) in maternal circulation (20 FNCs/mL). Following this diagnostic, a medical termination of pregnancy was decided at the 23rd week of gestation. The fetal autopsy showed dysmorphic findings represented by microretrognathia, hypertelorism, posterior cleft palate, widened and short neck, single transverse palmar crease on the left hand, agenesis of the right kidney and glomerulomegaly of left kidney. The examination of muscular tissues revealed an anisomorphism of cardiac and muscular striated fibers. The severe and extensive autolysis of cerebral tissues didnt allow an analysis of specific nervous structures. The analysis of fetal fibroblasts-DNA and parental blood-DNA by PCR using a panel of polymorphic microsatellite markers showed an allelic distribution with deleted markers localized in the chromosomal segment 1q42-1qter. The deleted markers (D1S2847, D1S103, D1S2709, D1S2800, D1S446, D1S235, D1S2785, D1S2811, D1S2682) were confirmed to be of paternal origin. Moreover, this analysis allowed us to exactly localize the breakpoints on chromosome 1q42.12 sub-band distal to the D1S479 marker. The combined clinical phenotype and cytogenetic features along with literature involving similar cases suggest a novel syndrome involving 1q42-qter and phenotypic features. Our study localizes the genetic defect to 1q42.12-qter and provides specific DNA markers for the syndrome.

Human experimental treatment: report of an ethical dilemma. *V. Muñoz, M. Raymundo, T.A. Vieira, L.L.C. Pinto, A.C.M. Azevedo, A.C.S. Puga, I.V. Schwartz, L. Kalakun, R. Giugliani* Medical Genetics Service and Bioethics Commitee, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil.

Medical Genetics Service of HCPA consulted Institutional Bioethics Committee (IBC), searching for an alternative for an adult male patient with attenuated mucopolysaccharidosis Type I (MPS I) and cord compression due to glycosaminoglican deposits. Clinical manifestation included important and progressive gait disturbance, difficulty holding objects, paresia and paresthesia on four limbs. After complete evaluation, laminectomy was recommended. Nevertheless, patient did not agree on blood transfusion (BT) in case of need, due to religious beliefs (Jeovah witness) and neurosurgery team did not agree with surgical procedure under such circumstances. Management, considering that BT authorization would be refused, became a main issue and options were discussed with genetics medical team and IBC. A recent experimental study, on canine model for MPS I, using enzyme replacement therapy (ERT) with laronidase through intra thecal (IT) approach, had presented positive results. Symptomatic cord compression was rapidly progressive on this patient and clinical complications that could result in permanent disabilities or even death could not be ruled out. In such conditions, IT ERT was considered the only existing option for this patient refusing any possibility of BT, in a compassionate manner. After literature review, IBC suggestion was of recommending a compassionate use of IT ERT only for this patient. The most striking peculiarity on this case was the fact that the patient is a Jeovah witness, refusing any chance of BT on one hand and, on the other, the fact that neurosurgery team would not submit the patient to a surgical procedure without a signed informed consent for BT in case of extreme need. From ethics point of view the main purpose was to offer the best option available considering religious beliefs freedom. Compassionate protocols need not to be submitted to Research Ethics Committees in Brazil, albeit this compassionate treatment was submitted to, and approved by, Institutional Research Ethics Committee. Patient underwent IT ERT protocol.

Increased power of within study adjustment of standardized quantitative phenotypes in family-based genetic association analysis. *M.G. Naylor[1], B. Raby[2], S.T. Weiss[2], C. Lange[1, 2]* 1) Department of Biostatistics, Harvard School of Public Health, Boston, Massachusetts, USA; 2) Channing Laboratory, Harvard Medical School, Boston, Massachusetts, USA.

Many genetic association studies of complex traits rely on standardized quantitative phenotypes. For instance, forced expiratory volume in one second (FEV1) is a measurement of lung function commonly used in asthma studies. Body Mass Index has also been used as phenotype data in genetic association studies (Herbert, 2006). Such standardized quantitative phenotypes are appealing because they provide a simple way of comparing people in terms of the trait of interest. However, they may not be the most accurate way to assess subjects within a study. In 2005, Naylor, et al. suggested that using the residuals of predicted FEV1 regressed on the relevant covariates within the study population rather than indirect adjustment using percent of predicted FEV1 is the most useful measurement of FEV1 for large scale asthma studies. Here we use the Childhood Asthma Management Program (CAMP) data to show that this method of assessing complex traits by comparing residuals after within study adjustment yields greater power in family-based association tests. Simulations in which FEV1 is generated by equations derived from the CAMP data by Naylor, et al. result in increased power for family-based association tests when FEV1 is adjusted using this method rather than using percent of predicted FEV1. Power is the same or better even in simulations where FEV1 is generated using standard prediction equations. An application of family-based association analysis of FEV1 and obesity on CAMP data illustrates that more meaningful associations are found when adjusted phenotype residuals are used as opposed to typical standardized quantitative phenotypes.

Characteration of chlorambucil induced chromosome deletions in mice using BAC array CGH. *W. Liu, WW. Cai*
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Chlorambucil(CHL) is a biofunctional alkylating agent used in cancer chemotherapy. CHL has been reported to be a very efficient mutagen in mouse germ cells, causing primarily chromosomal deletion. We are reevaluating the CHL deletion efficiency in mouse germ cells under different dose conditions in an attempt to develop an efficient strategy for generating mapped mouse deletion mutant resources. We administered intraperitoneally to C57BL/6J and 129 males at a dosage of 0.75, 1.5, 3 or 6 mg/kg body weight. At the 3rd week after treatment, each 129 male was mated to two virgin C57BL/6J females and each C57BL/6J male was mated to two virgin 129 females. We then used 21,382 clone BAC whole genome tiling arrays to analyze chromosome deletions. Among 59 Mice, 29 deletions were detected and verified with PCR using polymorphic microsatellite markers in the respective regions. The size of deletions ranged from 640 kb to 36420kb. Until now, 4 deletions were found transmissible to the next generation. Thus far, we found no obvious phenotype in the heterozygous deletion mutants. We have bred 2 deletions to homozygosity and found no obvious phenotype. These results suggest that all the genes in these homozygous deletion regions are not essential for development.

Automated Identification of Rare Fetal Cells in Maternal Circulation. *M.W. Kilpatrick, T. Tafas, I. Ichetovkin, A. Seppo, Y. Kim, Y. Zhu, P. Tsipouras* Ikonisys Inc., New Haven, CT.

The presence of rare fetal cells in the maternal circulation is generally accepted but their number and the best approach for their enrichment remains unclear. Fetal cells can be indisputably identified through detection of Y FISH signals. We therefore developed an automated system to identify and enumerate cells bearing X and Y FISH signals. Ikonisys fastFISH is a fully robotic fluorescence microscopy platform. Target cells are screened at 10X magnification then verified at 100X. More than 4,000 optical fields per slide can be analysed. To minimize the false positive detection of XY cells, two independent Y-chromosome probes; one centromeric and one telomeric, were used. Putative fetal cells are identified at 10X magnification based on the presence of the X and telomeric-Y signals, and verified at 100X magnification based on the presence of a single X-signal and single signals for each of the Y-probes. Implementation of this approach produced a false positive rate for detection of XY nuclei below 0.00005%. Analysis of 26 maternal samples, 6 first trimester and 20 second trimester, identified fetal cells in 21 of the 23 samples from pregnancies with male fetuses. Samples were analysed both as whole blood, to determine a baseline for the number of detectable circulating fetal cells, and following simple density gradient centrifugation. For whole blood, analysis of a total of 103.4 million nuclei on 161 slides from pregnancies with male fetuses, identified 52 XY nuclei (0.5 XY nuclei per million, 0.3 per slide). Following simple density gradient enrichment, analysis of 64.7 million nuclei on 104 slides from pregnancies with male fetuses, identified 135 XY nuclei (2.1 XY nuclei per million, 1.3 per slide). A single XY nucleus was found in 13.3 million nuclei in samples from female fetus pregnancies. Use of embryonal globin specific antibody for initial identification of fetal cells in first trimester samples could allow analysis of both male and female fetus pregnancies. Our preliminary data suggests such an approach, integrating ICC and FISH, might facilitate non-invasive prenatal diagnosis of chromosomal aneuploidies.

Telomere length in the Amish: heritability and association with age and sex-specific parental effects. *O.T. Njajou[1], R.M. Cawthon[2], C.M. Damcott[3], S-H. Wu[1], S. Ott[3], E.H. Blackburn[1], A.R. Shuldiner[3], B.D. Mitchell[3], W-C. Hsueh[1]* 1) University of California, San Francisco, CA; 2) University of Utah, Salt Lake City, UT; 3) University of Maryland, Baltimore, MD.

Telomere length (TL) is a biomarker for aging and survival. Previous reports have indicated that TL is substantially heritable, and may be influenced through sex-chromosome effects. To further evaluate factors influencing this trait, we measured leukocyte TL in a large homogeneous Amish population. We estimated the heritability (h^2) of TL, and tested for the presence of parental effect on TL variation. Our sample included 954 healthy Amish who were connected within a 5-generation pedigree (378 men and 576 women, aged 18 - 100 yrs with a mean of 51 18 yrs). TL in leukocytes was measured by quantitative PCR (value range: 0.61 - 4.29 units, mean: 1.49 0.49). Information on parental lifespan was obtained from genealogical records maintained by the Amish community. The h^2 estimate of TL in this sample was 0.50 0.06 (p 0.001), after adjusting for age at blood draw, sex and plate batches. As expected, TL was negatively correlated with age (r = -0.40, p 0.001). There was no significant difference in TL between men and women, consistent with our previous findings that Amish men live just as long as Amish women. These findings are in contrast to other populations in which TL was reported to be longer in women than men, consistent with a longer lifespan in women than men. We also observed a positive correlation (r = 0.12, p = 0.06) and association (= 1.0, P = 0.04) between TL and paternal lifespan, but not with maternal lifespan (n = 276). Furthermore, there was a strong correlation between TL in the offspring and paternal TL (r = 0.46), which was even stronger between fathers and sons (r = 0.61) than between fathers and daughters (r = 0.36) (all p 0.001). We did not observe any correlation between TL in offspring and maternal TL. Our analyses were based on one of the largest samples for genetic studies of human TL. Our observations support a link between TL and paternal aging/lifespan, and provide motivation for efforts to map autosomal and sex-linked genetic determinants of TL variation.

High Density SNP Analysis of Ashkenazi Jews. *K. Offit[1], A. Olshen[1], B. Gold[2], J. Struewing[3], J. Satagopan[1], E. Eskin[4], T. Kirchhoff[1], J.A. Lautenberger[2], S. Stefanov[2], D. Goldgar[5], E. Friedman[6], L. Norton[1], N. Ellis[7], A. Viale[1], P. Borgen[1], K. Lohmueller[8], A. Clark[8], J. Boyd[1]* 1) Memorial Sloan-Kettering Cancer Center, NY; 2) Laboratory of Genomic Diversity, NCI; 3) Laboratory of Population Genetics, NCI; 4) University of California, San Diego; 5) University of Utah; 6) Sheba Medical Center, Israel; 7) University of Chicago; 8) Dept of Molecular Biology and Genetics, Cornell University, NY.

To analyze patterns of linkage disequilibrium (LD) in Ashkenazi Jews (AJ), we compared genotypes of 101 healthy New York AJ and 60 Utah CEPH (CEU) samples. 435,632 markers on the Early Access Version 3 (EAv3) 500k SNP array (Affymetrix) were matched to SNPs in the 500k genechip analysis of the CEU samples compiled by Affymetrix. Call rates were 98% for the CEU samples and 93-95% for the AJ samples. Screening out markers with <85% call rate, 379,401 SNPs were evaluable. The proportion of SNPs out of Hardy-Weinberg equilibrium (at $P < 0.05$, FET) was 5% in the CEU and 6-7% in the AJ set. A small but significant global difference in allele frequencies between AJ and CEU was demonstrated by a derived FST of 0.008 ($p<0.001$). Analysis of local LD decay revealed no global differences, but found some regions (e.g. HLA region on chromosome 6) with different decay rates by both D' and R^2 analysis. Haplotype blocks inferred from pairwise LD statistics (Haploview) as well as by EM haplotype phase inference (HAP) showed a greater number of haplotype blocks in the AJ; 41,913 in AJ vs 37,421 in CEU by Haploview, and 52,696 in AJ vs 49,996 in CEU by HAP. Haplotype blocks were smaller in AJ by both measures (e.g. average length 40,354 bp in AJ vs 42,899 bp in the CEU by HAP). These data would suggest that the LD structure of AJ may not provide any global advantage for whole-genome association mapping. On the other hand, a likelihood ratio approach showed that runs of homozygous SNPs were approximately 25% longer in AJ. These data support an emerging picture of the AJ that suggests lower measures of LD than expected by some measures, but also distinctive differences from northern European populations that remain to be fully resolved.

In silico analysis of histone deacetylases. *S. Khuri, G. Dimicco, L.J. Elsas* Center for Medical Genetics, University of Miami , Miami, FL.

Human histone deacetylases (HDACs) remove acetyl groups from histone tails and repress transcription. The 11 known HDACs differ in size, domain organization, and function. They are evolutionarily classified into four groups which do not correlate well with HDAC function nor regulation. HDACs have some tissue- and temporal- specificity, that have not been fully characterized, and little is known about the regulation of HDAC expression. We approached these problems using three in silico strategies. 1.Nucleosome positioning: HDAC promoter sequences were analyzed using the NXSensor software, which predicts nucleosome positioning, and which we had used to demonstrate a difference in nucleosome position between promoters of housekeeping genes and those of tissue-specific genes. The promoter sequence of HDAC4 is predicted to be free of nucleosomes, whereas that of HDAC9 can have several nucleosomes on it. This has important implications for the types of transcription factors that may regulate HDAC genes. 2.Transcription factor binding sites (TFBSs): TFBSs that are conserved (cTFBSs) between human, mouse and rat were collected from the UCSC Genome Browser. cTFBSs are more likely to be functional than the numerous sequence motifs detected by pattern recognition software. However, motif detection is useful for identifying unique TFBSs that may play a role in the tissue- or temporal- specificity of a gene; for this we used the AliBaba2 (TRANSFAC) program. The HDAC4 promoter has the highest number of potentially active TFBSs, but only one cTFBS. There were hardly any cTFBSs that were common among the HDACs. A number of unique TFBSs were found through motif detection, and these merit further investigation. 3.Alternative transcripts (AlTs): NCBIs AceView was data mined for records of HDAC AlTs, and we compared the transcripts against the functional domains of HDAC proteins. The majority of transcripts did not include enough of the catalytic domain to be functional as deacetylases, but they may have a regulatory role. In conclusion, there is a significant variation in the stringency of regulation among the 11 HDACs. Integrated bioinformatics analyses such as this are vital for future experimental analyses of human gene regulation.

***Tbx1*, a 22q11 deletion syndrome candidate gene, is a selector for myocardial cell fate specification in the secondary heart field.** *J. Liao, S. Nowotschin, B.E. Morrow* Department of Molecular Genetics, Albert Einstein College of Medicine, Bronx, NY.

Tbx1 is a member of the T-Box family of dosage sensitive transcription factors and implicated in the etiology of 22q11 deletion syndrome (22q11DS; velo-cardio-facial/DiGeorge syndrome). The 22q11DS patients and *Tbx1*-/- null mice have characteristic cardiac outflow tract (OFT) defects. Tbx1 in the secondary heart field (SHF), a cardiac progenitor population contributing to the anterior part of the heart, is required for OFT development. To determine the molecular pathogenesis of OFT defects, we performed Affymetrix gene expression profiling of the SHF in wildtype and *Tbx1*-/- embryos from E8.75 to E10.75. Surprisingly, except for *Nkx2-6* and *Hod*, two genes involved in myocardial differentiation, which were downregulated, most genes were upregulated in the SHF of *Tbx1*-/- embryos, suggesting Tbx1 may work as a transcriptional repressor. Expression changes of selected genes were validated by real-time RT-PCR and *in situ* hybridization. Among these genes, *Aldh1a2* (*Raldh2*), *Gata4* and *Tbx5* have been reported to play a role in the determination of anteroposterior patterning of the heart. More importantly, expression of cardiac atrial markers *Myh6* (*MHC*) and *Myl7* (*MLC2a*) were significantly increased in the SHF of the null mutants. However, ventricular markers, *Myh7* (*MHC*) and *Myl2* (*MLC2v*) were unchanged, indicating inhibition of the atrial-specific differentiation of SHF cells by Tbx1 is required for normal OFT development. This study shows that *Tbx1* is a selector gene for myocardial cell fate specification in the SHF. These genes we found here are good candidates for genetic modifiers for OFT defects in patients with 22q11DS.

Origin of IVS6-1g>t Allele in Fumarylacetoacetate Hydrolase Gene among Hispanic Patients with Tyrosinemia Type I. *J.-Y. Huang, D. Xu, C.R. Scott* Pediatrics Department, 359320, University of Washington, Seattle, WA.

Hereditary tyrosinemia type I (HT1) is an autosomal recessive disorder characterized by a deficiency in fumarylacetoacetate hydrolase (FAH) activity. At least over 40 different mutations within the FAH gene have been identified with several common mutations having specific geographical origins. IVS12+5g>a is the most common mutation in Northwestern Europe, whereas IVS6-1g>t occurs more frequently in Spain and other Mediterranean counties. We recently screened FAH gene mutations in forty-three U.S. HT1 patients and found at least one copy of IVS6-1g>t mutation in 7 out of 10 patients of Latin American ancestry. To determine whether the IVS6-1g>t allele in Hispanic patients was identical to its European counterpart, we performed single-nucleotide polymorphism (SNP) analysis. We genotyped a panel of 10 SNPs spanning over a 48 kb genomic region at the FAH locus in a total of 12 patients (7 of Hispanic, 4 of European, and one of unknown background) carrying at least one copy of IVS6-1g>t mutation and 12 normal controls of Hispanic ancestry. We found that the allele frequency of each SNP in the Hispanic HT1 patients is significantly different from that of the Hispanic controls due to strong linkage disequilibrium (LD). Pairwise LD analysis of the SNPs demonstrates complete linkage disequilibrium within this region in the patients. One common haplotype backbone projecting the single haplotype block can be defined using maximum likelihood algorithm for each patient. The three patients homozygous for IVS6-1g>t mutation (two Mexican, one European) are also homozygous for this common haplotype. This indicates that the IVS6-1g>t mutation co-segregates with the common SNP haplotype shared by each patient and is independent of their ethnic background. A total of three LD blocks are observed which is consistent with the LD data reported in the HapMap database. The data implies that the IVS6-1g>t mutation was most likely introduced into the Latin American population during the Spanish Conquest of the 16th century.

Cytoplasmic FANCC is not Required for a Normal Response to Interstrand Crosslink Damage. *A. Hemphill, L. Lucas, M. Al-Dhalimy, Y. Akkari, S. Olson, R. Moses* Dept Molecular & Medical Gen, Oregon Health & Science Univ, Portland, OR.

Fanconi anemia (FA) is a recessive disorder characterized by increased cellular sensitivity to interstrand crosslinking (ICL) agents. A core complex of FA proteins (A, B, C, E, F, G, L, M) forms in the nucleus following ICL damage, and is required for normal repair of ICLs. The complex acts to the modify FANCD2 by monoubiquitination. Patient-derived cells deficient in any of the FANC proteins show decreased survival after ICL damage and increased radial formation. Several FA proteins, including FANCC and FANCE, also exist in the cytoplasm of cells before and after DNA damage. We find that depletion of FANCC by siRNA results in loss of cytoplasmic FANCC, but not nuclear FANCC. The nuclear fraction persists for over 96 hours following siRNA transfection, whereas the cytoplasmic fraction is depleted in 24 hours, with recovery apparent at 72 hours. The nuclear fraction confers normal resistance to damage caused by ICLs, as measured by cell survival or radial formation. The persistence of the nuclear FANCC protein may be due to association with other specific FANC proteins. To test this, FANCE, which is known to complex directly with FANCC, was depleted. The depletion of FANCE leads to overall decreased stability of the FANCC protein, allowing nearly complete depletion of nuclear FANCC. We conclude that cytoplasmic FANCC is not required for a normal response to ICLs, and that nuclear complex formation is the basis of stability for nuclear FANCC protein.

First trimester nuchal translucency and maternal ethnicity. *T. Huang[1], C. Meier[1], K. Boucher[1], F. Wang[2], A.M. Summers[1]* 1) Genetics Prog, North York Hosp, Toronto, ON, Canada; 2) Health Surveillance, Alberta Health and Wellness, Edmonton, Alberta, Canada.

Nuchal translucency (NT) is a first trimester ultrasound marker used in the prenatal screening for Down syndrome. Recent studies suggested there were possible ethnic related variations in the measurement of NT. We conducted a study among women who had the Integrated Prenatal Screening (IPS) in North York General Hospital (NYGH) to assess the influence of maternal ethnicity on NT. Between 11/1999 and 8/2004, 27,365 non-diabetic women who had an unaffected singleton pregnancy were screened at NYGH - 15,804 Caucasian, 4,305 Chinese, 4,178 Asian, and 1,286 Black women. Chinese women were identified from the ethnicity category of Asian using a published Chinese surname list. NT was measured between 10 and 14 weeks of gestation and was expressed as multiple of median levels (MoM) of unaffected pregnancies at the same gestational age based on sonographer specific NT curves. The sonographer specific NT curves were established after the initial assessment of 50 NT images from each sonographer before they can enroll in the program and subject for ongoing review. During the study period, 173 sonographers were enrolled in the screening program. Since the NT curves were based on all screened women rather than Caucasian and the proportions of non-Caucasian women each sonographer screened varied substantially, an adjusted NT MoM (Observed NT MoM/sonograper specific median NT MoM for Caucasian) was calculated for this study to reset the median NT MoM for Caucasian to 1.0 MoM. Compared with the median NT MoM for Caucasian, the median NT MoM for Chinese is 4% higher, for other Asian is 1% higher and for Black is 2% lower (p0.05). The differences were similar across most of the weight groups and consistent during the gestational weeks when NT is measured. Although ethnic differences in NT were small, NT is an influential marker in risk estimation. Screening programs may choose whether to made adjustment based on local requirements and the ethnic composition of screening population. The results of this study also provided useful information for genetic counseling.

Prenatal testing and screening in Northern British Columbia: Question of access or personal preference? *J. Nuk[1], J. Atkinson[2], M. Pearson[1], K. Kelly[3], L. Arbour[1]* 1) UBC Medical Genetics, Children's and Women's Health Centre, Vancouver, BC; 2) IWK Health Centre, Halifax, NS; 3) University of Northern BC, Prince George, BC.

BACKGROUND Access to specialty services is challenged in rural and northern areas. As prenatal testing and screening options are often considered standard of care, access is an issue for women in rural and northern Canada. In BC, a discrepancy in the uptake of the triple marker screen (TMS) is seen between the more densely populated Vancouver Coastal Health Authority (69%) and the less densely populated Northern Health Authority (NHA) (20%). To determine if the discrepancy results from difficulty in accessing screening and follow-up services, or reflects informed personal choices, an exploratory survey was distributed in the NHA to pregnant and postpartum women. **STUDY DESIGN** Surveys were distributed at hospital registration and at prenatal and neonatal classes. The survey collected demographic information, explored health care practices and documented experiences and beliefs related to prenatal testing and screening, community beliefs and telemedicine. Quantitative and qualitative data were collected. The majority of questions were categorical and analyzed using the ^{2} statistic. Trends were noted as the proportion of women who chose each multiple choice option. **RESULTS** From a total of 92 respondents, over 75% were offered TMS with 36% electing to be tested. The most influential factors on decision making were understanding of the test and the amount and quality of information available. Most felt well informed of their options (p0.001). Of least importance were culture and ethnicity (p0.035) and the cost and inconvenience of travel for follow-up services. **CONCLUSIONS** Our results suggest the lower uptake of TMS in the NHA is primarily based on informed personal choices. Though considered inconvenient, women in this northern community did not indicate that access to follow-up services influenced their decisions. This exploration may have relevance for health care planners concerned with access to genetic services. Further research is underway exploring the same questions in a high uptake region.

A novel g.-1285C>T substitution in a conserved putative regulatory element of *PAX9* is associated with autosomal dominant molar hypodontia. *G.A. Mendoza[1], J.M. Gee[1,2], C. Gonzalez-Quevedo[1], K. Lee[3], J. Hartiala[1], S.M. Leal[3], H. Allayee[1], P.I. Patel[1,2,4]* 1) Institute for Genetic Medicine, University of Southern California, Los Angeles, CA; 2) School of Dentistry, University of Southern California, Los Angeles, CA; 3) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 4) Center for Craniofacial Molecular Biology, University of Southern California, Los Angeles, CA.

The transcription factor PAX9 plays a critical role in the switch of odontogenic potential from the epithelium to the mesenchyme during tooth development. Twelve mutations in the coding region of *PAX9* have been previously reported to cause non-syndromic autosomal dominant hypodontia. We ascertained a family of European American descent that segregated a non-syndromic autosomal dominant form of hypodontia involving primarily molar teeth. Three microsatellite markers, D14S1462, D14S1463, and D14S1464 located 39, 49 and 72 kb, respectively upstream of the *PAX9* gene and a SNP within exon 3 of *PAX9*, were tested for linkage. A maximum multipoint LOD score of 3.6 was obtained with all four markers demonstrating linkage between the disease locus and the marker loci within and flanking the *PAX9* gene. There were no recombination events identified between these markers and the disease locus, and all affected individuals shared the same marker haplotype. Every exon of the *PAX9* gene was sequenced twice and no mutation was found in the coding or 5- and 3- untranslated regions. A novel g.-1285C>T sequence variant within a multiple species conserved sequence element upstream of the *PAX9* promoter, was identified in all affected family members, but not in the unaffecteds or within 3088 control chromosomes (95% confidence interval 0 - 0.0012) of the same ethnic background. The impact of the variant on *PAX9* expression is being examined. We have previously shown that haploinsufficiency for PAX9 is the underlying basis for molar hypodontia. The present data in conjunction with these earlier findings suggests that the g.-1285C>T variant may be located within a putative regulatory element that causes reduced *PAX9* expression leading to the hypodontia phenotype in this family.

A new model for South American origins inferred from hierarchical modeling of mtDNA variation. *C. Lewis, J. Long* Dept. Human Genetics, U. of Michigan, Ann Arbor, MI.

Genetic diversity in native South Americans forms a complex pattern at continental and local levels. Western populations have higher diversity within groups and smaller genetic distances among groups when compared to eastern populations. From this pattern, it was proposed that a larger, more genetically diverse, founding population entered the West than the East. After the initial settlement, the West harbored a larger effective size with higher gene flow. Here, we question the pattern and interpretation of South American genetic diversity because regional diversity was previously inferred from diversity within local populations without considering the diversity among local populations within regions. This potentially biased the continental pattern by underestimating eastern diversity. We analyzed mtDNA sequences from positions 16040-16361 for 892 people which represent 27 populations from North (2), Central (3), and South America (22). A series of nested models were fit to diversity estimates within and between all pairs of local populations. Each model added a level of nesting to the previous model. We tested three hypotheses: i) that diversity is patterned into West and East regional populations ii) that there is more genetic diversity in the West iii) that a single source population is sufficient to explain the continental pattern of diversity. By considering the diversity within and between populations, we found that i) the diversity in the East is not reduced relative to diversity on the continent as a whole, ii) that the West possesses a subset of the diversity on the South American continent, and iii) that diversity in the South American populations is reduced relative to the total for North, Central and South America. These findings suggest that South America forms a single meta-population with only one regional cluster, the West. This leads us to propose a new scenario for the peopling of South America: The continent was peopled from a single source population that became subdivided; the western region was founded by one subpopulation, while many subpopulations characterized by frequent fissions and bottlenecks persist in the eastern region.

Mathematical Methods for Analysis of Protein Arrays. *A. Pirozhenko[1], B. Love[3], G. Michaud[4], K.P. Clancy[2]* 1) Bioinformatic Sciences, Invitrogen Corp, 1600 Faraday Ave, Carlsbad CA; 2) Bioinformatic Sciences, Invitrogen Corp, 7305 Executive Way, Frederick MD; 3) Corporate Research Laboratory, Invitrogen Corp, 7305 Executive Way, Frederick MD; 4) Microarray R&D Protometrix, Invitrogen Corp, 688 E. Main St. Branford CT.

Microarray technologies permit investigators to investigate genes of interest in a global fashion. Performing such experiments with protein arrays provides investigators with a much broader understanding of the function of their protein of interest than is possible with conventional technologies. The Protoarray chip provides more than 5000 biochemically active proteins as well as appropriate negative and positive controls. These arrays provide analysis support for a number of proteomics based methodologies, including the global profiling of kinase or other enzymatic activities; identification of protein-protein interactions (PPI) between arrayed proteins and a protein of interest, and; profiling of serum antisera for reactivity to the arrayed proteins. Although the Protoarray has several similarities to more conventional microarray strategies, the more complex biological nature of proteomics applications means that different statistical methods for array analysis have had to be devised and tested. For instance, Protoarrays may be probed using either fluorometric or radiometric labelled analyses, so different statistical approaches were utiliized to discern signal from array background. Z-scores, representing the deviation of the signal for a given protein feature from the mean of the signal of all other protein features were found to maximize signals while minimizing Type I and Type II errors. Serum profiling was best analysed using a combination of quantile normalization and M-statistics to maximize signal while reducing errors. Sharing and reproduction of protein array experiments will require the development of suitable standards for data analysis and exchange. Changes to the oligo microarray stadard, MIAME, will be necessary to manage the more complex analyses of protein arrays.

Power loss in structured populations. *Y.G. He[1], R.H. Jiang[1], G.E. Swan[1], L. Jin[2,3]* 1) Health Center, SRI International, Menlo Park, CA; 2) School of Life Sciences, Fudan University, Shanghai, China; 3) CAS-MPG Partner Institute for Computational Biology, Shanghai, China.

Large scale association study holds promise to disclose genetic mechanisms for complex traits. However, loss of statistic power was still not well known for population-based association study with population stratification. Further investigation in this power loss would help us to figure more powerful approach for association studies.
We generalized an existed genetic model, which was proposed by Devlin et al in 2001, to more common genetic scenarios. The generalized model provides us a powerful tool to look into the power loss in structured populations. Our result shows that correlation between disease prevalence and suspect allele frequency among subpopulations plays critical roles in power change of statistic in association study. While there is a negative correlation between them, statistic power will reduce with an increase in genetic divergence. The power loss would be very severe in structured populations with moderate genetic divergence, Fst0.001. The change could be more than 20% if prevalence was obviously different among subpopulations. Power loss became more serious to the suspect loci with lower relative risk or lower allele frequency. Parts of suspect loci could be undetectable, even with extremely large sample size. Due to severe bias in estimation for relative risk of suspect loci, genetic contribution from these loci would be difficult to be evaluated accurately. Statistic power will increase with enhance of genetic divergence when disease prevalence and suspect allele frequency is correlated positively among subpopulations. However, the improvement in power disappeared under circumstance where false positive rate was well controlled.
All of the results indicated that control for false negative result is as important as control for false positive result in population-based association study for complex trait.

Novel mutations in the *MFN2* gene causing familial and sporadic axonal Charcot-Marie-Tooth disease type 2 (CMT2). *M. Milani, D. Pareyson, V. Seveso, C. Gellera, F. Taroni* Division of Biochemistry and Genetics, IRCCS Istituto Neurologico Carlo Besta, Milan, Italy.

Axonal CMT (CMT2) is genetically highly heterogenous, with at least 14 loci and 10 genes identified thus far. Mutations in the gene encoding the mitochondrial protein mitofusin-2 (*MFN2*) have been shown to be responsible for autosomal dominant CMT2 type A2 (CMT2A2). The *MFN2* gene maps to chromosome 1p36.2 and encodes a 757-amino acid protein which is an essential component of mitochondrial fusion in mammalian cells. The CMT2A2 phenotype is largely indistinguishable from that of CMT2A1 (*KIF1B*), CMT2E (*NEFL*), and CMT2F (*HSPB1*). However, in a subset of CMT2A2 patients, pyramidal involvement and visual impairment have been reported. The disease exhibits reduced penetrance: studies in large families have shown that individuals with *MFN2* mutations may present no signs of disease even at the electrophysiological examination. We studied 99 unrelated CMT2 index patients, including 82 isolated cases and 17 familial cases. Nine heterozygous mutations (8 novel) were detected in 10 unrelated patients but in none of >200 control chromosomes. Mutations were found in 6/82 (7.3%) sporadic cases and in 4/17 (23.5%) familial cases. Similar to previous reports, the majority (7/9) of the mutations cause an amino acid change. Two mutations were unusual: a frameshift mutation predicted to cause an early truncation of the protein and a synonymous mutation predicted to abolish an exon splicing enhancer (ESE). Onset in infancy was reported in 3 cases (1 sporadic and 2 familial). Pyramidal involvement was present in 2 cases. Optic atrophy was observed in 2 cases. In one family, CMT2 was associated with tremor. In conclusion, our results indicate that: 1) *MFN2* mutations are not rare causes of axonal CMT; 2) genetic testing for CMT2 should begin from the *MFN2* gene; and 3) *MFN2* mutation analysis should be performed in both familial and sporadic cases. [Partly supported by grants FIRB RBNE014HJ3010, Telethon-UILDM GUP04009, and Fondazione Mariani R0544 to FT].

Familial Translocation with Partial trisomy 21 and Partial trisomy 18, manifesteing as ADHD. *C. Trujillo[1], N.M. Erfan[2], S.B. El Badawi[3], A. Hasanat[1]* 1) Genetics Unit, Dr. Erfan & Bagedo Hospital, Jeddah, Saudi Arabia; 2) Jeddah Child and Adolescent Psychiatric Services, Jeddah; 3) Laboratory Department, Dr. Erfan & Bagedo Hospital, Jeddah, Saudi Arabia.

We report a case of a partial trisomy 21 and partial trisomy 18, in a 5 year old boy, referred to us with the clinical diagnosis of Atention Deficit Hyperactive Disorder (ADHD). The patient does not have mental retardation, with IQ in the normal range; initially presented speech and language delay, but now is normal. He had no major malformations, but have mild dysmorphic features.

G and R banding, coupled with FISH using probes WCP 21, WCP 18 and TelVysion 21q (Vysis), revealed a karyotype 47,XY,der(21),t(18;21)(18q22qter::21q2121pter). His mother and aunt had a balanced translocation 46,XX,t(18;21)(q22;q21)that was absent in all other family members. The aunt had 4 previous miscarriages but cytogenetic analysis was only performed as a consequence of proband findings.

The extra derivate chromosome of the proband is clearly missing the DSCR (Down Syndrome Critical Region) located between 21q22.1 to 21q22.3 explaining the absence of phenotypic features of Down Syndrome.

Major features of trisomy 18, such as congenital heart disease, early death, and external malformations, appear to be consistently related to the trisomic state of 18q12 to 18q21 but, in this case, partial trisomy 18 starts at 18q22, which may explain the mild phenotype.

Our results seems to confirm previous findings, that the smallest extra region causing serious anomalies of trisomy 18 appears to be 18q12.1-q12.2 segment being the "critical" zone.

The mild manifestations of our patient are probably due to the absence of critical regions on translocated segments of the 21 and 18 chromosome.

The genes associated so far with ADHD lie outside chromosome 21 and 18. We suggested that a third copy of a gene or genes in 18q22qter::21q2121pter regions, may be associated with ADHD.

Simvastatin lowers plasma 24S-hydroxycholesterol in Smith-Lemli-Opitz syndrome. *L.S. Merkens[1], J. Jordan[2], J. Penfield[1], D. Lutjohann[3], K. von Bergmann[3], W.E. Connor[2], R.D. Steiner[1,4]* 1) Dept Pediatrics, Oregon Health & Science Univ (OHSU), Portland, OR; 2) Dept Medicine, OHSU, Portland, OR; 3) Dept Clin Pharm Rheinische Fredrick-Wilhelms, Univ Bonn, Bonn, Germany; 4) Dept Mol & Med Genetics, OHSU, Portland, OR.

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive disorder caused by a defect of the enzyme in the final step of cholesterol (CH) synthesis. Mental retardation is a nearly universal feature. CH deficiency and accumulation of potentially toxic CH precursors such as 7- and 8-dehydrocholesterol (7- and 8-DHC) may be causative. One treatment objective of SLOS is to raise CH concentrations and lower 7- and 8-DHC. Dietary CH is widely used a potential treatment, but it does not cross the blood brain barrier (BBB), so it is unlikely to impact neurological development in affected children. Statins, inhibitors of a key regulatory enzyme of CH synthesis (HMG CoA reductase), have been shown to reduce plasma and cerebral spinal fluid 7- and 8-DHC in SLOS. Lipophyllic statins, like simvastatin (simv), cross the BBB and may be effective in reducing 7- and 8-DHC in the brain. 24S-hydroxcholesterol (24S) is formed from CH in the brain, and it is able to cross the BBB. Almost all of the 24S in plasma originates from the brain and is therefore a marker of brain CH turnover. We investigated the effect of simv on brain CH turnover as measured by plasma 24S in children with SLOS. Most of the children without simv treatment consumed a high CH diet (n=18,samples=27), a few were on a low CH diet (n=6,samples=6). All of the children receiving simv (0.2 to 0.5 mg/kg/d) consumed a high CH diet (n=5,samples=16). We measured plasma CH by GC and plasma 24S by GCMS. Plasma 24S was corrected for CH (g/mg) and analyzed on the natural logarithmic scale by multiple linear regression. Simv was estimated to lower 24S relative to CH by approximately 25%(P0.001). The decreased plasma 24S in response to simv may reflect decreased CH turnover and flux through the CH synthetic pathway in the brain. This could have a beneficial clinical effect by reducing brain 7-and 8-DHC.

The Prdm8 transcription factor is essential for the development of rod bipolar cells in the mammalian retina. *C. Jung*[1,2], *R.R. McInnes*[1,2] 1) Prog. in Developmental Biology, Hosp. for Sick Children; 2) Dept. of Molecular & Medical Genetics, Univ. of Toronto, Toronto, ON, Canada.

In mammals, disruptions of over 25 retinal transcription factor genes are associated with developmental eye defects or retinal degeneration. In a screen for retinal developmental regulators, we identified PRDM8 as a zinc finger putative transcription factor likely to be important for retinal formation, since at least 5 of the 17 predicted mammalian PRDM proteins are essential regulators of cell fate or maintenance. *Prdm8* mRNA is expressed broadly in the developing nervous system. In adults, expression is most prominent in hippocampus, cerebellum and retina; retinal Prdm8 protein is most abundant in the interneurons of the inner nuclear layer (INL) and in ganglion cells, with lower expression in photoreceptors. To define the roles of *Prdm8* in the developing and mature brain, we generated a mouse *Prdm8* protein null allele, replacing part of the ORF with an *eGFPnls* (nuclear localized eGFP) knock-in. Homozygous *Prdm8* null mutants are viable and express eGFP in retina, hippocampus and cerebellum. *Prdm8* null retinas have specific morphological defects: there is striking INL hypocellularity, with a complete absence of rod bipolar cells, while cone bipolar cells and other major retinal cell types appear normal. The regions of interneuron synaptic contacts are abnormal: the inner plexiform layer is thin, and the outer plexiform layer is disorganized. *Prdm8* null mice also groom excessively, leading to skin lesions in the groin and chest in 80% of outbred *Prdm8* null mice, with 20% displaying no increased grooming. We conclude that i) *Prdm8* is critical for the formation or maintenance of rod bipolar cells, a major class of retinal interneurons; ii) the loss of *Prdm8* function is associated with an unusual and specific behavioural phenotype that is influenced by the genetic background; iii) given the strong conservation of the Prdm8 protein sequence (90%) and of rod bipolar cell function between human and mouse, *PRDM8* (chr. 4q21) is a candidate gene for congenital stationary night blindness, a condition associated with disordered rod signaling in humans.

Rare missense polymorphisms: the good, the bad and the ugly. *G.V. Kryukov, S.R. Sunyaev* Division of Genetics, Brigham & Women's Hospital, Harvard Medical School, Boston, MA.

Several recent reports showed that common complex phenotypes can be caused by multiple rare non-synonymous variants, and proposed association studies based on complete re-sequencing of candidate genes. In a study of this design, a cumulative frequency of rare deleterious mutations in a candidate gene, rather than individual SNPs frequencies, is compared between disease and control cohorts. The success of such approach critically depends on the proportion of deleterious mutations among all detected missense polymorphisms and on our ability to distinguish deleterious amino acid substitutions from neutral ones. If the majority of amino acid substituions detected in the study are neutral, then, the power of the method will be low because of the low signal to noise ratio.

It is not known what fraction of missense substitutions among *de novo* mutations and polymorphisms are strongly detrimental, mildly deleterious or effectively neutral. We estimated these values by comparing expected and observed numbers of nonsense, missense and synonymous changes among disease mutations, human SNPs identified by systematic re-sequencing projects and substitutions fixed in the human lineage after divergence from chimpanzee. As expected, fraction of deleterious mutations among common polymorphism was extremely low. However, despite commonly held belief that even among rare missense SNPs most are effectively neutral, our results indicate that the majority of human missense polymorphisms with detected frequency below 1% are, in fact, deleterious. This suggests that allele frequency alone can serve as a strong predictor of functional significance of missense polymorphic variants. We estimated that, on average, each human genome has approximately 600 deleterious missense SNPs associated with selection coefficients in the range of 10^{-2}-10^{-3}.

Our results suggest that association studies aimed to detect enrichment in rare non-synonymous variants might be efficient tool to study genetics of complex diseases and our work serves as a theoretical foundation for this approach.

Individualized Bayesian updating of genetic risk prediction models. *E. Iversen[1], Y. Tao[2], G. Parmigiani[3]* 1) Dept Biostatistics & Bioinformatics, Duke University; 2) Dept Biostatistics, Johns Hopkins University; 3) The Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University.

Individuals at high familial risk of disease are frequently counseled on basis of predictions from a genetic model for the suspected syndrome. For example, an individual with a family history of breast cancer may be given BRCA1/2 mutation carrier probability estimates from a prediction model that makes a specific set of assumptions about the genetic model and related parameters that describe the syndrome. Given the inherent difficulty in characterizing allelic and other sources of family-specific heterogeneity in penetrance, the assumed penetrance models tend to characterize a typical mutation carrier.
We describe modifications to standard genetic models to address familial heterogeneity in penetrance of a disease gene or family of disease genes. The approach uses Bayesian model averaging, a technique that incorporates uncertainty in parameters of the genetic model and, to the extent possible, learning of family--specific values of those parameters. Bayesian model averaging has been shown to improve predictions over assuming a fixed model; we show that this result applies to carrier probability and disease risk prediction. We review Bayesian model averaging and what is necessary to adapt genetic models for family data to the approach and describe a method for updating model weights and making weighted predictions in light of family data. We restrict the collection of possible models to a discrete set with each representing a distinct penetrance scenario and describe an approach for estimating its members given a training dataset of family data on individuals genotyped at the gene(s) involved. We report on an analysis of family data from a multi-center study of familial breast and ovarian cancer. The data set includes results of genetic assays at BRCA1 and BRCA2 and detailed family histories. Using this data, we demonstrate that a typical family history collected in a high risk clinic carries enough information to tailor the genetic model to the individual's family history and that this approach results in more accurate predictions.

Methylation-dependent fragment separation: Direct detection of DNA-methylation by capillary electrophoresis of PCR products from bisulfite-converted genomic DNA. *A.E. Karger[1], B. Finkelnburg[2], A. Rico[3], K.I. Moody[1], K.J. Livak[1], G. Zon[1], V.L. Boyd[1]* 1) Applied Biosystems, MB-Genetic Analysis R&D, Foster City, CA; 2) Applera Deutschland GmbH, Darmstadt, Germany; 3) Applera France S.A., Courtaboeuf Cedex, France.

We report a novel method for methylation detection by denaturing capillary electrophoresis (CE) using standard fragment analysis conditions. Bisulfite treatment of gDNA will selectively deaminate C but not 5-methylcytosine (5mC). Amplicons generated from bisulfite-converted gDNA are analyzed immediately after PCR using a 6-carboxy fluorescein (6-FAM) dye-labeled primer. The amplicons from methylated and unmethylated gDNA separate based solely on base composition due to the presence of multiple C versus thymine (T) differences. By direct detection of PCR amplicons following PCR using primers that anneal independent of methylation status, the overall workflow from gDNA sample input to data analysis is relatively simple. Furthermore, the same PCR product is suitable for additional analyses such as direct sequencing, cloning and sequencing, single-base extension, and post-PCR incorporation of a modified dCTP, the latter of which allows resolution of amplicons with as little as a single C/T difference. We show the utility of this novel CE detection assay by analyzing the hypermethylated region of the fragile-X FMR1 locus.

A comprehensive analysis of LRRK2 gene in patients with Parkinsons disease (PD). *Y. Liu, K.M. Bond, G. Mayhew, J. Gibson, D. Daniel, M. Noureddine, M.A. Hauser, J.M. Vance* Center for Human Genetics, Duke University Medical Center, Durham, NC.

Genetic studies of PD have contributed significantly to our understanding of the pathogenesis of neurodegeneration. Leucine-rich repeat kinase 2 (LRRK2) gene has been identified as a cause of the late-onset PD. Due to the size of LRRK2 gene, most studies have only investigated a few primary exons. However, through our ongoing autopsy program for PD, we were able to sequence the cDNA of LRRK2 (NM_198578) from 35 brain autopsy samples with Parkinsonism, identifying 18 coding variants. Two variants were novel, but led to synonymous changes. Eight non-synonymous SNPs were previously reported, but in small studies that precluded a determination whether they were mutations or polymorphisms. Therefore, we genotyped these and the one novel SNP in 760 PD families to determine their frequency. Preliminary results demonstrate that at least seven are in fact polymorphisms, with two still in progress. As LRRK2 becomes increasingly screened, it is important to test all variations in large, well characterized populations to determine their value in diagnosis of PD.

Initial results from a Phase II clinical trial of migalastat hydrochloride in patients with Fabry disease. *P. Huertas[1], R. Schiffmann[2], G. Pastores[3], W. Wilcox[4], D. Palling[1], B. Wustman[1]* 1) Amicus Therapeutics, Cranbury, NJ; 2) National Inst. of Neurological Disorders and Stroke, Bethesda, MD; 3) NYU School of Medicine, New York, NY; 4) Cedars-Sinai Medical Center, Los Angeles, CA.

We report initial results from a Phase II clinical trial of migalastat hydrochloride (Amigal; Amicus Therapeutics, Inc.) in patients with Fabry disease. The trial is designed to enroll Fabry patients with a missense mutation, greater than 3% residual -galactosidase A (-GAL) activity, and an in-vitro increase of -GAL activity in lymphocytes treated with migalastat hydrochloride of more than 20%. Patients enrolled in the trial receive migalastat hydrochloride 25 mg PO BID, 100 mg PO BID and 250 mg PO BID in an ascending fashion every two weeks, followed by an additional 6 weeks of 25 mg PO BID. At the beginning of the trial and during each escalation, safety parameters, -GAL activity in white cells, globotriaosylceramide (GL-3) in plasma and urine, and migalastat hydrochloride pharmacokinetics are determined. Additionally, at the beginning of the trial and at the conclusion of 12 weeks of treatment, a skin biopsy is collected for analysis of -GAL activity, GL-3 levels, EM scoring of lysosomal inclusions, and IHC determination of -GAL and GL-3 localization. The initial data from the first four patients enrolled in the trial showed that migalastat hydrochloride was well-tolerated with no reported serious adverse events, and that the -GAL enzyme activity in patient white blood cells after six weeks of treatment was on average more than five-fold higher than before treatment. After 12 weeks of treatment, enzyme activity in skin was increased in two out of two patients with assessable biopsies. GL-3 levels in patient plasma, urine and skin were in the normal range of healthy individuals both before and after 12 weeks of treatment. Migalastat hydrochloride belongs to a new class of drugs known as pharmacological chaperones, which selectively bind to a target protein, increasing stability and trafficking to the proper location, thereby increasing activity and improving biological function. We will present an update on all available interim data from our ongoing Phase II clinical trials.

Sodium phenylbutyrate decreases steady-state plasma concentration of branched-chain amino acids without altering their appearance rate or protein turnover as measured by stable isotope tracers in healthy human subjects. *B. Lanpher[1], J. Marini[2,3], F. Scaglia[1], S. Carter[1], P.J. Garlick[2], F. Jahoor[3], B. Lee[1,4]* 1) Baylor College of Medicine, Houston, TX; 2) University of Illinois at Urbana-Champaign; 3) Children's Nutrition Research Center, Houston, TX; 4) Howard Hughes Medical Insitute.

Sodium phenylbutyrate (Buphenyl) is used commonly in the pharmacologic management of urea cycle disorders. The primary active metabolite, phenylacetate, conjugates glutamine to form phenylacetylglutamine which is readily excreted in the urine. We have observed that patients taking sodium phenylbutyrate have decreased circulating levels of branched chain amino acids. To examine this further, 7 healthy controls were studied using a multiple primed-constant infusion of stable-isotope tracers at baseline and after 2 days of sodium phenylbutyrate treatment. In each case, after two days of dietary stabilization (0.6 g protein/kg/day), infusions were conducted over 10 hours. Blood, urine, and breath samples were collected just prior to and during the infusions. Urea synthesis decreased by 247 mol/kg/hr($p<0.05$) in treated subjects, while glutamine flux increased by 409 mol/kg/hr($p<0.05$). Also, BCAA levels decreased in subjects on phenylbutyrate. Fasting leucine levels dropped from 10633 to 6418 mol/L($p<0.01$). Isoleucine dropped from 549 to 3310 mol/L($p<0.01$). Valine dropped from 20424 to 13932 mol/L($p<0.01$). There were no significant changes in the concentration of other amino acids, including threonine, serine, methionine, phenylalanine, and lysine. There was no change in the appearance rate of leucine (104.94 mol/kg/hr at baseline, 110.021 mol/kg/hr on treatment, $p=0.53$). There was no change in whole-body protein oxidation as measured by leucine oxidation($p=0.27$). Hence, at this level of protein intake in control subjects, sodium phenybutyrate achieves its desired effect on urea synthesis, while the decrease in BCAA does not appear to adversely affect total body protein catabolism. An important question will be whether this will similarly hold true in UCD patients who may have less capacity for adaptation to a nitrogen load.

Human neocentromere formation at a large variable-copy number tandem array in 8q21. *D. Hasson[1], A.L. Alonso[1], F. Cheung[1], J.H. Tepperberg[2], P.R. Papenhausen[2], J.J. Engelen[3], P.E. Warburton[1]* 1) Human Genetics, Mount Sinai School of Medicine, NYC, NY; 2) Laboratory Corporation of America, Research Triangle Park NC; 3) Clinical Genetics, Academic Hospital, Maastricht, the Netherlands.

Neocentromeres are variant centromeres that have arisen epigenetically in ectopic chromosomal locations. Neocentromere are devoid of alpha satellite DNA found at endogenous centromeres, and therefore permit analysis of the underlying DNA and chromatin structure important for centromere formation and function. We have investigated two independent neocentromeres from band 8q21, 1) a neodicentric chromosome 8 with an 8q21 neocentromere and an inactivated endogenous centromere and 2) an ~10Mbp neocentric ring chromosome largely derived from band 8q21. We have used FISH with ordered BACs from 8q21/q22 to localize the constriction on the neodicentric chromosome to an ~5Mbp region and CENP-C immunofluorescence to localize the kinetochore to ~1Mbp within this constriction. At the proximal edge of the CENP-C domain, the human genome sequence (hg18) showed a large tandem array, consisting of ~6 copies of a 12kb repeat which spanned an 87kb unbridged gap. Each repeat contains an mRNA that includes the Gor1 RNA exonuclease-like gene and an L1PA5 line element. Arrays were sized by pulsed field gel electrophoresis of genomic DNA digested with restriction enzymes that do not cut within the repeat unit. 13 independent arrays, from both the neocentromere lines and two families, ranged in size from ~700kb to ~1500kb (mean = ~950kb). Thus, this gap contains ~60 to ~125 additional copies of the 12kb repeat units in a large variable copy tandem repeat and represents a significant portion of genomic DNA. The arrays in the neocentromere lines were among the smallest observed (~700 to ~900kb), but similar sized arrays were also seen in non-neocentric lines. The neocentric ring chromosome contains an intact array with the 8q21 breakpoint mapping just a few kb proximal to it. We are investigating the hypothesis that these tandem arrays may provide good substrates for cis-transcribed RNA-based heterochromatin and neocentromere formation, analogous to alpha satellite DNA at endogenous centromeres.

Common leukemia-associated genetic alterations are present in healthy individuals: Silent micro-mosaicism or latent predisposition? *D. Mercer*[1], *M.M. Li*[1, 2] 1) Human Genetics Program,; 2) Dept Pediatrics, Tulane Univ Medical Sch, New Orleans, LA.

Leukemia-associated genetic alterations, such as the t(9;22) BCR/ABL fusion gene in CML and ALL, play important roles in leukemogenesis. However, recent studies have indicated that some leukemia-associated genetic alterations may present in up to 100% of healthy individuals when evaluated with nested RT-PCR. To further delineate these genetic alterations in healthy individuals and their significances, we studied MLL partial tandem duplications (PTDs) in 44 healthy individuals aged from newborn to 55 years old and BCR/ABL p190, BCR/ABL p210, AML1/ETO, and MLL/AF4 in 22 healthy individuals aged from newborn to 27 years old. Among the 44 individuals studied for MLL PTD, 24 showed positive (55%). Sequencing studies demonstrated that the MLL PTD is characterized by an in-frame repetition of MLL exons in a 5 to 3 direction. The most common duplication was PTD e9/e3, followed by e11/e3. Other duplications observed were e9/e4, e10/e3, and e10/e4. The MLL PTDs were also detected in genomic DNA samples. Among the 22 patients studied for BCR/ABL p190, BCR/ABL p210, AML1/ETO, and MLL/AF4, 13 were positive for BCR/ABL p190 (59%), 20 positive for BCR/ABL p210 (91%), 6 positive for MLL/AF4(27%), and none positive for AML1/ETO. These data demonstrated that many leukemia-associated genetic alterations are commonly present in healthy individuals. Since the frequency of healthy carriers is a few magnitudes higher than the incidence of the diseases, most healthy carriers will never suffer from the leukemia corresponding to their mutation, maintaining their silent micro-mosaic status for life. However, previous studies did show that some leukemia-associated fusion genes were detected in neonatal blood spots of children who developed related leukemia with more than 10 years latency, indicating a higher risk in at least some positive individuals. Carefully designed long term follow-up studies of positive individuals may help to determine criteria for subgroups that may be at a higher risk and to identify genetic or environmental factors that may promote malignant transformations.

Array-CGH analysis of cell-free fetal DNA in amniotic fluid detects human aneuploidies. *K.L. Johnson[1], O. Lapaire[2], X.Y. Lu[3], H. Stroh[1], J.M. Cowan[1], U. Tantravahi[4], D.W. Bianchi[1]* 1) Division of Genetics, Department of Pediatrics, Tufts-New England Medical Center, Boston, MA; 2) University Womens Hospital, University of Basel, Switzerland; 3) Spectral Genomics, Inc., Houston, TX; 4) Department of Pathology, Women and Infants Hospital, Providence, RI.

Background. Previously we showed that comparative genomic hybridization (CGH) analysis of cell-free fetal (cff) DNA isolated from amniotic fluid (AF) allows for prenatal molecular karyotyping and fetal gender identification (Am J Hum Genet 75:485-91). Subsequent technical advances have increased the yield of extracted cffDNA to permit CGH analysis of 10 mL of AF (Clin Chem 52:156-7). Here we aimed to determine whether a variety of fetal aneuploidies can be identified thorough the analysis of cffDNA from AF by array-CGH.

Methods. CffDNA was extracted from 10 mL of residual AF supernatant using AVL buffer and maxi columns (Qiagen, Valencia, CA). In all cases, test cffDNA and reference DNA were labeled with different fluorescent dyes and applied to Spectral Constitutional Chip 2.0. The following fetal abnormalities were analyzed: trisomies 13 (n=1), 18 (n=3) and 21 (n=2), as well as cases of mosaicism (47,XX,+9[18]/46,XX[2]) and triploidy (69,XXY).

Results. CffDNA isolated from aneuploid fetuses showed increased hybridization signals on the majority of the affected chromosome markers compared to euploid reference DNA. Aneuploid fetuses were correctly identified in all cases.

Conclusions. These results indicate that cffDNA extracted from 10 mL can be analyzed using array-CGH to correctly identify human autosomal and sex chromosome aneuploidies. In conjunction with previous work, we show that this technology allows for rapid screening of AF samples for whole chromosomal changes without interfering with current standard of care and may augment standard karyotyping techniques by providing additional molecular information.

Tumor marker in the serum proteins of Xiphophorus. *A.I. Islam* Ophthalmology, MEEI, Harvard Medical School, Boston, MA.

Xiphophorus serum proteins were investigated for the establishment of tumor marker in human being. Serum proteins of tumor free and tumor bearing Xiphophorus were conducted in 5% native-PAAG (Polyacrylamide Gel Electrophoresis). Serum proteins of tumor free (Xtf) and tumor bearing (Xtb) Xiphophorus were compared. Both of the strains have 48 chromosomes with similar genetic backgrounds. There have revealed polymorphism in the serum proteins of normal (Xtf) and tumor effected (Xtb) animals. Albumin peaks (molecular wt. - 65 kDa) of tumor free species have high peaks and concentration than Xtb. Lipoproteids peaks (molecular wt. - 24-25 kDa) were more or less similar in patterns in both of the strains. Transferrin peaks and globulin spectra were declining in tumor bearing Xiphophorus. Acute Xtb bear low peaks and contents of lipoproteids than the Xtf fish. Globulin and albumin spectra were more distinct in Xtf than tumor bearing offspring. The quantity of albumin patterns changes in relation to the globulin patterns. Globulin peaks were depressed in the normal and more declining in the acute and tumor-bearing melanoma formed baby Xiphophorus. The tumor free species have the up-rising peaks of globulin and transferrin spectra in comparison to Xtb. From the different patterns of serum proteins, different polymorphic structures were revealed in both Xtf and Xtb strains. This could be an ideal marker for human cancer research.

Restricted DNA Copy Number Variation in CNS Versus Lymphocyte Derived DNA. *R.A. Holt*[1,2], *G. Turecki*[3], *G.M. Wilson*[2] 1) Department of Psychiatry, University of British Columbia, Vancouver, British Columbia (BC), Canada; 2) Canada's Michael Smith Genome Sciences Centre, BC Cancer Agency, Vancouver, BC, Canada; 3) McGill Group for Suicide Studies, Douglas Hospital, McGill University, Montreal, Quebec, Canada.

DNA copy number differences are now recognized as an important source of genetic variation, and have been implicated in both mendelian and complex genetic disorders. However, the extent of somatic copy number variation remains unexplored. In the present study we used Quantitative Real Time PCR (Taqman, ABI) to screen a small cohort of 16 subjects (8 suicide, 8 control) for copy number differences. DNA was derived from both post mortem brain tissue (Brodmans Area 10) and peripheral blood lymphocyte (PBL) from each subject. We interrogated three genes involved in glutamate transmission in the CNS that had previously demonstrated DNA copy number aberrations in post mortem brain; GRIK3, AKAP5, and CACNG2. DNA copy number aberrations were observed at all three loci in the present study, with mean copy number variation significantly restricted in brain derived DNA relative to lymphocyte DNA (GRIK3, P=0.001; AKAP5, P=0.002; CACNG2, P=0.0001 (two-tail, paired t-test, n=16)). No significant difference was observed between diagnostic groups (suicide versus control). Observations at these three loci suggest that copy number variants may be less common in post mitotic cells (e.g. CNS) where fewer cell divisions means fewer chances for an aberration to arise. Further, genes with important roles in a given tissue (e.g. glutamate transmission in the CNS) may have restricted copy number variation due to functional constraint. We are currently estimating the genome-wide copy number variation in these same samples using microarray methods.

Cytochrome P450 CYP3A4 expression as a diagnostic biomarker in breast cancer. *D. McDaniel[1], A. Lewis[1], C. Berry[1], W. Barber[1], P. Smith[2], S. Bigler[3]* 1) Dept Surgery/Neurology, Univ Mississippi Medical Ctr, Jackson, MS; 2) Medicine; 3) Pathology.

This study is to identify variants of the Cytochrome P450 (CYP) 3A4 gene that might be used as diagnostic biomarkers in studies of breast cancer susceptibility. CYP3A4 plays an important role in cell regulation via its involvement in the metabolism of a wide variety of endogenous metabolites active in cellular signaling. Biochemical pathways involved in hormone metabolism have been implicated in the etiology of breast cancer and the CYP 3A enzymes are involved in such metabolisms. To date biopsy results are the most useful means of determination of clinical outcome of breast cancer. There is an urgent need for identification of patients who will benefit from early diagnosis and less aggressive postoperative therapy. Multiple SNPs have been identified in the regulatory region of the CYP 3A4 gene that has been associated with disease conditions. We sought to determine the frequency distribution of the CYP3A4 variant and its effect on gene expression in breast cancer. Clinical conditions including tumor stage, receptor status, previous breast cancer and family history were evaluated for genotype and phenotype association. The homozygous AA allele was present with higher frequency (71.0%) in Caucasian (Cau) group as compared with African American (Af Am) (29%) patients and controls (15%). Homozygous GG alleles were absence in Cau patients and the frequency was increased 4.8-fold in cancer patients as compared with controls ($p<0.05$). A vs. G genotypes were inversely associated with tumor staging in Af Am patients. Patients with stage 0 tumors 50% carry the AA alleles as compared with 33% in stage II, whereas patients with stage III and IV, 67 to 100% carry AG or GG alleles. Expression levels of CYP 3A4 transcript was higher in PBMCs of patients with benign tumors as compared with malignant tumors ($p<0.03$). In summary, Af Am patients with homozygous CYP3A4 GG genotype were at higher risk of developing cancer as compared with Cau and controls. In addition, expression levels of CYP3A4 gene may have implications for both the development and the therapy of breast cancer.

Copy Number Variation Analysis on the HapMap DNA Samples Using TaqMan Assays for CYP2D6, CYP2E1, CYP2A6, GSTM1 and GSTT1. *K. Li[1], K.A. Haque[2], R.A. Welch[2], K. Lazaruk[1]* 1) Applied Biosystems, Foster City, CA; 2) SAIC-Frederick Core Genotyping Facility (CGF), NCI/Advanced Technology Center, Bethesda, MD.

Gene copy number variation is now recognized as an important type of polymorphism in the human genome. Gene duplication/deletion can have a significant impact on phenotype. Copy number polymorphisms have been associated with genetic diseases such as cancer, immunological and neurological disorders, and also associated with variations in drug response as previously demonstrated. Measuring copy number variation in these genes is an important complement to genotyping for two reasons. First, falsely assuming diploidy can lead to inaccuracy in determining the genotype. Second, copy number can interact with genotype to influence phenotype of a variant. The availability of robust and validated genotyping and copy number variation assays allows for full characterization and understanding of genotypes of the genes of interest. Accurate detection of copy number changes is critical for understanding how gene copy number variation plays a role in disease or drug response. Here we report the development of an application using real-time PCR assays to quantify gene copy number and the results of copy number for 5 important drug metabolism genes (CYP2D6, CYP2E1, CYP2A6, GSTM1 and GSTT1) using DNA samples from the International HapMap Project. The gene copy number data is useful in understanding the SNP genotype data from the HapMap samples. For example, our copy number analysis showed high percentage of individuals from the HapMap panel had the null allele(s) for GSTM1 and GSTT1 genes. These observations suggest why many of the SNPs in GSTM1 and GSTT1 SNPs failed QC (were qc-) as reported by the HapMap Project, as they failed due to a low pass rate <80%, a low Hardy-Weinberg p-value, or to Mendelian inconsistencies. All of these inconsistencies within the SNP genotyping data can be explained when the gene dosage data are factored into the analysis, since departures from diploidy can cause apparent genotyping failure, a high proportion of outliers, departures from expected genotype frequencies and aberrant inheritance.

Neural crest derived osteochondroprogenitor cells predominantly contribute to cranial skeletal repair. *P. Leucht, J.-B. Kim, J.A. Helms* Department of Surgery, Stanford University, Stanford, CA.

The cranial skeleton has a complex developmental history. Whereas the appendicular skeleton is derived solely from mesoderm, the cranial skeleton is a patchwork of bony elements, derived from paraxial mesoderm as well as from the cranial neural crest (NC). These differences in embryonic origin raise the possibility that NC-derived and mesoderm-derived skeletal tissues may regenerate using distinct pathways. Since bone repair can accurately recapitulate skeletogenesis, we began to investigate the differences in cranial versus appendicular skeletal repair by creating a mouse model of mandibular/tibial defect healing. By using genetically engineered mice whose NC cells are indelibly labeled with Green Fluorescent Protein (GFP) and whose mesodermal cells are permanently labeled with LacZ (Wnt1Cre::Z/EG mice), we were able to understand the origin of the cells that contribute to the skeletal healing process. In addition, by transplanting cells from one origin into a skeletal element of a different origin, we were able to analyze to what extent adult NC-derived cells contribute to mesoderm bone (tibial) repair, and conversely, whether mesoderm-derived cells participate in NC bone (mandibular) healing. We found that, at a histological level, cranial skeletal repair is indistinguishable from appendicular skeletal repair. When the NC-derived mandible was injured, the callus was composed entirely of NC-derived cells. In contrast, the tibial defect site was occupied entirely by mesoderm-derived cells. These data suggest that injured skeletal elements apparently use cells of their own embryonic origin for repair. The potential clinical impact from this finding is significant, because if bones preferentially heal using cells that share the same embryonic origin then reparative strategies may also have to take this variable into account.

A screen for novel microdeletions/duplications in fetal samples with multiple congenital anomalies. *H.C. Mefford[1,2], A.J. Sharp[1], R.P. Kapur[3], D.G. Albertson[4,5], D. Pinkel[4], E.E. Eichler[1,6]* 1) Genome Sciences, University of Washington, Seattle, WA; 2) Medical Genetics, University of Washington, Seattle, WA; 3) Pathology, Children's Hospital and Regional Medical Center, Seattle, WA; 4) Comprehensive Cancer Center, UCSF, San Francisco, CA; 5) Cancer Research Institute, UCSF, San Francisco, CA; 6) Howard Hughes Medical Institute, Seattle, WA.

The estimated incidence of genomic disorders due to recurrent chromosomal structural rearrangements is 1/1000 live births. We have identified 130 regions in the genome flanked by large blocks of highly homologous segmental duplications whose structure predicts a susceptibility to rearrangements resulting in microdeletion/duplication (Sharp et al., Am J Hum Genet 77:78). Despite an architecture that predisposes to rearrangement, some regions show no evidence of copy number variation in 316 controls or 330 children with mental retardation and dysmorphic features. We hypothesize that these regions contain genes that are critical for normal development and that *de novo* deletion or duplication events will be found in individuals with multiple congenital anomalies (MCA). Using a custom BAC microarray targeted specifically to the 130 regions, we are analyzing 200 individuals with MCA who underwent autopsy after intrauterine demise or therapeutic termination for anomalies; autopsy reports provide detailed phenotypic information. Analysis of the first 38 samples identified two individuals with a potential duplication of approximately 2.5 Mb on 16q24. We are currently performing validation and further characterization of these findings and will report additional findings on the first 100 individuals in our series. Our previous studies and preliminary results of this study suggest that the regions we have targeted undergo recurrent rearrangement and that our strategy provides a powerful tool for identifying novel genomic disorders and genes that are critical in normal human embryonic development.

Agenesis of lateral superior incisors: novel and reappraised evidence for familial aggregation. *P. Maciel*[1], *C. Lemos*[2,3], *T. Pinho*[4], *A. Sousa*[2,3] 1) Life and Health Sciences Res. Inst.(ICVS), School of Health Sciences, Univ. of Minho, Braga, Portugal; 2) UnIGENe-IBMC, Univ. of Porto, Portugal; 3) Dept. Pop. Genet., ICBAS, Univ. of Porto, Portugal; 4) ISCS-Norte, Paredes, Portugal.

The first evidence for a genetic cause for hypodontia came from the identification of significant familial aggregation of congenitally missing teeth in the Swedish population, in the study by Grahnen (1956). The identification of phenotypes of tooth absence in several mutant mice, together with the identification of mutations that cause non-syndromic hypodontia in human families, confirmed the relevance of the genetic determination of tooth development. However, the role of genes in sporadic and less severe forms of hypodontia is not clarified, and there is evidence for an extensive clinical and genetic heterogeneity within the hypodontia phenotype. Further definition of clinical and genetic subtypes within hypodontia is thus warranted, and familial aggregation studies are needed in order to establish a measure of the genetic contribution in each subtype. In this study, we investigated the familial aggregation of the non-syndromic agenesis of lateral superior incisors (LSIA). For this, we obtained novel data in the Portuguese population, and re-assessed data within the Grahnen study, focussing only on LSIA. In the Portuguese sample, we performed a clinical evaluation of all available first and second degree relatives(n=698)of 111 probands, 62 with and 49 without LSIA. The risk for a relative of a proband with LSIA to have the same kind of agenesis was about 13 times greater than that for a relative of a proband without that agenesis. Interestingly, the relative risk (RR)of LSIA increased with the degree of kinship: RR was 8.5 for parents, 7.7 for sibs and 4.7 for cousins. When using the estimated prevalence of LSIA in the Portuguese population (1.3%), the RR for any relative of an affected proband was 15. Parents and sibs had a RR of 14. Analysis of data from the Swedish study, using the estimated prevalence of the trait in the Swedish population (1,6%), revealed a RR of 24 for parents and 15 for sibs. The results obtained with both data sets support a significant familial aggregation of LSIA.

Integrating Gene Arrays, Breast Cancer Families, and Evolutionary Genomics to Identify New Disease Genes for Risk Assessment. *G. Larson[1], D. Smith[2], O. Snove[3], C. Lundberg[1], G. Rivas[1], J. Weitzel[1], C. Glackin[1], T. Krontiris[1]* 1) Div Molecular Medicine, Beckman Res Inst City of Hope, Duarte, CA; 2) Div Information Sciences, Beckman Res Inst City of Hope, Duarte, CA; 3) Div Molecular Biology, Beckman Res Inst City of Hope, Duarte, CA.

Gene expression microarrays provide a quantitative tool (e-QTL) to measure differences in RNA expression patterns in breast tumors versus normal tissue. Specific target genes from these expression signatures are emerging as useful clinical tools in diagnosing the likelihood of disease recurrence. We believe array studies also offer the opportunity to identify a new class of heritable mutations in genes that lead to their abnormal expression. These variants may provide diagnostic value allowing one to identify both women predisposed to disease and those demonstrating altered response to therapy. To find these mutations we are adopting a three-part strategy. First, we employ meta-analysis to compensate for the frequent variability seen in gene expression portraits from multiple studies to identify a set of consistently dysregulated candidate genes in breast tumors. Second, a subset of these candidates, guided by bioinformatics, are being tested for linkages in a cohort of affected sibling pair families with breast cancer. Third, we are comparing orthologous genes from the genomes of primates and mammals to identify putative control elements phylogeneticially conserved through evolution that occasionally contain putative disease SNPs in humans. To date, data indicate that the mutations modulating gene expression in combination with expression profiles may provide a more specialized risk profile for patients and lead to a more detailed understanding of epistatic interactions in the genome.

Human SNM1 protein is a single-strand 5'-3' exonuclease acting in DNA crosslink repair. *J. Hejna, S. Philip, C. Faulkner, A. Hemphill, J. Ott, R. Moses* Molec & Med Genetics, L103, Oregon Health Sciences Univ, Portland, OR.

DNA interstrand crosslinking agents are particularly potent DNA damaging agents because they constrain DNA topology, impeding replication and transcription. Interstrand crosslinks (ICLs) must be removed by a multi-step process, and in yeast three independent pathways are required for optimal repair of ICLs. The yeast SNM1 protein is a 5'-3' exonuclease, operates in the Rad3 pathway, and is needed for normal ICL repair. In human cells, five SNM1 homologs (SNM1, SNM1B, SNM1C/Artemis, ELAC2, and CPSF73) have been identified, raising the question of whether the enzymological function of the protein has been conserved, or whether different homologs have acquired specialized functions. In order to characterize hSNM1 activity, recombinant protein was expressed and purifed from KC167 insect cells. It possesses a strong 5'-3' exonuclease activity, using a single-stranded DNA oligonucleotide substrate. A 5'-phosphate was strictly required for the hSNM1 exonuclease. Gel filtration indicated that hSNM1, like ySNM1, functions as a monomer. Mutation of a conserved aspartate residue (D736 and D252, in hSNM1 and ySNM1, respectively) within the -CASP domain abrogated the activity in both proteins. Whereas ySNM1 efficiently digests both single-stranded and double-stranded substrates, hSNM1 shows a marked preference for single-stranded substrates. Depletion of hSNM1 by siRNA leads to increased chromosomal breaks and radial formations, and decreased cell survival after treatment with mitomycin C. We conclude that hSNM1 is a 5'-3' exonuclease required for normal ICL repair.

Results from a study evaluating the ex vivo effect of AT2101 in blood cells derived from Gaucher patients and phase 1 results of AT2101 in healthy volunteers. *K. Ludwig[1], G. Grabowski[2]* 1) Amicus Therapeutics, Inc. 6 Cedar Brook Drive Cranbury, NJ; 2) Children's Hospital Research Foundation Division and Program in Human Genetics Cincinnati, Ohio.

AT2101 (isofagomine; Amicus Therapeutics, Inc.) is an investigational, orally administered small molecule for the treatment of Gaucher disease (GD). In most GD patients, missense mutations result in the production of beta-glucocerebrosidase (GCase) with reduced stability that may not be properly trafficked from the ER to the lysosome. AT2101 belongs to a new class of drugs known as pharmacological chaperones, which selectively bind to a target protein increasing stability and trafficking to the proper location, thereby increasing cellular activity. We are conducting a non-interventional survey study to characterize the ex vivo effects of AT2101 on lymphoblast and macrophage cells derived from GD patients. Different effects of AT2101 on the mutant GCase protein will be evaluated, including enzymatic activity, protein half-life and trafficking. In addition, effects of AT2101 on cellular physiology will be examined, including changes in substrate levels and cellular stress associated with retention of misfolded GCase in the ER. A variety of mutations associated with types I, II and III GD will be studied. Clinical data will be collected retrospectively from patient medical records. We are also conducting Phase I studies of AT2101 in healthy volunteers to assess safety and pharmacokinetics. In addition, we are studying the ability of AT2101 to enhance GCase activity in white blood cells in healthy volunteers. In a previous study of migalastat hydrochloride, a pharmacological chaperone in development for the treatment of Fabry disease, significant enhancement of -galactosidase activity was observed in a Phase I trial. These data showed that pharmacological chaperones can enhance enzyme activity even for wild-type enzyme in healthy volunteers. We will present data from the ex vivo survey study and results from the AT2101 Phase I studies in healthy volunteers.

Pathogenicity of Lrrk2 R1514Q variation in Parkinsons disease. *K. Haugarvoll[1, 2], M. Toft[2], I.F. Mata[1], O. Ross[1], M.J. Farrer[1]* 1) Neuroscience, Mayo Clinic Jacksonville, Jacksonville, FL; 2) Department of Neuroscience, NTNU, Norway.

Pathogenic evidence for Lrrk2 R1514Q remains equivocal after it was identified in an affected pair of Norwegian concordant twins. To clarify the role of this variant we performed a screening of sixty-six dominant families and three case-control series from Norway, Ireland and Spain. We identified a large kindred harboring the R1514Q, however the variant did not segregate fully with disease. The relatively high frequency in elderly controls in two of the series also supports the hypothesis that this non-synonymous substitution is not pathogenic. These findings highlight the importance of using family-based studies and large multiple population screenings when examining the association of polymorphic variants in LRRK2 with Parkinsons disease.

Increased Expression of a Neuron-Specific Isoform of the *TAF1* Gene with Mouse Development and aging. *S. Makino, G. Tamiya* Division of Human Molecular Genetics, Dept of Neurology and Neuroscience, Tokushima University Graduate School of Medicine, Japan.

The *TAF1* (TATA-binding protein-associated factor 1) gene encodes the largest component of the TFIID complex involved in RNA polymerase II-mediated expression of genes related to cell division and proliferation. We found the limited expression of an isoform of the *TAF1* gene in human brain and neuronal cell line, SH-SY5Y, but not in glial cell line. We cloned the full length of the neuron-specific isoform of the *TAF1* gene, named *N-TAF1*, from human CapSite cDNA library. We also cloned mouse and rat homologues of the *N-TAF1* and found the limited expression in murine brains as well as the human *N-TAF1*. To investigate the detailed expression of the *N-TAF1* gene, we carried out probe-based quantitative RT-PCR analysis in mouse embryo (10.5 and 17.5 dpc), newborn and adult brain (8, 15 and 43 weeks). The *TAF1* mRNA was gradually decreased with mouse development and aging, whereas the *N-TAF1* was rapidly increased after birth and stable until 43 weeks. Our result suggests that the *N-TAF1* gene has an important role in non-dividing neuronal cell after birth rather than in cell division and proliferation during neurogenesis.

Genotyping by Whole Genome Hybridization. *K.W. Jones, P.-H. Wang, J. Yang, J. Huang, G. Fu* Affymetrix, Inc, 3420 Central Expressway, Santa Clara, CA.

Recent technological advances in genotyping methodologies have led to the ability to genotype hundreds of thousands of SNPs in a rapid and cost-effective manner. This in turn has led to fixed marker panels which have successfully been used in whole genome association studies. Recent studies have shown that commercially available panels capture the majority of common SNPs in the HapMap collection either directly or through linkage disequilibrium. However, methods which have based their SNP selection criteria on maximizing genetic information within the HapMap dataset have been shown to overestimate their ability to extract information from hidden SNPs (the estimated 5.3M common SNPs that are not part of the HapMap dataset). In contrast, methods that use an unbiased approach to SNP selection (i.e., Affymetrix GeneChip Human Mapping 500K arrays) have balanced coverage among hidden and non-hidden SNPs. As a means to generate a generic target preparation which maximizes the number of SNPs interrogated and does not bias the genetic information content of the SNP panel, we enabled whole genome hybridizations (WGH) in which unprocessed genomic DNA is fragmented and hybridized directly to arrays containing hundreds of thousands of SNPs. Samples with limited amounts of DNA can also be Whole Genome Amplified prior to hybridization. The WGH assay was tested on 49 HapMap samples using arrays containing greater than 113,000 SNPs that were not preselected based on their performance on other platforms or their presence in the HapMap collection. Genotypes for greater than 50% of the tiled SNPs were called with high consistency and accuracy across the 49 samples. For instance, SNPs with a minor allele frequency >2% were 99.5% concordant with HapMap reference genotypes. Additional data on the ability to extract information from hidden SNPs will also be presented. The simplicity of the WGH assay and its ability to accurately genotype vast numbers of SNPs should prove useful for developing whole-genome genotyping tools and other high complexity DNA hybridization assays.

Population genetics of EWSR1, a highly conserved chromosome 22 breakpoint region. *N. Orr[1], S. Savage[2], G. Thomas[2,3], S. Chanock[1,2,3]* 1) Pediatric Oncology Branch, NCI, Bethesda, MD; 2) Division of Cancer Epidemiology and Genetics, NCI, Bethesda, MD; 3) Core Genotyping Facility, NCI, Gaithersburg, MD.

Ewings Sarcoma (ES) is characterised by the presence of a small number of highly specific somatic translocations resulting in fusion of the transactivation domain of the *EWS* gene with the RNA binding domain from one of five members of the ETS gene family. Distributions of *EWS* breakpoint junctions are confined to a 6 kb region termed EWSR1. The incidence of ES is significantly higher in Caucasians than in African Americans suggesting that population specific susceptibility and/or protective factors are implicated. We present a detailed population genetics analysis of EWSR1 as a step towards understanding the ethnic bias of the genetic lesion driving these pathogenic translocations.

We detected a 3.7 kb region of EWSR1 whose evolutionary conservation between 17 species was estimated at 0.752 using the phastCons17way algorithm, suggesting an area of functional or structural significance. We characterised common genetic variation within the EWSR1 region by resequencing in the SNP500 Cancer population (32 Caucasians, 24 African Americans, 23 admixed/Hispanic and 23 Pacific Rim individuals). A total of 16 SNPs were detected; of these, 14 were population private, 11 were singletons, only three had a minor allele frequency of greater than 5% and all were intronic. Nucleotide diversity, as calculated using the pi statistic, was $4x10^{-5}$ and the population mutation frequency, theta, was $42x10^{-5}$. We observed the same predominant ancestral haplotype in all populations accompanied by a low number of singletons. Tajimas D statistic was marginally significant ($p<0.05$) in the African American (-1.866) and admixed (-1.820) populations, suggesting recent selection. Fst, a measure of genetic distance between populations was unremarkable (0.019). Comparison of HapMap phase II data indicates that there may be a region of high recombination in *EWS* in the Yoruba which is not present in Caucasians. These data form the foundation for further genetic studies of ES in order to investigate the marked population bias of this early onset malignancy.

Identifying and comparing sequence determinants of mammalian recombination hotspots. *S.R. Myers[1], A. Kirby[3], C. Wade[1,2], G.A.T. McVean[4], M.J. Daly[1,2,3], P.J. Donnelly[4]* 1) Broad Institute of MIT and Harvard, Cambridge, MA; 2) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA; 3) Department of Medicine, Harvard Medical School, Boston, MA; 4) Department of Statistics, Oxford University, Oxford, UK.

It is now well understood that most human recombination is concentrated into narrow hotspots, of width one to two kilobases, and there is substantial evidence for a similar pattern in other mammalian species. However, the nature of the relationship between the location of such hotspots, and primary DNA sequence, has remained mysterious. We employ new variation datasets to investigate this relationship, and to compare results between humans and other species. A new set of more than 25,000 human hotspots, identified using variation data, provides overwhelming evidence that DNA sequence has a strong local effect, that certain short, specific sequence motifs influence human hotspot activity, and that the same motifs are active in many different genomic contexts. Using mouse polymorphism data, we identify a second new set of potential hotspot locations, within the mouse genome, and use these to compare the two species. As in humans, mouse recombination shows a strong dependence on specific local sequence features - but although some associations are in the same direction as in humans, there are also some intriguing differences. Such cross-species comparisons should help to answer important evolutionary and biological questions relating to recombination and hotspots.

Combined ancestral linkage analysis and whole genome association analysis of migraine with vertigo and aura.

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Migraine is a common disorder affecting at least 15% of the adult population world-wide. Several linkage studies and population based family studies demonstrate that migraine is a genetically complex disorder involving multiple genetic factors as well as environmental factors. The International Headache Society (IHS) classifies migraine into the most common forms of migraine without aura (MO) and migraine with aura (MA). However, in order to generate a potentially more homogeneous case group, we have restricted the migraine phenotype to include only individuals who manifest recurrent migrainous headaches, recurrent visual aura and episodic vertigo (MAV). In total 96 samples with MAV were included within the case group and 104 genetically matched samples were selected for the control group. Affymetrix Mapping 250K NspI arrays were used for the genotyping, and 170K snps passed our quality control check. 258 SNPs are associated with MAV in the initial screening set at a $p0.001$, and 4 SNPs are associated with individual $p10^{-5}$. Although none of the SNPs is significant if a conservative Bonferroni correction is applied, several candidate genes are identified. We overlay this association information with the identification of identical by descent intervals between the affected individuals to search for families that share the same ancestral haplotypes at the associated loci.

Cytokine SNPs and Preterm Birth. *M.V. Hollegaard[1,2], J. Grove[2], P. Thorsen[2], D.M. Hougaard[1]* 1) Department of Clinical Biochemistry, Statens Serum Institut, Copenhagen, Denmark; 2) NANEA at Department of Epidemiology, Institute of Public Health, University of Aarhus, Aarhus, Denmark.

Several observations support the idea of a genetic influence on the risk of Preterm Birth (PTB, birth before 37 weeks of gestation (w.g.)). E.g.: the leading risk factor for PTB is a previous preterm birth and mothers themselves born preterm also have an increased risk of a PTB. PTB is most likely to be caused by multiple genetic and environmental factors. Many environmental factors that are known to activate the cytokine mediated inflammatory pathways have been associated with PTB. The aim of this pilot study was to elucidate if any of 289 selected SNPs distributed in 47 selected cytokine loci could be associated with PTB. The SNPs found interesting here will be tested in a cohort study consisting of app. 3,900 pregnant women. Three groups of 40 patients, all Caucasian women, from the Danish National Birth Cohort were included into this study. The three groups were; controls 37 w.g., PTB >34 w.g. (moderate preterm) and PTB 34 w.g. (very preterm). DNA was extracted from dried blood spots (Guthrie cards) and used for whole genome amplification using Genomeplex (Sigma). Samples were shipped to Illumina Inc., USA for genotyping. In time of writing, preliminary statistical analysis of the two PTB subgroups against controls has revealed a total of 31 different SNPs associated with PTB. Moderate PTB was associated to 16 SNPs located in 8 different loci, IFNA1, IFNGR2, IL1A, IL1B, IL2, IL26, IL2RB, and IL7. Very PTB was found associated to 15 SNPs located in 8 loci, IFNA1, IFNGR2, IL18, IL1A, IL2RB, IL2RG, IL6 and IL7. When analyzing all PTB cases against controls, there were 15 SNPs associated in 8 loci, IFNA1, IFNGR2, IL1A, IL2, IL26, IL2RB, and IL7 remained in the list and IL4 was added. The difference in loci associated to moderate and very PTB, is consistent with the idea of different causal pathways are involved in PTB. This pilot study suggests that SNPs in and surrounding the genes IFNA1, IFNGR2, IL18, IL1A, IL1B, IL2, IL26, IL2RB, IL2RG, IL4, IL6 and IL7, should be investigated further both in a larger study as well as in populations with other ethnicities.

Allele frequency estimates from DNA pools for 317,000 SNPs for multiple European and worldwide populations and discovery of Ancestry Informative Markers for Europe. *J. Li[1], D. Absher[1], A. Southwick[1], M.D. Shriver[3], M. Bauchet[3], P.A. Underhill[2], L.L. Cavalli-Sforza[2], G. Barsh[2], R.M. Myers[2]* 1) Stanford Human Genome Center; 2) Department of Genetics, Stanford University, Stanford, CA; 3) Department of Anthropology, Penn State University, University Park, PA.

The identification of Ancestry Informative Markers (AIMs) and inference of individual genetic history is useful in many applications, including studies of geography and evolution of human populations, forensic sciences, pharmacogenomics, admixture mapping and association studies of complex diseases. While many AIMs have been reported that define strong genetic differences between major continents, it is more difficult to identify markers that reflect subtle, within-continent diversity, such as the heterogeneous ancestry of European Americans contributed by different populations within Europe. We have analyzed DNA pools, each for a different population, on Illumina HumanHap300 BeadArrays to estimate allele frequencies for ~317,000 Single Nucleotide Polymorphisms for 9 European, 6 African, and 2 Amerindian populations in the Human Genome Diversity Project collection. We have also evaluated the performance of this method by analyzing three HapMap pools (YRI, CHB, and JPT), for which the true allele frequencies are already known from the International HapMap Project. We found that the allele frequency estimates differed between replicate chips by less than +/-5% for 95% of the SNPs, and that the estimated frequencies and the true frequencies differed by +/-5-10% for 90% of the SNPs. The data for nine European populations, from western Caucasus, Scotland, Tuscany, Sardinia, France, Iberia, Russia, Northern Italy, and a Basque region, showed a clear excess of SNPs having large allele frequency differences (e.g. >30%) between most pairs of populations, compared to what would be expected given the sample sizes. These results provide a valuable resource of European AIMs for monitoring within-continent stratification in association studies. We are currently validating the most informative SNPs by individually genotyping samples that formed the pools as well as those from additional European populations.

Patterns of sequence variation in the human pigmentation candidate gene SLC24A5. *H. Norton, M. Hammer* ARL-Biotechnology, Univ Arizona, Tucson, AZ.

Human skin pigmentation shows a strong correlation with the intensity of ultraviolet radiation (UVR), suggesting that variation in pigmentation is the result of natural selection. Recent work has demonstrated that a derived functional allele at the pigmentation gene SLC24A5 is fixed in Europeans and absent or at very low frequencies in other populations typed in the International HapMap Project. These results argue for an independent evolution of lighter skin in European and East Asian populations. To further test the hypothesis that SLC24A5 has been shaped by positive directional selection in European (but not East Asian) populations we sequenced 4.8 kb of SLC24A5 spanning a 20 kb region in a geographically diverse panel of human populations representing Europe, Asia, Africa, and the Americas. We compare levels of nucleotide diversity (and) at SLC24A5 to four X-linked loci sequenced in the same panel of individuals. While overall levels of diversity at SLC24A5 are only slightly lower than those reported for these comparison loci, Europeans show a marked reduction in diversity (, = 0.000). Further, all Europeans share a single haplotype that is defined by the SLC24A5 111*A functional allele. This haplotype is rare or absent outside of Europe. HKA tests comparing ratios of polymorphism and divergence in Europeans between SLC24A5 and the X-linked loci indicate a departure from neutrality at 2 of the 4 loci (DMD intron 44, $p < 0.01$; AMELX $p < 0.01$) and ratios at a third locus are suggestive of reduced European polymorphism (APXL, $p = 0.057$). Taken together these results suggest that patterns of variation at SLC24A5 in Europeans are due to selection rather than to demographic processess. Finally, we examine variation at increasing distances from the functional polymorphism to assess the extent of linkage disequilibrium around the selected haplotype.

Extended family relationships in idiopathic adolescent scoliosis pedigrees: a founder effect. *M.C. Meade, L.M. Nelson, V. Argyle, T. Berry, S. Murphy, J. Braun, J.W. Ogilvie, K. Ward* Axial Biotech, Inc., Salt Lake City, UT.

Introduction: Our aim was to study the extended relationships in families with adolescent idiopathic scoliosis (AIS) using the unique genealogy data available for the Utah population. Methods: Using IRB approved protocols, four-generation pedigrees were obtained from 85 probands with AIS, 83 of which were treated surgically. Descriptive statistics and standard pedigree analyses are presented in an accompanying abstract. In order to understand extended relationships between these families, a proprietary genealogy database (GenDB) was searched for the names, birth dates, and birthplaces of the four grandparents for each of the affected probands. GenDB documents the relationships between approximately 17.5 million ancestors and 3.5 million descendants of the Utah founder population (founders are approximately 10,000 individuals). Results: In 69 of the 85 families, probands reported a positive family history of AIS within the 4 generations (n=314 affected individuals). GenDB found 426 familial connections with a range of 0 to 23 connections in the 85 families. GenDB showed a mean of 5 common ancestors between any pair of probands. Familial connections ranged from the year 1460 to 1858 with a mean connection year of 1645. In 15 of 16 families in which the proband did not know of a family history of AIS within 4 generations, GenDB showed a distant connection with one of the 85 probands. There were consanguineous relationships found in 3 probands families. 15 proband families had affected or unconfirmed individuals on the unreported side (other side) of the family. 33 of the 69 families had genealogy connections found through an ancestor on the unreported side of the family. Conclusions: This study confirms the familial nature of adolescent idiopathic scoliosis and expands our understanding of the genetic parameters. This cohort of patients supports a founder effect in this population. A genome wide scan for the loci involved in these affected families is reported separately.

***CRTAP* is required for collagen 3-prolyl hydroxylation and loss of its function causes recessive osteogenesis imperfecta.** *R. Morello[1], T.K. Bertin[1], P. Castagnola[2], H.P. Bachinger[3], F.H. Glorieux[4], P.H. Byers[5], D.R. Eyre[5], B.F. Boyce[6], B. Lee[1,7]* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Istituto Nazionale per la Ricerca sul Cancro, Genova, ITALY; 3) Shriners Hospital for Children, Portland, OR; 4) Shriners Hospital for Children, Montreal, CANADA; 5) University of Washington, Seattle, WA; 6) University of Rochester Medical Center, Rochester, NY; 7) Howard Hughes Medical Institute, Houston, TX.

Osteogenesis Imperfecta (OI) is caused by mutations in the *COL1A1* or *COL1A2* genes. Although its clinical spectrum of severe to mild OI (clinical types II, III, IV, and I) has been well described, new types of OI (types V, VI, and VII), defined either genetically or histologically, have emerged. We hypothesized that this group of OI phenotypes may include recessive forms due to dysregulation of components of the collagen processing machinery. We generated *Crtap* null mice and found that they exhibit osteochondrodysplasia characterized by rhizomelia, kyphosis, and severe osteopenia. In vivo histomorphometric analyses showed significantly decreased bone mass and bone formation rate, but normal numbers of osteoblasts and osteoclasts. Moreover, *Crtap-/-* mice synthetize very little osteoid and have decreased mineralization lag time. We demonstrate that CRTAP exists in a protein complex with prolyl-3-hydroxylase-1 protein and *Crtap-/-* mice lack fibrillar collagen prolyl 3-hydroxylation by tandem mass spectrometric analysis. Finally, we identified *CRTAP* mutations in two families with recessive OI. In the original family with OI type VII, we detected a CG transversion in intron 1 that created a new splice donor site and activated a cryptic exon in most transcripts. However, normal residual CRTAP mRNA and protein were still detected suggesting the hypomorphic nature of this mutation. In the second, consanguineous family with a very severe form of OI (type II), we identified a single nucleotide deletion in exon 4 that led to a premature termination codon, and virtually complete lack of mRNA and protein. Our data demonstrate that loss of function of *CRTAP* causes recessive OI and show the biological importance of collagen prolyl 3-hydroxylation for bone formation.

Fine-mapping of *INSIG2* variation associated with obesity. *H. Lyon[1,2,3], J. Butler[1,2], T. Bersaglieri[1,2], E. Speliotes[1,2,4], X. Zhu[5], R. Cooper[5], J. Hirschhorn[1,2,3]* 1) Div Genetics, Boston Children's Hosp, Boston, MA; 2) Broad Institute, Cambridge, MA; 3) Harvard Medical School; 4) Mass General Hospital, Boston, MA; 5) Loyola University Medical School, Maywood, IL.

Background: Obesity is a heritable trait causing substantial morbidity and mortality, yet the common genetic determinants are largely unknown. **Objective:** Reproduce an association between common genetic variation and obesity, and fine-map the nearby gene to find a causal variant. **Method:** We and collaborators identified a SNP (rs7566605) 10 kb upstream of the *INSIG2* gene (insulin-induced gene) reproducibly associated with obesity.(1) The recessive genotype (frequency 10%) was significantly associated with obesity and was reproduced in 4 out of 5 large populations tested (N>10,000). The pooled odds ratio of the recessive genotype for BMI >30 was 1.26(95% CI 1.09-1.47 p=0.002). It is possible that rs7566605 is not the causal SNP but rather is in linkage disequilibrium (LD) with the causal variant. We further sought a possible causal variant in the 72 kb region (30kb upstream and 20kb downstream of *INSIG2*) by testing 58 tagSNPs, 7 SNPs highly correlated with rs7566605(R2>0.8), and 3 missense SNPs in the gene. TagSNPs were chosen using HapMap data to include a set of SNPs predictive of the LD structure in Yorubans with additional SNPs to capture the variation in Europeans resulting in a superset of tagSNPs likely to capture the variation in an African-American population. All SNPs were genotyped in the African-American population from the Chicago area used in our original report, comprised of 735 people in families and 182 lean and obese people. **Result:** While the SNPs correlated with rs7566605 demonstrated the association as expected, none of the correlated, missense or tagSNPs showed a stronger association to obesity than rs7566605, suggesting that they are not in strong LD with the causal variant. Haplotype analysis and further replication in additional populations are underway. **Conclusion:** Variation upstream of *INSIG2* is associated with obesity in African-Americans. Fine-mapping has not revealed stronger association evidence than the rs7566605 association. (1)Science 2006, 312(5771):279-83.

The influence of *YY1* and retinoic acid in the Treacher Collins syndrome gene (*TCOF1*) regulation. *C. Masotti, E. Kague, M.R. Passos-Bueno* Inst de Biociencias, Univ de Sao Paulo, Sao Paulo, Brazil.

Treacher Collins syndrome (TCS) is an autossomal dominant craniofacial malformation caused by null mutations in the *TCOF1* gene, most of them small deletions or duplications causing a premature stop codon. The hypothesis for clinical manifestation is haploinsufficiency of treacle during embryonic development. High inter and intra-familial clinical variability, ranging from mild malar hypoplasia to perinatal death due to airway collapse is observed, but, to date, no genotype-phenotype correlation has been reported. Interestingly, phenocopy of the syndrome was produced by acute exposure to retinoic acid on the ninth day postfertilization in mice. We have previously hypothesized that mutations in elements of the promoter region of the gene, in trans with a pathogenic mutation, could modulate the phenotype. Therefore, we have delimited the minimal promoter and identified a functional single nucleotide polymorphism that impairs DNA-binding to the *YY1* transcription factor (Masotti et al. *Gene*, 2005,359:44-52). Considering that in silico analysis predicted binding sites for both *YY1* and retinoic acid receptors in this promoter, the aim of the present work is to characterize the role of *YY1* and retinoic acid in the regulation of *TCOF1* expression. We performed co-transfection assays with an *YY1* expression vector and the *TCOF1* promoter constructs bearing the luciferase reporter gene that were described by Masotti et al.(2005). The results demonstrated that *YY1* acts as a repressor element in a dosage-dependent manner. In addition, to study the effect of retinoic acid, we have treated HEK293 cells with 10^{-6}M of all trans retinoic acid (ATRA), for 24h, in three independent experiments. The *TCOF1* and the *SDHA* (succinate dehydrogenase complex, subunit A) and *HPRT1* (hypoxanthine phosphoribosyl transferase 1) endogenous controls expressions were measured by real time quantitative PCR in treated and non-treated cells. We did not observe any up or down regulation of *TCOF1* in treated cells. We are currently testing different concentrations of ATRA and another ligand, the 9-cis-retinoic acid, as well as initiating the characterization of *YY1* binding sites. FAPESP/CEPID/CNPq.

Variants in FOXE1 are Highly Associated with Non-syndromic Cleft Lip with or without Cleft Palate. *MA. Mansilla[1], LM. Moreno[1], M. Johnson[1], SA. Bullard[1], A. Jugessur[2], RT. Lie[2], A. Wilcox[3], K. Christensen[4], TH. McHenry[5], BS. Maher[5], ME. Cooper[5], P. Chines[6], AC. Lidral[1], ML. Marazita[5], JC. Murray[1]* 1) U of Iowa, Iowa City, IA; 2) U of Bergen, Bergen, Norway; 3) NIEHS, Durham, NC; 4) U of Southern Denmark, Odense C, Denmark; 5) U of Pittsburgh, Pittsburgh, PA; 6) NHGRI, NIH, Bethesda, MD.

Non-syndromic cleft lip with or without cleft palate (NSCL/P) is a common birth defect that results from genetic and environmental factors. We are investigating genetic contributors to NSCL/P by using linkage and association approaches. Our linkage scan meta-analysis from 7 different NSCL/P populations identified a major locus within the 9q22-q33 region(HLOD=5.3). For fine mapping, association was performed on extended families and nuclear triads from Southeast Asia to map and identify gene(s) contributing to this signal which spans 50 Mb and contains 104 genes. 330 SNPs in that region were genotyped by CIDR on 220 multiplex families and 44 additional SNPs were genotyped in specific genes on 300 nuclear trios. Modest association was seen for SNPs in the FOXE1 region (p=0.029, rs3758249). To confirm this finding the same SNPs were genotyped in 922 nuclear triads from Northern Europe (NE) and 203 from North America (NA)revealing significant results in all tested populations with the strongest evidence found at rs37582493 with a combined p-value of 2 X 10x-6. Individual populations, NA(p=0.0002) and NE (p=0.0008), were significant by themselves even when adjusted for multiple comparisons and estimates of the attributable risk for FOXE1 ranged from 5% to 22%. Haplotype analysis showed a p-value of 7 X 10x-6 (rs3758249-rs894673). Sequence analysis of 60 NSCL/P cases with evidence of linkage to 9q failed to disclose any common etiologic coding variants. But FOXE1 lies in a 150 Kb LD block that could harbor one or more regulatory regions in which a common variant could explain this association. Mutations in FOXE1 cause the Bamforth-Lazarus syndrome that includes cleft palate. This report provides evidence for a major role for FOXE1 in NSCL/P and adds to the number of specific transcription factors playing a role in facial morphogenesis in humans.

Concordance Measurements and Genotyping Error Rate in the HapMap Dataset. *J. Huang, J. Yang, K. Hao, S. Cawley, K.W. Jones* Affymetrix Inc, 3420 Central Expressway, Santa Clara, CA.

The International HapMap Consortium has generated data for over 3M SNPs in 270 individuals from 4 distinct ethnic populations. This data has been used for both identifying patterns of common genetic variation and as a framework for genetic association studies. Although an initial set of SNPs was genotyped on multiple platforms to assess reliability of the different methodologies, once the project moved into a high-throughput mode the vast majority of SNPs were genotyped on only a single platform. Because SNPs on the Affymetrix GeneChip Human Mapping 500K arrays were selected based on their ability to generate robust genotype calls, and not as a function of reducing the overlap with previously genotyped SNPs, there is a significant number of SNPs (>300,000) on this platform which overlap SNPs genotyped in the context of the HapMap project. Here we present data demonstrating the overall concordance of genotype calls seen between the different platforms. Overall, the Mapping 500K arrays display a 99.4% concordance of calls with those generated in the HapMap project. Further analysis involving assessment of the raw intensity measurements used to make the genotype calls suggests that a small, but measurable, error rate exists in the HapMap dataset. For instance, some platforms have shown a susceptibility to allelic dropout which can manifest itself as monomorphic sequences which are not filtered out based upon Mendelian inconsistencies or deviation from Hardy-Weinberg equilibrium. Lastly, we describe a method which utilizes linkage disequilibrium between markers as a means to determine the true genotypes when calls from two platforms are discordant. The true positive and false positive rate for this analysis will be presented.

Capturing Common, Multi-Ethnic Human Variation On A Single Microarray. *S.S. Murray, P.C. Ng, K. Kuhn, D. Peiffer, L. Zhou, L. Galver, C. Taylor, K. Gunderson, R. Shen* Illumina, Inc, San Diego, CA.

Many of the common SNPs in the genome are known and the results of the International HapMap Project have shown that the information from a majority of these SNPs can be captured by genotyping 250,000-500,000 well-chosen tagSNPs (International HapMap Project, 2005). We have developed a standard tagSNP panel of over 555,000 SNP loci that capture the majority of common variation in CEPH (Caucasian), Han Chinese/Japanese, and Yoruba populations.

The CEPH, Han Chinese/Japanese, and Yoruba populations have approximately 2 million common SNPs each (minor allele frequency 0.05) identified from the HapMap Phase I+II data. To capture this variation, tagSNPs were chosen by an algorithm utilizing the linkage disequilibrium statistic r^2 (Carlson, et al. 2004). An r^2=0.8 threshold was used for common SNPs in or within 10kb of genes or in evolutionarily conserved regions. For all other regions, an r^2=0.7 threshold was used. This panel captures 90%, 87%, and 57% of the HapMap Phase I+II variation in CEPH, Han Chinese/Japanese, and Yoruba populations using pairwise tests at r^2 0.8, respectively. Ninety-six percent, 90% and 92% of all SNP loci are polymorphic in the CEPH, Han Chinese/Japanese and Yoruba populations, respectively, with average minor allele frequencies of 0.23, 0.21, and 0.22, respectively, in these populations. The average spacing between common SNP loci (MAF 0.05) is 5.5, 6.5, and 6.2kb in the CEPH, Han Chinese/Japanese and Yoruba populations, respectively. We have also included over 4,000 SNPs from recently reported LOH/copy number (CN) regions of the genome for more comprehensive coverage for LOH/CN applications and have confirmed several hundred of these regions using this panel. In addition, we have also included 180 mitochondrial SNPs and over 7,000 non-synonymous SNPs. This tagSNP panel is a valuable resource for both genome-wide association and CN studies and will help identify genetic variation affecting both human health and disease.

Mutations in the monogenic diabetes gene HNF4alpha are a novel cause of macrosomia and neonatal hyperinsulinsim. *A.T. Hattersley[1], A. Steele[1], T. Barrett[2], K. Stals[1], J.P. Shield[3], S. Ellard[1], E.R. Pearson[1,4]* 1) Peninsula Medical School, Exeter, UK; 2) Birmingham Childrens' hospital, Birmingham, UK; 3) Bristol University, Bristol, UK; 4) Ninewells Medical School, Dundee, Scotland.

Heterozygous mutations in the genes encoding the beta-cell transcription factor Hepatocyte Nuclear Factor (HNF)-4alpha are known to cause maturity onset diabetes of the young (MODY). These patients have progressive reduction in insulin secretion and present with diabetes in early adulthood. Recently a mouse with a beta cell specific deletion of HNF-4 was surprisingly shown to have lower glucose and increased insulin. We examined whether HNF-4 mutations could cause hyperinsulinaemia in man.

Reported hypoglycaemia was assessed in 106 members of 14 HNF-4 families. We also collected birth weight as insulin is a growth factor in utero and so birth weight can be considered a bioassay of insulin secretion in utero. We compared these results with 134 members of 32 HNF-1 MODY families. Comparison was by Mann-Whitney U, Wilcoxon Signed rank and fishers exact tests.

8 of 58 patients with heterozygous HNF-4 mutations had documented severe (<2 mmol/l) transient hypoglycaemia in infancy, with established hyperinsulinaemia in three cases. In contrast none of the 52 non mutation carriers had hypoglycaemia($p<0.01$). Birth weight was increased by 790g ($p<0.001$) in all mutation carriers (NM) (median corrected birth weight 4450g, IQ range 3880 to 4890g) compared to non mutation (NN) family members (BW 3660g, 3340 to 3900g). 59%NM patients were macrosomic compared with 13%NN ($p<0.001$). In contrast there was no documented severe hypoglycaemia in HNF-1 mutation carriers and the birth weight is not increased (NM 3700g (3330 to 4200g); NN 3720g (3330 to 4360g); $p=0.86$).

We conclude that HNF4- mutations are a novel cause of both transient hyperinsulinaemia of infancy and macrosomia as well as causing early-onset diabetes. This is the first beta-cell gene where a single mutation results in both increased insulin secretion in the fetus and early life and reduced insulin secretion latter in life.

Support for the homeobox transcription factor, ENGRAILED 2, as an Autism Spectrum Disorder susceptibility gene. *J.H. Millonig[1,2], R. Benayed[1,2], P.G. Matteson[1,2], N. Gharani[3], S. Kamdar[1,2], G. Lazar[1,2], I. Rossman[1], E. DiCicco-Bloom[1], L.M. Brzustowicz[3]* 1) Department of Neuroscience & Cell Biol, UMDNJ-RWJMS, Piscataway, NJ; 2) Center for Advanced Biotechnology and Medicine, UMDNJ-RWJMS, Piscataway, NJ; 3) Department of Genetics, Rutgers University, Piscataway, NJ.

We have demonstrated that the homeobox transcription factor ENGRAILED 2 (EN2) is a likely Autism Spectrum Disorder (ASD) susceptibility gene (ASD [MIM 608636]; EN2 [MIM 131310]). EN2 is encoded by two exons and a single intron, spanning 8.1kb. Two intronic SNPs (rs1861972 and rs1861973) are consistently and significantly associated with ASD in 3 separate datasets (A-C rs1861972-rs1861973 haplotype; P=0.0000004; 518 families)(Gharani et al., 2004; Benayed et al., 2005). LD mapping for 16 additional EN2 polymorphisms demonstrated that only the intronic SNPs are in strong LD with rs1861972 and rs1861973. Re-sequencing and association analysis identified the A-C rs1861972-rs1861973 haplotype as a candidate disease allele. This hypothesis is supported by current HapMap data in which rs1861973 is not in strong LD with any other SNP within 2Mb of EN2. Since disease alleles for other common complex disorders can affect the transcriptional regulation of the associated gene, we investigated whether the EN2 intron is capable of acting as a cis-regulatory element. Human intronic sequences containing either the associated A-C or the non-associated G-T haplotype were cloned in front of a luciferase reporter. These constructs plus appropriate controls were transiently transfected into 3 different cell types: HEK-293T cells (a human embryonic kidney cell line), PC12 cells (a rat neuronal cell line) and primary cultures of P6 mouse cerebellar granule cells. These experiments have demonstrated that the EN2 intron acts as a neuronal-specific transcriptional repressor (P= 0.0000055, Students T-test). Preliminary data has also revealed a difference between the A-C and G-T haplotypes. These data provide further genetic evidence that EN2 acts as an ASD susceptibility locus and suggests that the A-C rs1861972-rs1861973 haplotype is the disease allele responsible for our observed association of EN2 with ASD.

High-resolution mapping of partial trisomy breakpoints in Down syndrome patients using oligonucleotide tiling arrays. *J.O. Korbel[1], A.E. Urban[1], X.N. Chen[2], E. Stein[1], M.B. Gerstein[1], S. Weissman[1], M. Snyder[1], J.R. Korenberg[2]*
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Amplifications and deletions of chromosomal regions are involved in many genetic disorders. Down syndrome is caused by one of the most common chromosomal abnormalities in liveborn children, trisomy 21, associated with a variety of medical conditions including mental retardation and congenital heart disease. A fraction of patients carry partial trisomies involving distinct regions of chromosome 21, instead of an extra chromosome. This phenomenon is expected to provide a valuable entry point for analyzing the molecular etiology of the disease and its associated developmental malformations. In particular, precise mapping of the partial trisomies may substantially advance the analysis of phenotype-genotype relationships. Recently, we have developed ultra-high-resolution comparative genomic hybridization (UHR-CGH) and shown that it is capable of detecting accurately the extent of chromosomal aberrations on human chromosome 22; in several cases, breakpoint sequences were identified by PCR following data analysis (see e.g. Urban *et al., PNAS* 2006; 103:4534-9). Here, the same technology was used to construct chromosome 21 arrays containing 385,000 isothermal oligonucleotide probes (probe lengths: 45-85 nt; uniform hybridization temperature: 76C). All except the very highly repetitive regions of chromosome 21 are covered with a 122 nt tiling path. Using a custom Hidden-Markov-model based segmentation algorithm this allowed breakpoint-mapping at unprecedented level. The breakpoints and duplicated regions of two partial trisomies were determined by Southern blots and high resolution multicolor FISH with BAC arrays, and this could be refined using chromosome-wide UHR-CGH to below exon-level resolution. This represents a 100-1000 fold enhancement in resolution over conventional methods and provides firm evidence for sensitive detection of copy number variation in the range of 3:2 for unique and repeated genomic sequences.

How to associate the somatic mutations and a specific cancer. *K. Li[1], L. Xiao[2], Y.F. Yin[1], J. Zhang[2]* 1) Molecular Genetics, City of Hope, Duarte, CA; 2) SNP Institute, Nanhua University, Hengyang, China.

We observed that the incidence of small genetic variations between human and chimpanzee genomic sequences decays rapidly according to the size of the inserted or deleted fragments during evolution. Quantitative analysis with double-regression of small mutations deposited in the Human Genomic Mutation Database and in the human p53 somatic mutation database of nearly eighty thousands mutations revealed that the decay of small insertions and small deletions is proportional with the square of the number of base pairs inserted or deleted, which is thus described as M(i/d)N = M(i/d)1 x N2. Here M is the number of mutations with N nucleotides or 1 nucleotide inserted or deleted. This characterized decay pattern of small insertions and small deletions have immediate applications in distinguishing neutral drift and positive selections in phylogenetic studies and in association analyses for somatic mutations identified in cancers. When mutations are identified in one gene in a given cancer of a specific tissue a strict and unique association between the mutated gene and the cancer can be conclusively ruled out if the small deletions or insertions are distributed following the decay pattern as described. Otherwise, an association relationship is generally accepted to the non-randomly occurring mutations. Additionally, the decay pattern can be applied to illuminate the biochemical basis of a newly associated gene in carcinogenesis: Significantly more inframe or frameshift deletion/insertions indicate gain-of-function or loss-of-function respectively. The latter is invaluable for genes with unknown functions.

High heritabilities of metabolites in families from the GENECARD Study of early onset coronary artery disease.

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Introduction. Metabolomic profiling of small-molecule metabolites holds promise for integrating genetic and biochemical data to give a more complete picture of human systems biology. Profiles can predict presence of CAD, a complex disease with a known genetic basis. However, the genetic basis of these profiles has not been established.

Methods. We identified five families from the GENECARD study of early-onset CAD. Blood was collected on family members with CAD and unaffected offspring (N=80 members in 5 families; 20 with CAD). Gas chromatography/mass spectometry (MS) with isotope-labeled internal standards were used for targeted quantitative measurements of ~60 metabolites (free fatty acids, acylcarnitines, amino acids, conventional metabolites). Variance components as implemented in SOLAR was used to calculate heritabilities (h^2).

Results. Multiple metabolites showed high heritabilities. Among acylcarnitines, C18s (steroyl, oleyl, linoleyl) and C16 (palmitoyl) were highly heritable (h^2 0.42-0.63, $p<0.002$), as were C5 (h^2 0.35, $p=0.007$) and C2 (h^2 0.63, $p=0.0003$). Among amino acids, alanine, serine, proline, valine, leucine/isoleucine, methionine, glutamic acid/glutamine, ornithine and arginine were heritable (h^2 0.33 - 0.88, $p=0.02$ - 9E-13). CRP showed high h^2 (0.56, $p=0.008$), as did conventional metabolites (0.34 - 0.57, $p=0.02$ - 0.0002).

Conclusions. We found strong heritabilities of metabolites in families with a common, high propensity for early-onset CAD. The genetic architecture underlying these heritabilites remains to be elucidated.

Protein walking of hnRNP E1 with a yeast n-hybrid system. *L.R. Huo[1], W. Ju[2], N. Zhong[1,2]* 1) Peking University Center of Medical Genetics, Beijing, China; 2) New York State Institute for Basic Research, Staten Island, NY.

hnRNP E1 is the family member of hnRNPs, which comprise of RNA-binding proteins with three KH domains. hnRNP E1 plays a pivotal role in post-transcriptional regulation for RNA metabolism and RNA function in gene expression. Alteration of cellular hnRNP E1 level may change the global transcriptional and translational profiles (Zhong et al., ASHG 2006 annual meeting). We hypothesized that the regulating function of hnRNP E1 is performed along with other proteins, with which a protein network is formed. To test our hypothesis, we have initialized a study to investigate this network. An approach of protein walking with the yeast n-hybrid system is employed for this study. The hnRNP E1 is used as the initial walker to search for its contactor(s), which could be protein molecules or RNA molecules. Candidate contactors are identified with selection mediums, reporter gene, and co-immunoprecipitation. With these candidate RNA and protein molecules, an hnRNP E1 network can be established, which may contribute to a better understanding of physiological functions of hnRNP E1.

Allelic heterogeneity in autosomal dominant hereditary motor and sensory neuropathy with proximal dominancy (HMSN-P). *K. Maeda[1], R. Kaji[1], S. Makino[2], K. Yasuno[3], H. Takashima[4], M. Nakagawa[5]* 1) Dept of Neurology,Tokushima Univ. Graduate School of Medicine,Japan; 2) Division of Human Molecular Genetics,Dept of Neurology and Neuroscience,Tokushima Univ. Graduate School of Medicine,Japan; 3) Division of Genetic Diagnosis,The Institute of Medical Science,Tokyo Univ.,Japan; 4) Dept of Neurology and Geriatrics, Kagoshima Univ.Graduate School of Medicine,Japan; 5) Dept of Neurology,Graduate School of Medical Science,Kyoto Prefectural Univ.of Medicine,Japan.

Hereditary motor and sensory neuropathy with proximal dominancy (HMSN-P; OMIM% 604484) is endemic in Okinawa Islands, 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. All affected individuals share an identical haplotype within the disease locus, suggesting that the undiscovered mutation of HMSN-P originated in a single founder in Okinawa. In the middle of Japan, we newly found a large family with many members developed the similar symptoms of HMSN-P, which had no record of affinal connection with Okinawan. Physical and electrophysiological examinations in a total of 41 members, including 13 affected individuals, revealed the disease is neurogenic but not myogenic as well as HMSN-P. Our linkage study in the large family using 15 microsatellite markers around the HMSN-P locus identified a 4.6-Mb interval in 3p13 cosegregated with the disease (maximum multipoint lod score of 5.08 at theta=0.0). The candidate region was identical to the HMSN-P locus recently refined by Takashima et al, but the disease haplotype in the large family was different from that in the Okinawa families. Our results suggest that HMSN-P show allelic heterogeneity in Japan. Such allelism would facilitate the identification of the disease-causative mutation in the candidate region. Moreover, the allelic heterogeneity of HMSN-P in Japan implies the possibility that HMSN-P is common across other ethnic groups but erroneously classified into other disease categories, such as HMN, CMT, and LGMD.

Haploview: A computatioonal tool for analysis and visualization of whole genome association data. *J. Maller[1,2], D. Bender[1,2], J. Barrett[1,3], S. Purcell[1,2], M. Daly[1,2]* 1) Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, MA; 2) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA; 3) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford UK.

In the whole genome association era there are orders of magnitude more SNP data being collected than had previously been feasible. This huge increase necessitates computational tools which can efficiently analyze and effectively visualize these data sets. The linkage disequilibrium and haplotype analysis package Haploview has been extended to be a part of a complete set of tools for such whole genome association analysis. Here we describe some of the new functionality and integration with other tools which enable this type of analysis. This includes the ability to load genome wide association results generated by other software packages in order to view the results and pick tag SNPs for follow up genotyping. Additionally, Haploview can now automatically load phased HapMap data over the internet, which greatly simplifies LD analysis and tagging of HapMap data.

Genomic analysis of large tandem arrays in the human genome. *C. Lescale, D. Hasson, S. Gmuca, P.E. Warburton*
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Copy number variation (CNV) in the human genome is important in phenotypic variation and genome dynamics. Comparative genomic microarray analysis of CNVs does not easily distinguish between low copy duplications/deletions and variation between highcopy numbers of tandem repeats. Therefore, a genome-wide bioinformatic analysis was performed that identified 18 distinct tandem repeat families with a 1.5kb or greater repeat unit in arrays of at least 4 tandem copies. All but three of these families are included in the Database of Genomic Variants (Univ. Toronto). At least five families are found in inter- and/or intrachromosomally dispersed multiple arrays, and altogether represent over 45 large tandem arrays in the genome. Arrays are often embedded within larger segmental duplications, and represent regions prone to chromosomal rearrangments such as 8p23. Repeat units usually contain multiple fragments of interspersed transposable elements. 5 arrays contain a gene in each repeat unit, while 7 arrays spanned by a single large gene contain a spliced exon in each repeat unit. The sequence assembly of many of these tandem arrays does not accurately reflect copy number, with 7 arrays spanning a gap and 4 with assembled BAC sequences ending within the repeat units. For example, ~6 copies of a 12kb repeat unit spanning an 87kb gap in band 8q21.2 in hg18 was shown by pulsed field gels to vary from ~700kb to ~1500kb (~60 to ~125 repeat units) in 13 sized arrays. The largest tandem array family seen in hg18 consists of ~3kb repeat units made up of fragments of MaLR MSTA retrotransposons, which are found in ~25 arrays on 9 different chromosomes. The largest arrays of 60kb, 80kb and 54kb are found in the pericentromeric regions of chromosomes 13q, 18p and 21q, respectively, embedded in regions of related segmental duplications. However, distinct higher-order repeat structure suggests unequal crossing over events have occurred within the arrays since these duplications. FISH analysis confirms the arrays in hg18 and shows additional large arrays on chromosomes 15, 20, and 22. Multicopy tandem arrays represent great potential for variability between individuals, and may have distinct functional roles in chromosome structure or segregation.

KAL1 mutation in two brothers with Kallmann syndrome and previously unreported concurrent deafness and renal agenesis. *D.M. Niyazov[1], D.J. Gruskin[1], P.M. Fernhoff[1], E.M. Dystrka[2], L.P. Chorich[2], L.C. Layman[2]* 1) Human Genetics, Emory University School of Medicine, Atlanta, GA; 2) Obstetrics & Gynecology, Section of Reproductive Endocrinology, Infertility & Genetics, Institute of Molecular Medicine and Genetics, Medical College of Georgia, Augusta, GA.

Kallmann syndrome (KS) is characterized by anosmia and hypogonadotropic hypogonadism (HH). KS has sporadic, X-linked (XLKS), autosomal dominant and autosomal recessive inheritance patterns. XLKS may be due to mutations in the KAL1 gene on Xp22.3. Renal agenesis (RA) has been reported in 30-50% of XLKS patients and hearing loss of varying degrees in 5-10%, with bilateral sensorineural deafness (SD) documented in only three patients. However, the occurrence of both RA and SD in one patient has not been previously described. We report a family with a KAL1 point mutation in a carrier mother and two of her sons with XLKS, one of whom had concurrent RA and SD. The proband presented at 16 years of age for an evaluation of HH, anosmia and left cryptorchidism. His chromosomes and FISH for KAL1 deletion were negative. His parents then brought his 22-year-old full brother who was never seen by a clinical geneticist. In addition to HH, anosmia and left cryptorchidism, he had SD and left RA which was discovered incidentally by ultrasound for a sports-related abdominal injury. The probands ultrasound showed bilateral kidneys. Both brothers had pes cavus and no synkinesia. Both parents denied any history of hearing loss or anosmia, however the mother also had pes cavus. To determine if a KAL1 mutation was present, the coding regions of exons 2-13 and splice junctions were amplified by PCR and sequenced. Both affected brothers were hemizygous for an R257X mutation in exon 6, which was present in the mother but not the father. This mutation within the first fibronectin III repeat region is predicted to truncate the protein, removing 27 AA from this repeat and the remaining three FNIII repeat regions. Although this nonsense mutation was previously reported, it was never associated with SD as in one of the brothers. We postulate possible molecular mechanisms to explain the different effects of the same mutation in the two brothers.

Provision of Genetic Services in the aftermath of Katrina: The LSU Experience. *Y. Lacassie[1,2], M. Marble[1,2]* 1) Dept Ped/Div Clin Genetics, LSU Health Sciences Ctr, New Orleans, LA; 2) Children's Hospital, New Orleans, Louisiana.

The occurrence of hurricanes Katrina and Rita in 2005 produced a profound impact on all medical services in New Orleans and other cities along the Louisiana, Texas and Mississippi coastline. The LSU Division of Pediatric Genetics was no exception. These catastrophic events not only jeopardize the lives of patients with inborn errors of metabolism, but also the care of patients and families with other genetic disorders. The Division of Genetics from LSU/Childrens Hospital stayed in South Louisiana in the storm aftermath providing as much care as possible to our patients. The metabolic specialist stayed at Childrens Hospital until mandatory evacuation. Thereafter, he moved to an area close to Baton Rouge and Lafayette, where the Genetics Division staffs our most important satellite clinics. Through our satellite clinics system we were able to provide uninterrupted genetics/metabolic services in South Louisiana until Childrens Hospital reopened on October 10, 2005. Numerous factors were crucial to providing direct, fast, effective, continued and appropriate medical care. Among the most important were to stay in the area. This allowed to communicate with peer physicians, university and hospital administrators and to see patients in outreach clinics. Crucial too were communications with patients and their families, satellite clinics, the Office of Public Health, the laboratory where neonatal screening was deferred, and geneticists from other cities or states. Another important factor was the availability of a system of records available to the geneticists. With all of these basic action blocks in place, our group was able to provide medical care to the genetics population of South Louisiana. We provided care to established patients, to 8 new patients with metabolic disorders detected through the newborn screening, and also to some patients from other institutions. Our experiences regarding these challenges, and how clinical, social and even legal issues can become a problem in catastrophic situations will be discussed.

Contactin-associated protein-like 2 is a positional candidate gene for idiopathic adolescent scoliosis. *L.M. Nelson, J. Braun, J.W. Ogilvie, K. Ward* Axial Biotech, Inc., Salt Lake City, UT.

Introduction: Our aim was to map the loci causing or contributing to adolescent idiopathic scoliosis (AIS). Methods: Using IRB approved protocols, parametric linkage analysis was performed in 35 families with multiple affected individuals using 750 microsatellite markers spaced at approximately 5 cM. Genome-wide association was tested using the Affymetrix HuSNP 100K array on 204 cases and 145 controls Results: Three neighboring microsatellite markers had individual LOD scores greater than 4. Location scores maximize in a 4.3 cM interval. Several single nucleotide polymorphisms (SNPs) within the region were highly associated with AIS as well ($p<0.00001$). Locus heterogeneity was evident; LOD scores increased to 7.3 when considering the subset of pedigrees most likely to be linked to this locus. These polymorphisms lie within a very large gene (over 2.5 million basepairs)the contactin- associated protein-like 2 gene. This gene codes for a neurexin-like protein expressed in the central nervous system which plays a role in the formation of functional distinct domains critical for saltatory conduction of nerve impulses in myelinated nerve fibers. Mutation scanning is underway in cases and controls. Conclusions: These data indicate the presence of an important AIS susceptibility locus that occurs at 7q35. The contactin-associated protein-like gene 2 is a candidate gene for adolescent idiopathic scoliosis. A truncating mutation in this gene was recently implicated in Old Order Amish patients with cortical dysplasia-focal epilepsy syndrome, but it has not been associated with scoliosis in animals or humans prior to this work.

The omega-6/omega-3 fatty acid ratio in transgenic animals. *J.X. Kang* Medicine, Massachusetts General Hospital , Charlestown, MA.

An important nutritional question as to whether a balanced n-6/n-3 fatty acid ratio can reduce risk of modern diseases needs to be addressed in well-qualified experimental models. The recent development of genetic approaches to balancing n-6 to n-3 fatty acid ratio by expressing the fat-1 gene (encoding an omega-3 fatty acid desaturase) in cultured mammalian cells and animals provides a new opportunity to address this issue. Our studies have demonstrated that mammalian cells or animals engineered to express the humanized fat-1 gene can effectively produce n-3 fatty acids from the n-6 type and have a balanced tissue ratio of n-6 to n-3 fatty acids, without the need of exogenous n-3 supplementation. So far, transgenic fat-1 mice and pigs rich in n-3 fatty acids have been successfully generated. As well presenting a technology to optimize fat composition in animal products by genetic means, these transgenic animals serve as novel and useful models for studying the roles played by n-3 fatty acids in the body because they have a number of advantages over the conventional feeding methods, including being free of the confounding factors of diet. We have performed a series of experiments in both fat-1 transgenic cells (in vitro) and animals (in vivo). Our data obtained so far support the notion that a reduced or balanced ratio of cellular n-6 to n-3 fatty acids can alter gene expression and cell function, and may reduce the risk of certain diseases, including cardiovascular disease, inflammatory disorders and cancer. This presentation will provide some perspectives on the impact of genetic technology on nutrition as well as the effect of lipid nutrients on gene expression.

A Statistical Method for Assessment of the Extent and Statistical Significance to which an Association Accounts for a Linkage Signal for a Quantitative Trait. *R. Hanson* Diabetes Epidemiology Clin Res, NIDDK, Phoenix, AZ.

Association studies are often used to finely map loci identified by linkage analysis of a quantitative trait. Once an association is identified, a method to determine the extent to which it accounts for linkage is desirable. To accomplish this we propose bivariate analysis of the trait and its residual after regression on associated marker genotypes. Both original and residual traits are included in a bivariate extension of the variance-components method for quantitative trait linkage analysis. The model includes nine variance-covariance components representing effects of the quantitative trait locus, along with residual genes and environment; one of the components represents the effects of the quantitative trait locus on the original trait that have been regressed out of the residual ($^2_{QO}$). $^2_{QO}$ quantifies the contribution of the associated marker to the linkage and its statistical significance is assessed by a likelihood ratio test. We conducted a simulation study to determine power with respect to extent of linkage disequilibrium and similarity in frequency between functional and marker alleles. Families consisted of 264 nuclear families, containing 1004 offspring who had participated in a linkage study. Genotypes were simulated for these individuals at: 1) a diallelic functional locus with a high-risk allele with frequency=f_A; 2) a diallelic marker locus closely linked (=0.00) to the first with an allele of frequency=f_1 associated with the high-risk allele at a specified degree of linkage disequilibrium (D'); 3) a perfectly informative marker linked (=0.05) to the other two loci. In the absence of linkage disequilibrium between functional and diallelic marker loci, type I error rates for the statistic were near nominal values; the test was also robust to simulated population stratification. Power depended on degree of concordance between alleles at functional and marker loci. For example, with $f_1=f_A$, power for $p<0.0001$ was 2% at D'=0.4, 15% at D'=0.6, 65% at D'=0.8 and 99% at D'=1.0. In fine-mapping studies, this procedure can be useful for prioritizing associations that are likely due to linkage disequilibrium with a functional locus.

Whole-Genome Association (WGA) Study for Multiple Sclerosis. *S.L. Hauser for The International Multiple Sclerosis Genetics Consortium (IMSGC)* Neurology, University of California, San Fancisco, CA.

Multiple sclerosis (MS)is a debilitating neuroimmunological and neurodegenerative disorder that affects more than 2.5 million people worldwide and has a complex and strong genetic component. The standard whole genome screening approach using genetic linkage has failed to conclusively identify any genetic susceptibility to MS apart from the well-established effect residing within the major histocompatibility complex (MHC) on chromosome 6p. The latest data from the HapMap project suggests that by genotyping a relatively small portion of the known variation across the human genome, >80% of all common variation can be interrogated. Given this, the IMSGC has designed a whole genome association (WGA) study, allowing for a much finer scale inspection of the genome for involvement in MS risk. Genotyping has been performed using the Affymetrix 500k SNP chip in 1,000 MS trio families (an affected individual and both parents) collected in the US and UK. The completed dataset will comprise approximately 1.5 billion genotypes. To date, the platform has proven very robust when using the newly released BRLMM algorithm, with genotyping efficiencies exceeding 99%. Because of the trio design, we can obtain accurate error estimations using Mendelian inheritance. The preliminary data suggest an average true error rate of approximately 0.2%. Furthermore, additional extensive quality control procedures are in place including: assessing SNP, sample, and trio genotyping efficiencies, sample contamination and relatedness tests, extensive tests of Hardy-Weinberg equilibrium, tests for population substructure, examination of linkage disequilibrium relationships, and correlation of SNP characteristics with p-values. After QC filtering, the remaining SNPs have error rates <0.1%. Preliminary analysis of a subset of these QC'd data provides the expected distribution of p-values, with an extended right-hand tail (0.09% of the markers have $p< 1x10^{-4}$). As a positive control, TDT analysis of the MHC region shows 22 markers with $p <1x10^{-6}$; the most extreme with $p <1x10^{-15}$. The results from the full 1,000 trio screen will be presented.

Use of Forensic Markers in the Assessment of Population Stratification. *C. Oddoux, S. Shajahan, D. Parrott, L. U., A. Pearlman, H. Ostrer* Div Human Genetics, New York Univ Sch Medicine, New York, NY.

Assignment of individuals to population groups is important to genetic case control association studies, admixture mapping, medical risk assessment, genealogy, and forensic studies. Polymorphic sequences can be used to infer ancestry but their utility for such an application is related to the number of alleles and relative frequency differences of these alleles between the population groups under study. Multiple study designs differing in numbers and types of polymorphic markers with differing levels of informativeness make comparison of studies difficult. The use of commercially-available highly-informative markers that are used internationally in forensic applications could provide a universal first tier analysis for assignment of individuals to population groups prior to inclusion in association and admixture studies. We evaluated the utility of the PowerPlex kit of 16 markers from Promega for this purpose. Multiple population groups including African, Bengalis, Chinese, Japanese, Koreans, Crypto Jews, Sephardic Jews, and Dutch were genotyped using the PowerPlex kit. The data were analyzed with STRUCTURE (Pritchard et al.) using an admixture model, correlated alleles and 3 clusters. Africans, Asians (Bengalis, Koreans, Chinese and Japanese), and Caucasians (Dutch, Sephardic Jews, and Crypto Jews) were clearly delineated. Individuals showing admixture were detectable and their removal resulted in more discrete clustering. An independently collected and genotyped set of Dutch individuals was indistinguishable from the original Dutch group providing reproducibility across data sets. The sensitivity conferred by the number of markers used in the analysis was assessed by removing markers. Delineation of population groups was apparent when 14 markers were used, although clusters were noisier; however it was not possible to delineate population groups when only 8 markers were used. The use of forensic markers is a promising strategy for clustering individuals into population groups and will be an inevitable outcome of their forensic use.

Adrenocortical hyperplasia or adenomas are associated with inhibition of phosphodiesterase 11A in carriers of *PDE11A* gene sequence variants and in *pde11a*$^{+/-}$ mice. *A. Horvath, C. Giatzakis, S. Boikos, K. Griffin, E. Stein, J. Surapisitchat, A. Robinson-White, I. Bossis, V. Kamvissi, P. Soni, J.A. Carney, J. Bertherat, P. Gregersen, E. Remmers, J. Beavo, C. Stratakis* SEGEN, DEB, NICHD, NIH, Bethesda, MD 20892, USA.

Increased cAMP signaling has been associated with mutations of the *PRKAR1A* and *GNAS* genes leading to adrenocortical tumors and Cushing Syndrome (CS). We recently published that the genomic area with the highest probability to harbor a susceptibility gene by LOH and other analyses was 2q31-35. Five different, previously undescribed sequence variants were identified in the coding sequence of *PDE11A* - a dual specificity PDE that is expressed in the adrenal cortex and other tissues; its activity is partially inhibited by tadalafil and other PDE inhibitors. Three of the mutations led to premature stop codon generation; the other two sequence variants are missense substitutions. At least one of them is located in a highly conserved region of the enzyme and was found to be associated with various forms of bilateral adrebnocortical hyperplasia in patients with mostly mild or cyclical CS. A panel of sporadic adrenocortical tumors showed decreased *PDE11A* expression, 2q31-35 LOH, and high cyclic nucleotide levels and CREB phosphorylation. *Pde11a* was found to be expressed widely in the developing adult mouse. A limited study of pde11a$^{+/-}$ mice confirmed the development of adrenocortical hyperplasia or adenomas in older mice. The two missense substitutions are found to be present in the general population with a frequency close to 2.5%. The three nonsense mutations were found rarely among individuals enrolled in the New York Cancer Project. These results indicate that *PDE11A* inactivation predisposes to the development of adrenocortical hyperplasia and suggest another means by which genetic dysregulation of cAMP signaling can cause endocrine tumors. We conclude that *PDE11A* - inactivating mutations are present in the population; their association with BACH and other adrenal tumors in both humans and mice points to their low penetrance but also possible involvement in predisposition to other tumors.

Agenesis of the corpus callosum with optic coloboma and colloid cyst: a possible new syndrome of cerebral development. *J. Li[1], S. Shivakumar[1], M. Wakahiro[1], S. Stefanos[1], A. Slavotinek[2], J. Barkovich[3], P. Mukherjee[3], E.H. Sherr[1]* 1) neurology, UCSF, San Francisco, CA; 2) pediatrics, UCSF, San Francisco,CA; 3) radiology, UCSF, San Francisco, CA.

Agenesis of the corpus callosum is a common brain anomaly with an incidence of approximately 1 per 4000 live births. There are only a few genes in humans that, when inactivated, result in a fully penetrant syndrome with complete ACC. In mice the corpus callosum is derived from axons arising from cortical neurons in layers 2, 3 and 5, and there is evidence that the cingulate cortex provides pioneer axons for midline crossing. Previous studies have shown that deletion of vax1 results in ACC and optic coloboma in mice. ASXL2 and KIAA1803 are linked by a balanced chromosomal translocation in a patient with ACC and optic colobomata. Here, we report a novel autosomal recessive condition with agenesis of the corpus callosum, unilateral coloboma, craniofacial and skeletal dysmorphisms, profound cognitive impairment and intractable seizures in a brother and sister of a consanguineous middle eastern family. Brain imaging showed complete ACC, colpocephaly, interhemispheric colloid cyst and probst bundles. This case presents many features similar to a syndrome, originally described by Dr. Temtamy, in which siblings from consanguineous parents presented with seizures, mild to moderate developmental impairment and bilateral optic colobomata. We performed homozygosity mapping, and this excludes the regions encompassing the genes Vax1, ASXL2 and KIAA1803 from causing this syndrome. Genome-wide mapping is ongoing and we hope the findings will help define the spectrum of Temtamy syndrome and shed light on cerebral development.

Identification of Polymorphisms with Remedial Metabolic Impact. *N. Marini[1], J. Ziegle[2], J. Gin[1], K. Hunkapiller[2], D. Ginzinger[2], D. Gilbert[2], J. Rine[1]* 1) Dept Molecular/Cellular Biol, Univ of California, Berkeley, CA; 2) Applied Biosystems, Inc, Foster City, CA.

Compelling data from human and bacterial genetics show that the phenotype of some mutations in vitamin-dependent enzymes can be suppressed by vitamin supplementation. Such vitamin-remedial mutations can affect the binding site for the vitamin cofactor, or can destabilize the protein in a way that higher vitamin concentration can remedy. The known cases from human genetics are rare and typically result in severe disease that is vitamin remedial. To test the hypothesis that there may be polymorphisms that more subtly affect enzyme activity, yet are vitamin-augmentable by the same principles, we have sequenced the coding exons in 4 prototypical genes in a large (564 individual), diverse population. All missense variations from Ornithine Aminotransferase, Thymidylate Synthase, Methylenetetrahydrofolate Reductase (MTHFR) and Glycinamide Ribonucleotide Transformylase are being evaluated in functional assays based on complementation in the yeast, Saccharomyces cerevisiae and in cell-free biochemical analyses. We have demonstrated that quantitative yeast growth assays accurately reflect vitamin-responsiveness and intrinsic activity of enzyme variants. From 32 total coding exons comprising the 4 target genes, we have identified 21 novel low frequency coding variants (allelic frequencies <1%) with at least two such variants seen in each gene. Functional analysis has centered on MTHFR variants (11 low-frequency, 3 common) and has demonstrated that nearly 50% of nonsynonymous substitutions affect enzyme function, most of which are augmented by elevated folate levels in the yeast assay. Furthermore, we have seen synergistic effects between variants in multiply-substituted versions of MTHFR indicating the need to determine all changes in a particular allele to estimate functional impact. Extrapolating these observations to the larger set of folate (or other cofactor)-dependent enzymes indicate that low frequency variants, either alone or in combination with common variants, may contribute significantly to inidividual heterogeneity in folate or other metabolic pathways.

Molecular analyses in a female with symptoms of Rett syndrome and Pelizaeus-Merzbacher disease. *G.M. Hobson[1], T. Alberico[1], K. Sperle[1], L. Banser[1], J. Taube[1], A.P. Davis-Williams[1], J.R. Jones[2], M.J. Friez[2], G. Bibat[3], S. Naidu[3]* 1) A I duPont Hosp Children, Wilmington, DE; 2) Greenwood Genetic Center, Greenwood, SC; 3) Kennedy Krieger Institute, Baltimore, MD.

We report a case of a 9-year-old female with symptoms of both Rett syndrome (RTT) and Pelizaeus-Merzbacher disease (PMD). RTT is caused by mutations in the methyl cytosine binding protein 2 gene (*MECP2*) at Xq28, and PMD is caused by mutations in the proteolipid protein 1 gene (*PLP1*) at Xq22. The patient's clinical phenotype includes rigidity, microcephaly, febrile seizures, handwringing and hand mouthing, vasomotor instability, feeding problems, and drooling, but without nystagmus. She is nonverbal and wheelchair bound. Nonprogressive hypomyelination and thin corpus callosum were observed on MRI at ages 2, 3, and 6 years. The white matter changes were inconsistent with RTT, but because of the possibility of two coexisting diseases, *MECP2* testing was performed by sequencing and multiplex ligation-dependent probe amplification (MLPA). Sequence analysis was normal and no deletions or duplications involving *MECP2* were detected in the MLPA analysis. However, one of the MLPA control probes, which hybridizes in exon 4 of *PLP1*, indicated the possibility of a deletion or mutation involving *PLP1*. Testing by semiquantitative multiplex PCR analysis confirmed that the patient has a deletion of one *PLP1* allele that extends from 980 kb proximal of *PLP1* to a region between 2.2 and 2.4 Mb distal. Sequence analysis indicated that she also has an 11 bp deletion in the promoter of her remaining *PLP1* allele in a region previously demonstrated by others to bind nuclear proteins. This 11 bp deletion is predicted to remove two Sp1 transcription factor binding sites and recreate one Sp1 site across the junction, which may affect *PLP1* expression levels. Thus, the molecular findings in this female patient are consistent with a diagnosis of PMD.

Multi-species conserved sequences within a chromosome 1q43 region linked to Multiple Sclerosis have reduced SNP density and polymorphism. *D.P. Mortlock[1], J.L. McCauley[1], S.J. Kenealy[1], E.H. Margulies[2], N. Schnetz-Boutaud[1], S.G. Gregory[3], S.L. Hauser[4], J.R. Oksenberg[4], L.F. Barcellos[5], M.A. Pericak-Vance[3], J.L. Haines[1]* 1) Center for Human Genetics Research and Dept. of Molecular Physiology and Biophysics, Vanderbilt University Medical Center, Nashville, TN; 2) Genome Technology Branch, National Human Genome Research Institute, Bethesda, MD; 3) Center for Human Genetics and Dept. of Medicine, Duke University Medical Center, Durham, NC; 4) Dept. of Neurology, University of California San Francisco, San Francisco, CA; 5) Division of Public Health-Epidemiology, University of California, Berkeley, CA.

Although genes play a key role in complex disease, the specific genes involved in the majority of complex diseases remain largely unidentified, and effective methods are needed for screening variants in candidate regions identified through linkage analyses. Evolutionary conservation can be used as a guide to indicate noncoding or coding regions that are likely to be functional and thus may be more likely to harbor SNP variants with functional consequences. Previously, a region of chromosome 1 was linked to Multiple Sclerosis (MS) in a genome-wide screen. We devised a process for prioritizing annotated SNPs for follow-up genotyping studies based on their location within Multi-species Conserved Sequences (MCSs) in the 1q43 linkage region. We then obtained genotypes for 768 SNPs in 989 individuals from MS families. While many SNPs within MCSs were monomorphic in our data set, many were validated as polymorphic. Our analysis of HapMap SNP data across the region confirmed that annotated SNPs in MCSs were more likely to be monomorphic than SNPs in non-conserved regions. We also verified that the density of annotated SNPs is much lower in MCSs, suggesting that a random selection of noncoding SNPs would bias toward variants in nonfunctional regions. We believe that this novel approach for follow-up analysis will increase the likelihood of successfully identifying a genetic factor for MS in the 1q43 region and demonstrates a paradigm for expediting the search for additional genes in MS and other complex diseases.

An accurate and robust high density SNP genotyping microarray platform. *K. Kuhn, P.C. Ng, S.S. Murray, L. Zhou, L. Galver, C. Tsan, D. Bullis, P. Merrit, F. Steemers, K. Gunderson, R. Shen* Biochemistry Development, Illumina, Inc., San Diego, CA.

We have developed a high density SNP genotyping platform that enables genotyping hundreds of thousands of loci on a single microarray accurately and efficiently. This platform utilizes a single base extension (SBE), which requires a single bead-type per assay and 2-color fluorescent imaging (Steemers et al. Nature Genetics, 2006). The assay is a single-tube, PCR-free protocol that yields extremely high data quality. We genotyped 120 HapMap DNA samples including 25 parent-child trios and 15 replicates in a panel of >550,000 SNP loci, generating over 66 million genotypes. From this dataset, we have observed >99% call rate, >99.9% reproducibility, >99.9% genotypes consistent with Mendelian inheritance, and 99.7% genotype concordance with the International HapMap Project. Because the assay uses a single-tube sample amplification process that does not require PCR or genome complexity reduction, it has virtually unconstrained SNP selection and unlimited multiplexing potential. In addition, the laboratory process workflow has been streamlined and includes automation enabling high sample-throughput, and a laboratory information management system (LIMS) enabling positive sample tracking, integrated project and workflow management, real-time quality metrics, data storage and analysis. The single slide substrate is highly flexible and can support a range of configurations including single-sample/slide whole genome content (100,000 - >550,000 SNP loci) or multi-sample/slide custom content (12,000 - 60,000 SNP loci). The assay and workflow process will enable researchers to quickly and inexpensively generate millions of genotypes to achieve their research objectives.

A genome-wide linkage scan of adiponectin levels in the GEMS study. *H. Ling[1], H. Stirnadel[2], N. Galwey[2], V. Mooser[2], D. Waterworth[2], B.D. Mitchell[1], The GEMS Investigators* 1) University of Maryland School of Medicine, Baltimore, MD; 2) Medical Genetics, Genetic Research, GlaxoSmithKline.

Adiponectin, a protein secreted exclusively by adipocytes, is the most abundant gene product in adipose tissue. Plasma adiponectin levels are negatively correlated with BMI, especially visceral adiposity, and are lower in subjects with type 2 diabetes (T2DM) and coronary artery disease. Metabolic studies in both animals and humans suggest it has a variety of metabolic effects, including anti-diabetic, anti-atherosclerotic, and anti-inflammatory and may mediate the relationship between obesity and insulin resistance or T2DM. At least 5 genome scans for adiponectin levels have been published to date. Chromosomal regions reported likely to harbor genes influencing variation in plasma adiponectin levels include chromosomes 5p, 14p, 9p, 15, 18 and 3q. We performed a genome-wide linkage scan of adiponectin levels in two different Caucasian populations, Mediterranean origin and non-Mediterranean origin, recruited as part of the GEMS study (3,069 participants from 450 families, 789 Mediterranean and 2,280 non-Mediterranean) aged 16-70 yrs. Quantitative trait linkage analysis was carried out using a variance components approach. Analyses were adjusted for age and sex. Plasma adiponectin level was significantly higher in females than in males. The heritability of adiponectin levels was 0.68 0.06 for Mediterranean and 0.67 0.04 for non-Mediterranean. The peak LOD score was 1.8 occurring at chromosome 3q27 (231 cM) in Mediterranean subjects and 2.78 occurring at chromosome 8p (8 cM) in non-Mediterranean subjects. The suggestive linkage at 3q27 replicates a previously reported linkage to 3q27 near the adiponectin structural locus. Though the linkage for adiponectin on chromosome 8 has not been reported so far, many significant linkages of obesity-related traits are found in this region. Our results support the linkage on ch3q27 and provide evidence for a locus on chromosome 8p influencing adiponectin concentrations in non-Mediterranean populations.

msHOT: Simulating crossover and gene conversion hotspots with Hudson's (2002) ms simulator. *G. Hellenthal, M. Stephens* Statistics, University of Washington, Seattle, WA.

Hudson's "ms" simulator (2002) is a widely-used program for simulating genetic variation data for randomly-sampled chromosomes from a population. Such data can be used to test new methodology or explore expected patterns of Linkage Disequilibrium (LD) (i.e. patterns of non-random associations among alleles on a chromosome) under a vast variety of scenarios. The program allows the user to specify various aspects of population demography (e.g. variable population sizes, migration patterns) and factors governing evolution (e.g. mutation, crossover, and gene conversion rates) when generating chromosomes. However, it presently does not allow for variation in recombination rates. In particular, ``hotspots," or areas of the genome in which crossover and/or (allelic) gene conversion occur at higher rates than the genome-wide average, appear to be common in humans. These "hotspots" can significantly alter patterns of LD, which can strongly affect the inference of methods that make use of such patterns (e.g. association studies). To address this, we have incorporated both crossover and gene conversion hotspots into a freely available, updated simulator called "msHOT." The program allows users to easily place in as many crossover and/or gene conversion hotspots (which can be of variable lengths and intensities) as they wish, in addition to the other parameters of the "ms" simulator.

Haplotype association with common and high CGG repeats at the *FMR1* gene in Thai subjects. *P. Limprasert, J. Thanakitgosate, T. Sripo* Pathology, Fac. Medicine, Prince of Songkla University, Hat Yai, Songkhla, Thailand.

Fragile X syndrome (FXS) is the most common inherited cause of mental retardation. Approximately 7% of Thai subjects referred for FXS testing showed CGG repeats expansion (> 200). The three most common CGG repeats in Thais are 29 (47%), 30 (31%) and 36 (10%). We described that there was no founder effect in Thai FXS but we found associated haplotypes in 29 and 30 CGG repeats using two microsatellites and two SNPs, DXS548-FRAXAC1-ATL1-IVS10 [Am J Med Genet 2001; 98: 224-229]. To further evaluation of this finding, we studied four old markers and three new SNPs in 134 normal males and 51 FXS males. We discarded DXS548 from haplotype analysis because it was not informative. The location of the markers related to CGG repeats (Kb) were described as 5-FRAXAC1-(7.2)-WEX5-(1.2)-CGG-(5.6)-ATL1-(13.6)-rs25731-(24.5)-IVS10-(29.1)-rs25702-3. We found 14 haplotypes in controls and 4 haplotypes in FXS group. Of 14, three common haplotypes were 18-G-G-A-T-A (HapA), 19-C-A-T-C-G (HapB) and 18-C-G-T-T-A (HapC), associated with 29 (22/31; 71%), 30 (21/30; 70%) and 36 (16/21; 76%) CGG repeats, respectively. Also, we found common haplotypes in FXS group (HapA = 18, Hap B = 20, HapC = 12, other = 1) with no statistically significant difference between controls. We found 11/14 (79%) chromosomes with HapA or HapC in 37-56 CGG repeats whereas we found only 3 chromosomes with HapB. In addition, we found all 36 CGG repeats with HapA or HapC. HapA and HapC might be evolutional derived since they have only two SNPs difference while HapA and HapB were different in all markers. This suggest that HapA and HapC were associated with 36-56 CGG repeats. However, we could not prove that HapA and HapC were susceptible to high risk for CGG repeat expansion since we did not see this association in the FXS group. Other alternative predisposing factors for repeat expansion need to be further elucidated.

Distinguishing single from multiple association signals within a gene. *K.L. Lunetta, J. Dupuis* Dept of Biostatistics, Boston University School of Public Health, Boston, MA.

When investigating an observed association between variation in a specific gene and a phenotype, for example, in the follow-up of a candidate gene or whole genome association scan, it is common practice to type multiple SNPs within the gene, many of which are in linkage disequilibrium (LD). Typically, we observe several SNPs to be associated with a trait when analyzing the SNPs individually (unconditional analysis). One goal is to determine whether all observed associations are due to a single variant, with LD explaining the rest of the observed associations, or whether evidence exists for multiple effects within the gene. One way to test for additional associated SNPs, assuming one Primary SNP is associated, is to test the secondary SNP in a model which contains the Primary SNP (conditional analysis, e.g., see Cordell and Clayton, 2002). We examine the effect of LD on power to detect primary and secondary associations under two scenarios: 1) the functional SNP or SNPs are genotyped and 2) the functional SNPs are not genotyped, but SNP(s) in LD with the functional SNPs are genotyped. We show that for the first scenario, analysis of a quantitative trait conditional on the primary SNP reduces power to detect the secondary SNP linearly with LD (r^2). For unconditional analysis, when coupled alleles both increase or decrease the quantitative phenotype, the expectation of the test statistic of the secondary SNP is inflated. When coupled alleles have effects of opposite direction, the effect on the expected test statistic of the secondary SNP is nonlinear in r^2; for a range of r^2 the secondary SNP has low power in unconditional analysis, but high power in conditional analysis. Under the second scenario, we show the power to distinguish a single functional SNP from two functional SNPs depends on the underlying LD structure. Our results show that power to detect secondary associations is strongly dependent on LD. Our findings can aid in determining how many and which SNPs should be genotyped in confirmation, in planning sample size requirements for these studies, and in planning studies to have power to detect multiple associated SNPs within a gene.

PseudoFinder: a genome-wide pseudogene finding method. *Y.T. Lu, D. Haussler* Center for Biomolecular Science & Engineering, University of California Santa Cruz, Santa Cruz, CA.

Pseudogenes are genomic debris derived from retrotransposed, incompletely duplicated, or inactivated copies of functional genes. Identifying pseudogenes allows us to better understand the evolution of genes and genomes and improve gene annotation. We developed a computational method, PseudoFinder, to identify pseudogenes in mammalian genomes. PsudoFinder finds homologues of functional genes in a genome and then classifies homologues into either pseudo or functional categories using Support Vector Machines (SVMs), a machine learn approach, based on a combination of features including sequence homology, similarity of gene structure, existence of stop codons and frameshifts, insertion of repetitive elements, and other features. The ten-fold cross validation test result showed that PseudoFinder was able to achieve more than 90% accuracy. Using PseudoFinder, we identified 16527 human pseudogenes and 18324 mouse pseudogenes.This is compatible or better than current pseudogene finding methods. There are 168 predicted pseudogenes in the ENCyclopedia Of DNA Elements (ENCODE) regions, where we have significant additional experimental information for further study.

Genetic analysis of acute necrotizing encephalopathy. *D. Neilson[1,2,3], M. Adams[2], D. Tefft[2], K. Trevarthen[2], D. Kerr[3], M. Warman[1,2,3]* 1) Ctr Human Genetics, Univ Hosps Cleveland; 2) Dept. Genetics, Case Western Reserve University; 3) Dept. Pediatrics, Case Western Reserve University, Cleveland, OH.

Acute necrotizing encephalopathy (ANE) typically affects children under 6 years of age. Following the onset of a febrile illness, such as influenza, affected children become progressively lethargic and progress to develop seizures or coma. Necrotizing lesions in the brainstem and thalamus are detectable by MRI. Although ANE usually occurs sporadically, we previously reported a family in which ANE segregates in an incompletely penetrant, autosomal dominant, manner (ADANE). We also reported the mapping of ADANE to 2q12.1-2q13, which encompasses 7.6 Mb, 36 known genes, and multiple duplicated regions. We assessed all coding exons of the known, non-duplicated genes in the region. This strategy revealed no mutations, although multiple intronic single nucleotide polymorphisms were observed. The nucleoporin gene RANBP2, which is contained within the candidate interval, has had its 5 sequence duplicated eight times across chromosome 2. However the 3coding portion of RANBP2 is unique. We used this unique 3 sequence to amplify RANBP2 cDNA from patient lymphoblast-derived RNA. Sequencing RANBP2 revealed a c.1754C>T transition, resulting in a threonine to methionine substitution (p.T585M), that segregates with the linked disease-associated haplotype. Duplicated portions of RANBP2 in mammalian genomes, coupled with the incomplete sequencing of many genomes, has made it difficult to identify RANBP2 orthologs across species; however, the disease-associated amino acid change does not appear to be present among known orthologs. To determine whether this coding change is a human polymorphism, 200 human control DNAs were sequenced and denaturing HPLC was used to screen additional controls from the CEPH genomic diversity panel. The allele was not observed in 2346 chromosomes. These results indicate that c.1754C>T is not a polymorphism and suggest it may be disease-causing. However, the demonstration of a functional consequence for this mutation or the identification of mutations in other patients or families will be needed to establish RANBP2s role in ADANE, and possibly ANE.

Nonsense-medicated mRNA decay is not a major contributor to downregulate the *Alu*-containing splicing variants. *K. Inoue, K. Takano, Y. Goto* Dept MR & BD Res, Natl Inst Neurosci, NCNP, Kodaira, Tokyo, Japan.

Alu is a primate specific family of short interspersed elements that is most abundantly present in primate genomes, accounting for >10% of the human genome. Genomic integration of *Alu* elements has been suggested to play multiple roles in the primate genome evolution, including the generation of new alternative exons utilizing splice donor/acceptor-like sequences present in *Alu* elements. However, only a small number of genes have been identified to carry *Alu*-containing splicing variants (ASV) despite the facts that *Alu* elements are abundantly present in genic introns, suggesting a presence of mechanisms that prevent exonization of *Alu* elements. Nonsense mediated decay (NMD) is an mRNA surveillance system by which mRNAs carrying premature termination codons (PTCs) are selectively detected and disrupted to prevent production of truncated proteins. Because the vast majority of potent ASV in the EST database carries PTC in its coding sequence, we hypothesized that NMD may play a role in suppressing the expression of ASV. To examine this hypothesis, we measured the expression level of ASV in 9 human genes in which EST database search and RT-PCR using HeLa cells confirmed the presence of ASV. Expression level of ASV was very low compared to wild type alleles. Similarly, expression of ASV appeared to be minor in multiple human tissues. Interestingly, removal of NMD in HeLa cells showed little effect in upregulating ASV in all genes examined, suggesting a limited role of NMD in suppressing ASV expression. To delineate whether this insensitivity to NMD is modulated by low efficiency of alternative splicing of ASV, we employed the ADAR2 gene, in which ASV became a major transcript with no premature termination, thus representing a suitable model to examine the effect of NMD on alternative ASV transcript. We generated a series of minigene expression vectors with PTCs in ASV, which were transiently transfected into HeLa cells. Unexpectedly, PTCs generated in the ASV failed to trigger NMD. These results suggested that NMD is not a major contributor to downregulate the alternatively spliced ASVs, despite carrying PTCs.

Non-Allelic Heterogeneity in Langer Mesomelic Dysplasia. *P.D.R.D. Nicola, A.B.A. Perez* morphology, Universidade Federal de São Paulo, São Paulo, São Paulo, Brazil.

The Langer Mesomelic Dysplasia (LMD; OMIM 249700), is a skeletal dysplasia characterized by severe short stature with hypoplasia/aplasia of the ulna and fibula. The envolved gene is located in the pseudoautosomal region (PAR1) of the X and Y chromosomes. The SHOX (short-stature homeobox-containing) gene is present in the locus Ypter-p11.2 and Xpter-p22.32. The SHOX gene encodes isoforms of a homeodomain transcription factor important in human limb development. SHOX haploinsufficiency has been implicated in three human growth disorders: Turner syndrome, idiopathic short stature, and Leri-Weill dyschondrosteosis. A partial deficiency of the SHOX gene in heterozygous individuals is responsible for Leri-Weill Dyschondrosteosis (LWD; OMIM 127300), which is characterized by disproportionate short stature with predominantly mesomelic limb shortening. The complete deficiency of the SHOX gene in homozygous individuals is responsible for a rarer and more severe form of osseous dysplasia, the LMD. We report a male patient, the only child of an incestuous relationship (father-daughter), presenting normal intelligence, short stature with disproportionate mesomelic of upper and lower limbs, frontal bossing, hypertelorism, low-set ears, long philtrum, micrognathia, normal thorax, scoliosis and bilateral Madelungs deformity of the forearm, a clinical phenotype that suggests LMD. X-ray shows mandibular hypoplasia, normal spine, bilateral short radii, bilateral short and broad ulna, bilateral short femoral neck, bilateral short tibia and bilateral short fibula, which are typical of mesomelic dysplasia. The reported case shows that in this patient the mutated gene cannot be in the pseudoautosomal region, since the degree of kinship between the parents is high and none of them presents other anomalies or Madelungs deformity, which is typical of LWD. Therefore, we can assume that the mutated gene is in an autosomal chromosome, suggesting an non-allelic heterogeneity in LMD. Another hypothesis is that the reported case is a new type of mesomelic dysplasia.

Influence of 2-adrenergic receptor (*ADRB2*) haplotypes on obesity-related traits and interaction with physical activity in adolescent Caucasians. *C.N. Moran[1], M.E.S. Bailey[1], A. Tsiokanos[2], A.Z. Jamurtas[2], Y.P. Pitsiladis[1], R.H. Wilson[1]* 1) Div. of Molec. Genet. and IDEAL, IBLS, Univ. of Glasgow, Glasgow, UK; 2) Dept of Phys. Education and Sport Sci., University of Thessaly, Trikala, Greece.

Genetic variation in human ?adrenergic receptor genes has been reported to be associated with obesity and degree of adiposity. We have carried out an association study of obesity-related phenotypes and the *ADRB2* gene in 1084 teenage Greeks. Three polymorphisms were investigated, p.G16R (c.46G>A), p.E27Q (c.79G>C) and the rarer p.T164I (c.491C>T). Ancestral and derived alleles are known from primate sequences and from existing haplotype data. The receptor encoded by the A-allele at codon 16 (p.16R) is known to desensitise less quickly in the presence of agonist, thus extending signalling half-life and probably affecting downstream lipolytic pathways in adipocytes. Associations (tested by ANOVA) were found only in males, there being a strong association between p.G16R genotype and both BMI (P=0.002), and skinfold thicknesses. These associations were best explained by an A-allele dominant model with homozygotes and heterozygotes for the derived A-allele exhibiting lower values of BMI. Investigating the influence of lifestyle on these associations, we found that the association was strongest in very active males, accounting for up to 9.4% of the phenotypic variance in BMI by ANOVA. Cumulative probability plots showed that the association acts across the full phenotype distribution. The three common haplotypes defined by the p.G16R and p.E27Q polymorphisms showed the same degree of association with BMI as p.G16R alone. p.T164I arose on a haplotype that contained the ancestral p.16G and derived p.27Q variants. When p.16G-27Q haplotypes were split into those with and without the derived p.164I variant (MAF=2%), the proportion of phenotypic variance in BMI explained rose from 3.2% to 5.2%, suggesting that the p.T164I polymorphism influences adiposity phenotypes through interaction effects with other coding or noncoding variants. These findings are commensurate both with a history of selection at this locus and an impact on body composition and response of adipose tissue to exercise in modern populations.

Delineation of breakpoints in chromosomal rearrangements: Continued examination of the mechanism and complexity of rearrangements. *H. Ho[1], C. Astbury[2], L. Christ[3], M. Falk[3], C. Fitzpatrick[1], M. Graf[4], H. Mashek[1], S. Schwartz[1]* 1) Human Genetics, Universitry of Chicaago, Chicago, IL; 2) Southern California Permanente Med Group, Los Angeles CA; 3) Case Western Reserve University, Cleveland, OH; 4) TGEN, Pheonix.

Our initial work showed that several patients with balanced rearrangements had previously undetected deletions with more complexity than originally thought. We have continued these studies to now include 32 patients with initially reported balanced rearrangements, including both de novo and familial cases. Using FISH with both BACs and fosmids, this work has identified over 80 breakpoints in these cases. Our findings show that 58% of the de novo cases had a deletion; however none of the 8 familial balanced rearrangements had a deletion. The vast majority of all cases involved either 2 or 3 breaks; however a subgroup (of 10 cases) involved multiple (5-12) breaks; 80% of these had a deletion. Results from these studies are interesting and reveal important information including that: (1) Patient ascertainment is correlated to the presence of a deletion; the more complex the phenotype and the involvement of multiple systems - the more likely that a deletion will be present; (2) Remarkably, 3 of the 14 cases with deletions had two different deletions; (3) Of the 14 cases with a deletion, many also had another breakpoint within a gene, which could confound the expected phenotype; (4) In the familial cases, gene disruption occurred as a result of the translocation in several cases; in two of these the gene and phenotype segregated together. However, in several cases both a normal parent and affected child had the translocation suggesting that either the gene causes no effect if haploinsufficient, and the phenotype is unrelated to the finding ,or the effect of the gene is more complex and requires careful interpretation; (5) No specific difference were seen between the de novo translocations with deletions, those without a deletion or the familial cases with respect to the involvement of low copy repeats, evolutionary breakpoints, or the recombination rates of the DNA segments involved in the rearrangements.

Elucidation of the Structure of Inverted Duplications: Heterogeneity in the Mechanism of Formation. *A.E. Murmann[1], C.A. Curtis[2], L.A. Christ[2], D.F. Conrad[1], H. Mashek[1], S. Schwartz[1]* 1) Human Genetics, University of Chicago, Chicago, IL; 2) Case Western Reserve University, Cleveland, OH.

The development of molecular technologies in cytogenetics has lead to a better understanding of the mechanism of the formation of a variety of chromosomal abnormalities. Inverted duplications (inv dup), although rare, comprise approximately 13% of all intrachromosomal rearrangements and broadly fall into four categories: inv dup; inv dup with concomitant deletion; accessory acentric inv dup (acentric inv dup); and accessory dicentric inv dup. We have acertained 12 different inv dups (most with a concomitant deletion) and an additional 14 acentric inv dups and have completed precise breakpoint analysis in 9 and 5 of these cases, respectively, using FISH analysis, qRT-PCR, and quantitative SNP-microarray analysis. These analyses were performed to determine if the same mechanism is involved in the formation of one category of inv dups or even shared between different categories. This study demonstrates a number of very intriguing findings regarding the mechanisms of formation on these inv dups: (1) There is no specific mechanism of formation leading to the different types of inv dups; (2) We found heterogeneity of the breakpoints localized to both chromosomes 13 and 15; (3) None of the five acentric inv dups were associated with the presence of low copy repeats (LCR); (4) The formation of one acentric inv dup of chromosome 9 was highly unique in that it did not start at the breakpoint on chromosome 9; but rather involved a deletion of material; (5) Four of the nine inv dups with deletion involved the fusion of two chromosomal segments that were nonsymmetrical leaving a deletion, a stretch of single copy material followed by the duplication; (6) Four of the inv dups were associated with LCR elements; however these were very chromosome specific (one on chromosome 22 and three on chromosome 8); (7) All three chromosome 8p abnormalities involved the same breakpoint; and (8) All 5 analyzed breakpoint regions of acentric inv dups coincided within regions of evolutionary breakpoints, as did half of the analyzed breakpoints of the other inv dups.

The complete set of designs for maximum power candidate gene association studies. *B. Han[1], N. Zaitlen[2], H. Kang[1], E. Eskin[1,2]* 1) Computer Science and Engineering, UC San Diego, La Jolla, CA; 2) Bioinformatics Program, UC San Diego, La Jolla, CA.

Discovering correlation between causal genetic variation and clinical traits through association studies is an important method for identifying causal variation. Ahead of performing a real genetic association study, a researcher should design a study that maximizes the power to detect causal variants while minimizing the cost of the study. The design of a study involves choosing a set of tag SNPs and the number of individuals to be genotyped. The recent release of reference data sets such a the HapMap provide an opportunity to evaluate the effectiveness of designs through simulations. These simulation studies allow us to empirically measure the power of a set of candidate designs. By evaluating candidate designs, we can choose the most efficient design that achieve the desired power. However, designing efficient association studies requires addressing many computational challenges including the exponential number of possible designs in a region as well as the computational costs of performing the simulations to evaluate their power. We introduce a new association study design methodology which allows us to efficiently design association studies. Our methodology allows the approximation of the power of an association study from the correlation structure estimated from a reference data set such as the HapMap data. This allows us to avoid evaluating the power by simulations. Our methodology also presents new efficient algorithms for choosing a set of tag SNPs which maximize power, improving on current approaches which choose tag SNPs based on a r-square criterion. Our method can either maximize average power for all SNPs in a region or maximize the minimum power for each SNP in the region. In our experiment with ENCODE region, our method significantly outperforms r-square based tagging in power, resulting in cost reduction. We have applied our method to the entire set of human genes and the resulting designs are available as a resource for the community.

BDNF haplotypes differentiate schizoaffective and affective disorders from schizophrenia. *T. Lencz[1,2,3], R.H. Lipsky[4], P. DeRosse[1], K.E. Burdick[1,2], J. Jaeger[1,2,3], J.M. Kane[1,2,3], D. Goldman[4], A.K. Malhotra[1,2,3]* 1) Dept. of Psychiatry Research, The Zucker Hillside Hospital, Glen Oaks, NY; 2) Dept. of Psychiatry, Albert Einstein College of Medicine, Bronx, NY; 3) Center for Neuroscience, Feinstein Institute for Medical Research, Manhasset, NY; 4) Laboratory of Neurogenetics, NIAAA, Bethesda, MD.

Background: Recent population genetic studies suggest a partial overlap in genetic transmission of schizoaffective disorder and mood disorders. Allelic variation in the gene encoding Brain Derived Neurotrophic Factor (BDNF) has been associated with affective disorders in several family-based studies, but studies of schizophrenia have been generally negative. It is hypothesized that BDNF allelic variation is associated with the mood component of disorder, and that haplotype frequencies will be similar in patients with schizoaffective disorder and primary mood disorders, as distinct from patients with schizophrenia and healthy volunteers.

Methods: We tested for an association between a 5-marker BDNF haplotype and SCID-based, consensus DSM-IV diagnosis in 220 Caucasian healthy subjects and 373 Caucasian patients diagnosed with schizophrenia (n=208), schizoaffective disorder (n=60), bipolar disorder (n=76), or major depressive disorder (n=29).

Results: There was a significant association between BDNF haplotypes and illness manifestation. The common haplotype (containing the valene variant of the Val66Met polymorphism) was overrepresented in patients with schizoaffective and primary affective disorders compared to healthy volunteers. Moreover, the common haplotype showed significantly greater frequency in schizoaffective patients compared to patients with schizophrenia. Patients with schizophrenia did not significantly differ from healthy volunteers.

Conclusions: This is the first candidate gene study to differentiate schizoaffective disorder from schizophrenia. BDNF genetic variation may be associated with the clinical phenotype of affective dysregulation across several DSM-IV diagnostic categories.

Surveyor nuclease scanning and DNA sequencing reveal a high frequency of von Hippel-Lindau gene mutations in sporadic renal cell carcinoma tumors from Eastern Europe. *M.L. Nickerson[1], E. Jaeger[2], J.A. Durocher[1], K.B. Walters[3], Y. Shi[1], S. Mahurkar[1], M. Smithhisler[1], L.S. Schmidt[4], J.R. Toro[5], B. Zbar[6], W.-H. Chow[5], G.F. Gerard[1], S. Lilleberg[7], F.M. Waldman[2], L.E. Moore[5]* 1) Transgenomic, Gaithersburg, MD; 2) Comprehensive Cancer Center, UCSF, San Francisco, CA; 3) Biological Sciences Program, GWU, Washington, DC; 4) SAIC, NCI-Frederick, Frederick MD; 5) Division of Cancer Epidemiology and Genetics, NCI, Bethesda, MD; 6) Laboratory of Immunobiology, NCI-Frederick, Frederick, MD; 7) Transgenomic, Omaha, NE.

Mutation of the von Hippel-Lindau (VHL) tumor suppressor gene has been implicated in the pathogenesis of clear cell renal cell carcinoma (RCC). A large case-control epidemiology study of kidney cancer is underway in Eastern Europe, which will examine how exposure history relates to the spectrum of VHL mutations. Three methods of mutation detection were initially compared: denaturing high performance liquid chromatography (DHPLC), double-stranded (ds) sequencing, and Surveyor endonuclease scanning. Surveyor nuclease scanning was as sensitive as DHPLC and provided additional detail about the location of a variant/mutation in an amplicon. An approach using both Surveyor nuclease and ds sequencing was then applied to analysis of VHL in 205 tumors from Eastern European patients with sporadic clear cell RCC. Using these two methods, 171/205 tumors (83.4%) were VHL mutation positive. An unexpectedly high proportion of mutations (45%) were difficult to detect by ds sequencing alone. PCR products were re-amplified from 91 tumors using a proofreading polymerase and Surveyor digests showed 100% agreement with the original data set. Analysis of Surveyor digests on a 96 well capillary electrophoresis instrument was amendable to high sample throughput that matched ds sequencing. Significantly, Surveyor scanning dramatically reduced staff time required for manual review of sequencing chromatograms. These two methods are complementary and sensitive, and can be used to meet current demand for large scale genetic analysis of patient tumor samples to detect somatic and inherited mutations relevant to personalized medicine.

Significant linkage of major depression on 15q25-q26 after SNP fine-mapping. *J. Knowles[1], D.F. Levinson[2], O.V. Evgrafov[1], J.B. Potash[3], M.M. Weissman[4], W.A. Scheftner[5], J.R.Jr. DePaulo[3], R.R. Crowe[6], K. Murphy-Eberenz[7], D.H. Marta[5], M.G. McInnis[8], P. Adams[4], M. Gladis[7], E.B. Miller[3], J. Thomas[3], P.A. Holmans[9]* 1) University of Southern California, Los Angeles, CA; 2) Stanford University, Stanford, CA; 3) Johns Hopkins University, Baltimore, MD; 4) Columbia University and NY State Psychiatric Institute, New York, NY; 5) Rush University Medical Center, Chicago, IL; 6) University of Iowa, Iowa City, IW; 7) University of Pennsylvania, Philadelphia, PA; 8) University of Michigan, Ann Arbor, MI; 9) Cardiff University, Cardiff, UK.

The authors studied a dense map of single nucleotide polymorphism (SNP) DNA markers on chromosome 15q25-q26 to maximize the informativeness of genetic linkage analyses in a region where they previously reported suggestive evidence for linkage of recurrent, early-onset major depressive disorder (MDD-RE). In 631 European-ancestry families with multiple MDD-RE cases, 88 SNPs were genotyped and multipoint allele-sharing linkage analyses carried out. Marker-marker linkage disequilibrium (LD) was minimized (r2 0.05), and a simulation study using founder haplotypes from these families suggested that linkage scores were not inflated by LD. The dense SNP map increased the information content of the analysis from around 0.7 to over 0.9. The maximum evidence for linkage was Zlr = 4.69 (Kong-Cox LOD = 4.78) at 109.8 cM (92.6 Mb, NCBI Build 34). The exact p-value (0.0000014) was below the genome-wide significance threshold (0.00002). By contrast, in the genome scan using microsatellite markers at 9 cM spacing, the maximum Zlr score for European-ancestry families was 3.43 (106.53 cM, LOD=2.56). It was estimated that the linked locus or loci in this region might account for a population-wide increase of 20% or less in risk to siblings of cases. This region has produced modestly positive evidence for linkage to depression and related traits in other studies. These results suggest that DNA sequence variations in one or more genes in the 15q25-q26 region can increase susceptibility to major depression, and that efforts are warranted to identify these genes.

A member of the immunoglobulin superfamily, CD96 contributes to cell adhesion and growth. *T. Kaname[1,2], K. Yanagi[1], H. Maehara[3], F. Kanaya[3], Y. Kubota[4], Y. Oike[4], K. Naritomi[1,2]* 1) Deptartment of Medical Genetics, University of the Ryukyus, Nishihara, Japan; 2) SORST, Japan Science and Technology, Kawaguchi, Japan; 3) Deptartment of Orthopedics, University of the Ryukyus, Nishihara, Japan; 4) Deptartment of Cell Differentiation, Keio University School of Medicine, Tokyo, Japan.

CD96 was identified as a human T-cell antigen, which expression was increased during 6th and 9th day after activation by IL-2. It is known that CD96 is expressed in several T-cell lines (e.g.; MOLT-15, PEER), naïve T-cells, and weakly in NK cells. However, its function and expression in other tissues are still unknown. Our aim is to understand the function of CD96 *in vivo* and *in vitro*. Fist, we investigated its expression in adult tissues and in embryos by using RT-PCR and whole mount *in situ* hybridization. In human, *CD96* is strongly expressed in the lung, spleen, and thymus, and moderately expressed in the brain, spinal cord, trachea, digestive tissues, prostate, and testis. In mouse embryo, the *Cd96* gene is expressed in the forebrain, cardiac jerry, endothelial cells, pharynx, and blood cells at 10 dpc. Next, we investigated function of CD96 *in vitro* by characterization of HT1080 cells or K562 cells stably expressed CD96. The HT1080 cells expressing CD96 increased adhesive activity to cell-culture plates and increased cell growth activity. The K562 cells expressing CD96 increased cell aggregation. Thus, we concluded that CD96 would be involved in cell adhesion and growth control.

Obstetrics and Obstetrical Anesthesia Issues in Women with Dwarfism. *J. Hoover-Fong[1], G. Oswald[1], D. Miller[1], J. Leadroot[1], H. Barnes[1], J. Rossiter[1,2], D. Krakow[3], D. Penning[1], I. Berkowitz[1]* 1) Institute of Genetic Medicine, Greenberg Center for Skeletal Dysplasias, Johns Hopkins University, Baltimore, MD; 2) St. Joseph Medical Center, Baltimore, MD; 3) Cedars-Sinai Medical Center, Los Angeles, CA.

OBJECTIVE:There is a paucity of medical literature regarding obstetrics and obstetrical anesthesia in dwarf women (aka Little People, LPs).This population has no recognized infertility predisposition.Thus, dissemination of information about pregnancy and delivery in dwarf women would be extremely useful to LPs and their healthcare providers. METHODS: A 2 part assessment; part 1 is a comprehensive survey of the Society for Obstetric Anesthesia and Perinatology (SOAP) members regarding formal training, clinical practice and opinions about 3 hypothetical case scenarios concerning anesthesia for pregnant dwarfs.Part 2 is a retrospective cohort study of LPs with 1 prior conception, regarding pregnancy and delivery experience, physical status pre- and post-partum, and general opinions about pregnancy in LPs. RESULTS: SOAP members were recruited via membership roster mailing; 149 completed the survey (99 male, 50 female) and 134 are board certified in general anesthesia or a related field.LPs were recruited via a gender- and age-specific mailing to members of the Little People of American,Inc.To date, 37 women with 10 different skeletal dysplasias (76 pregnancies) have completed the survey.There were 13 miscarriages, 1 termination, and 60 live infants delivered via cesarean section (31 general anesthesia-GA, 29 spinal and/or epidural anesthesia).Two live infants were delivered vaginally without anesthesia to a mother with hypochondroplasia. CONCLUSIONS: We do not promote or deter childbearing in LP women.However there is little formal training and no practice guidelines regarding pregnancy and delivery in dwarfs.Many have carried successful pregnancies and delivered with adequate pain control (under regional and GA).We present practical experiences with obstetrics and obstetrical anesthesia from the dwarf and healthcare provider perspective for others who choose to have children and those who provide their medical care.

Informed consent in the era of identifiable publicly released DNA data. *A.L. McGuire*[1], *R.G. Gibbs*[2] 1) Center for Medical Ethics, Baylor College of Medicine, Houston, TX; 2) Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX.

Increasing numbers of investigators are adding DNA banking and analysis to their research protocols, resulting in the rapid proliferation of DNA databases. These databases contain increasingly valuable information that will be used by scientists around the world to study the complex relationship between genetic variation and human health. To maximize the scientific and clinical utility of this information, existing research policy calls for the rapid public release of all generated data on the World Wide Web. Ethical concerns about subject privacy have largely been addressed by de-identifying sequenced data, but we now know that an individual can be uniquely identified from a limited amount of genetic information. Informed consent for the public release of sequenced DNA data is therefore required. Federal agencies have begun to address this problem by requiring informed consent for public data release, but the degree to which potential subjects are invited to participate in decision making about public data sharing and the amount of control that potential subjects are given over the release of their DNA data remain controversial. Draft consent documents for large scale sequencing projects such as the Human Genome Project, the International HapMap Project, and The Cancer Genome Atlas Project all adopt a traditional take-it-or-leave-it approach, where consent to public data sharing is required for participation in the study. This approach can be contrasted with a stratified consent model that gives potential subjects more authority to decide whether, how, and with whom they want their DNA data shared without effecting their participation in the primary research study. Whereas the traditional model promotes international coordination and collaboration, stratified consent is more responsive to individual preferences and judgments about the privacy-utility trade-off. Which approach to adopt will depend partly on the degree of variation in potential subjects' judgments about the privacy-utility trade-off and the impact of each approach on enrollment rates in genetic research.

Association Mapping in Model Organisms under the Effect of Population Structure. *H.M. Kang[1], N.A. Zaitlen[2], C. Kadie[4], C.M. Wade[3], A. W. Kirby[3], M.J. Daly[3], D. Heckerman[4], E. Eskin[1,2]* 1) Computer Science and Engineeing, UC San Diego, La Jolla, CA; 2) Bioinformatics Program, UC San Diego, La Jolla, CA; 3) Broad Institute of M.I.T. and Harvard, Cambridge, MA; 4) Microsoft Research, Redmond, WA.

Genetic association mapping in model organisms has tremendous potential to identify genetic determinants of complex traits related to human disease. A tremendous amount of genotype and phenotype information is available for in-slico association mapping in various model organisms such as inbred mice, Drosophila, or Arabidopsis thaliana. The mouse model is a very important model organism for many human diseases. These association mapping studies are often complicated by the complex population structure between the strains due to historical breeding patterns which can cause a significant amount of false positives. Traditional methods for coping with population structure assume the presence of a handful of populations within the data and can not correct for mutliple levels of complex population structure. We present a statistical test correcting for population structure in association mapping for model organisms by taking a phylogenetic tree as a confounding factor to correct for population structure effects. Our method can be extended to arbitary genetic correlation structure beyond phylogenetic tree, and the model is mathematically equivalent to unified mixed model proposed by Yu et. al. However, our method is more advantageous in the sense that it is computationally more efficient than the typical iterative procedure in mixed model and has better convergence properties, enabling us to perform genome wide association mapping much more efficiently. Our genome wide analysis with inbred mouse strains and Arabidopsis shows that our approach significantly reduces the inflation of false positives while identifying many associations consistent to previously known genes or QTLs.

Constructed eukaryotic expression plasmid of small hairpin RNA of PCNA and investigated the inhibitory effect on HeLa carcinoma cells. *Hao. Huang[1], Xin. Tu[2], Nancai. Yu[1], Wenli. Wu[1], Qian. Liu[1], Wei. Ma[1], Jianjun. Hao[1], Yandong. Yi[1]* 1) Center of Medicine, Wuhan first hospital, Wuhan, Hubei, China; 2) Human Genome Research Center, Huazhong University of Science & Technology.

Objective: To evaluate a new plasmid mediated RNA interference (RNAi) system and nvestigate whether knock-down of PCNA by short hairpin RNA (shRNA) can inhibited proliferation of human on HeLa cell carcinoma (HeLa) line in vitro. Methods: The pGenesil-1 plasmid containing mU6 promoter was digested by Bam HIand HindIII enzyme ,recombinant plasmid expressing shRNA targeting PCNA gene was designed and constructed, and were co-transfected cells with green fluorescence protein expressing plasmid. Flow cytometry was used to analyse the cell cycle and Western blot were applied to analyze PCNA mRNA and protein levels, and MTT test were applied to estimate the inhibition of growth, Single cell gel electrophoresis was used to analyse apoptosis ,respectively. Results: The shRNA expressed by the recombinant plasmid efficiently suppressedPCNA gene expression and induced apoptosis of HeLa cell carcinoma cell in vitro Conclusion: The recombinant plasmid can sufficiently mediate RNAi in HeLaarcinoma cells, and knock-down of the PCNA expression by shRNA significantlyinhibited the proliferation and induced apoptosis in HeLa cells. The results suggest this new system, mediated RNAi can be used as a tool for the study of gene function and gene therapy.

Application of whole genome tiling arrays to formalin-fixed paraffin embedded tissue: Opening up the archives to cancer gene discovery. *E.A. Maher[1], R.R. Selzer[2], D. Castrillon[1], P.S. Eis[2]* 1) University of Texas Southwestern Medical Center, Dallas, TX; 2) NimbleGen Systems, Inc.

Study of the human cancer genome has largely been based on DNA extracted from tumor tissue frozen at the time of operative resection. While even in the most committed academic centers, relatively few tumors are saved in accessible tumor banks, thus limiting the potential value of these samples for cancer gene discovery. Historically, archival tissue has been inaccessible to high resolution genomic technologies, including array comparative genomic hybridization (aCGH), since the quality of DNA extracted from formalin-fixed paraffin embedded tissue (FFPE) has been poor. Fragment lengths are generally 50-150 bp, well below minimum length necessary for gene-specific array CGH. However, recent modifications to DNA extraction methods has enabled extraction of long intact fragments of DNA (>15 kb) from FFPE tumors (Maher et al., submitted) providing an opportunity to profile these specimens. Using DNA from FFPE glioblastomas and other solid tumors (archival time ranged from 1-10 years), we performed whole genome analysis using 25 kb-resolution tiling-path aCGH (NimbleGen Systems, Inc.). Full complexity genomic DNA from FFPE was labeled and analyzed in a 2-color format (Cy3/Cy5). The FFPE data were compared to data generated from frozen tumor DNA. No differences were detected among the profiles when examined for signal-to-noise ratios and a series of quality control parameters and, most importantly, detection capability of both large and small copy number alterations was similar for FFPE and frozen tumor DNA. Indeed, in a small number of glioblastomas, the classical amplifications including EGFR, MDM2, CDK4, PDGFRA and classical deletions including p16, PTEN, and RB were easily detected. In addition, many novel focal loci were identified. This study demonstrates the feasibility of using FFPE DNA for high resolution genomic analysis. Applying this technology to archived cancer specimens that have full clinical annotation and are readily available in most pathology departments provides an unprecedented opportunity for comprehensive investigation of the cancer genome.

Browsing Associations. *W.J. Kent, D. Haussler, UCSC Genome Bioinformatics Group* Biomolecular Engineering, University of California, Santa Cruz, CA.

The UCSC Genome Browser at genome.ucsc.edu is a popular and useful tool for finding genes and other functional areas within a region of a chromosome. We are adding several new features that make it even easier to use the genome browser to analyze the results of SNP association studies and other genome-wide scans. Weve designed a genome-wide view that displays graphs of LOD scores and other numerical values across all chromosomes in a single page. From the genome-wide view you can go to a candidate region view that contains a list of all regions above a scoring threshold on the left, and the classic genome browser on a selected region on the right. There is also a candidate-gene view that lists all known genes in the candidate regions in a table with hilights of what is known about each gene. Clicking on an individual gene takes you to a page with a great deal of additional gene-specific information. The system is designed so that you can upload the scores on pre-publication data in such a way that you can view it just in your own web browser. After publication we encourage you to contribute the scores to our public database, where others working on the same disease processes can find it.

How well do the HapMap SNPs tag functional variants in 217 Drug Metabolizing Enzyme genes? *F.C.L. Hyland[1], K. Lazaruk[1], K.A. Haque[2], R.A. Welch[2], F.M. De La Vega[1]* 1) Applied Biosystems, Foster City, CA; 2) Core Genotyping Facility, Division of Cancer Epidemiology and Genetics, SAIC Frederick, National Cancer Institute, Gaithersburg, MD.

We examined patterns of linkage disequilibrium (LD) among putatively functional polymorphisms in drug-metabolizing enzyme (DME) genes, many of which have previously been shown to alter drug responses in individuals. We have developed and wet-lab validated 2,394 specific and robust assays in 217 DME genes. Functional, especially rare, alleles may be less likely to be in high LD with a set of common tagging SNPs, such as those selected using HapMap data. We thus investigated the degree of pairwise LD among these putative functional DME SNPs, and between these putative functional variants and the HapMap SNPs (release 19). In collaboration with National Cancer Institutes Core Genotyping Facility we genotyped these polymorphisms on the HapMap samples (n=270). Few putative functional SNPs are in high or perfect linkage disequilibrium with each other: among 1702 SNP pairs within 50 kb both polymorphic in CEU, only 10.6% of the pairs have pairwise $r^2 > 0.85$. Low frequency variants (MAF < 0.05) showed less LD with the others. About 40% of the DME putative functional SNPs were found in HapMap. However, of these about 34% failed the internal HapMap quality control metrics, probably reflecting the difficulty in developing robust assays for variants in challenging, highly homologous genes such as these. 23% of DME genes have none of their non-HapMap SNPs tagged by any HapMap SNPs. By determining how many of the non-HapMap DME SNPs are tagged by HapMap SNPs, a predictive model can be generated to assess the power of an association mapping study to capture functional variants in genes. Of the SNPs polymorphic in CEU and not found in HapMap, about 38% were not effectively tagged by any combination of HapMap SNPs ($R^2 < 0.8$). Our results suggest that investigators interested in DME putatively functional SNPs should exercise caution in relying solely on tagging SNPs for disease association, drug efficacy, toxicity, and metabolism studies, and should consider typing directly the relevant variants when feasible.

Increased Specificity for the Detection MSUD by the Addition of a 2nd-tier LC-MS/MS Assay of Alloisoleucine.

D. Oglesbee[1], J.M. Lacey[1], C.D. Spolar[1], K.A. Strauss[2], B. Casetta[3], S. Tortorelli[1], S.H. Hahn[1], P. Rinaldo[1], D. Matern[1] 1) Mayo Clinic, Rochester, MN; 2) Clinic for Special Children, Strasburg, PA; 3) Applied Biosystems, Monza, Italy.

Alloisoleucine (Allo-Ile) is a metabolite of L-isoleucine. Increased Allo-Ile levels are pathognomonic for Maple-Syrup Urine Disease (MSUD), a defect of branched-chain amino acid (BCAA) metabolism. Classic MSUD presents with feeding intolerance, vomiting, lethargy and maple syrup odor in urine and cerumen. If untreated, it can progress to mental retardation, cerebral edema, and death. Newborn screening includes leucine (Leu), isoleucine (Ile), and valine (Val) quantification in dried blood spots (DBS). However, current screening methods are unable to distinguish between elevations of isobaric amino acids, Allo-Ile, Ile, Leu, and hydroxyproline (OHPro). This is problematic as high BCAA are suggestive of MSUD while a OHPro elevation occurs in hydroxyprolinemia, a benign disorder. Increased levels of BCAA are also frequently observed in newborns given total parenteral nutrition, results that can lead to further testing. We developed an LC-MS/MS method for the detection of Allo-Ile in a single dried blood spot (DBS) punch. Allo-Ile and other BCAA are extracted from DBS with methanol/H_2O, dried under nitrogen, and reconstituted into mobile phase. Chromatographic separation of Allo-Ile is achieved within 10 min. Inclusion of isotopically labeled Ile (^{15}N-Ile) internal standard permits Allo-Ile, Leu, and Ile quantification. The assay is calibrated for BCAA concentrations up to 1250 mol/L ($y=3586x+30048$; $R^2=0.9919$). In a blinded cross-center study, 100% of MSUD-positive cases (N=16) were correctly identified from collection of DBS (N=37). Allo-Ile concentrations ranged from 0.00-984.64 mol/L. In 2005, 0.1% of newborns screened at the Mayo Clinic required follow-up due to multiple AA abnormalities including BCAA elevations. Allo-Ile detection in DBS performed as a second-tier assay when elevated BCAA are encountered can aid in reducing false-positive cases requiring unnecessary follow-up. In addition, this method can resolve isolated OHPro elevations, leading to increased specificity of MSUD detection.

Molecular cytogenetic analysis of complex chromosome rearrangements. *E.N. McDonald, G.S. Sekhon, J.B. Ravnan, D.M. Stenberg* Cytogenetics Laboratory, Genzyme Genetics, Santa Fe, NM 87505.

Complex chromosome rearrangements (CCRs) are extremely rare but are usually ascertained in patients with phenotypic abnormalities or in phenotypically normal patients presenting with recurrent miscarriages or infertility. Conventional karyotyping is usually difficult and often does not completely characterize the rearrangement. Molecular cytogenetic methods can reveal subtle abnormalities not evident in the G-banded karyotype. We present two patients with CCRs. A four year old patient was referred due to developmental delay and dysmorphic features. G-banding showed a CCR involving three chromosomes (3, 7, 12) however, the precise nature of the rearrangement was not clear. Fluorescence in situ hybridization (FISH) testing, analyzed in conjunction with the G-band karyotype, revealed a more complex rearrangement with multiple insertions and translocation involving 11 chromosome breakpoints and resulting in a deletion of the long arm of chromosome 3 from 3q12 to 3q21. In a second patient, G-band analysis showed that a 37 year old female with diabetes mellitus, obesity, sleep apnea, coronary artery disease, and mental retardation had a CCR involving chromosomes 3, 7, 10, 11, and 17. FISH analysis showed the presence of a translocation and insertion between one chromosome 3 and one chromosome 10, a reciprocal translocation between one chromosome 7 and one chromosome 17, and an intrachromosomal rearrangement of chromosome 11, consistent with an inversion. We demonstrate the value of painting probes and subtelomere probes as a useful complementary tool to cytogenetic analysis for detecting complex chromosome rearrangements.

A Robust and Sensitive Method For Detection of exonic deletions in the critical gatekeeper genes: VHL, APC, and RB1 By Multiplex Polymerase Chain Reaction (Mpcr) And High-Sensitivity High-Performance Liquid Chromatography (HS-HPLC). *S. Lilleberg, J. Hempel, S. Edstrom, M. Nickerson* Translational & Clinical Res, Transgenomic, Omaha, NE.

Reliable and sensitive screening for large gene rearrangements is a vital part of molecular genetic testing, however it can be technically challenging. A variety of robust methods can detect whole-gene deletions, but fail to detect more subtle rearrangements such as single exon deletions. Here we describe the implementation of a versatile and robust technique to assess exon copy number, which utilizes a multiplex polymerase chain reaction (mPCR) and high sensitivity (HS) post-column fluorescence detection of the amplicons after separation by nondenaturing high performance liquid chromatography (HPLC). The relative peak intensity for each target exon is associated with the exon copy number present in the sample. This novel approach was used to screen various patient samples for exon deletions in the VHL,APC and RB1 genes. The use of multiplex PCR coupled with HS-HPLC allows for analysis of small gene rearrangements in biological samples with limited source DNA (e.g. biopsy samples) on an automated platform without the use of fluorescent probes.

The experience of a newborn screening referral/treatment site with expanded newborn screening. *P. Levy* Dept Pediatrics,Albert Einstein College of Medicine,Children's Hosp Montefiore,Bronx,NY.

NY State had two recent expansions of the newborn screening (NBS) program with the introduction of MS/MS technology. The second expansion occurred in February 2005. Our center's experience for 2005 was reviewed and compared with NY State totals, and recent published data. We had 48 presumptive referrals with an estimated 20,000 births per year in the Bronx. The true positives (4) from these presumptive results were GALT (0 of 2), PKU (1 of 1), HCY/Methionine (0 of 3), SCAD (1of 2), Carnitine uptake (C0) (0 of 8), CPT II/CACT (1 of 1) , CPT I (0 of 16), 3-MCC (1 of 11), and MMA/PA/MCD (0 of 3). NY State had 242,184 births in 2005. 26 of the 37 disorders tested were Inborn Errors of Metabolism. These 26 tests had 3133 presumptive positives (1:77) which resulted in the following confirmed cases (67): 5 of 11 GALT, 4 of 250 PKU, HCY (2 of 290), 7 of 128 SCAD, 5 of 501 Carnitine Uptake, 1 of 50 CPT II/CACT, 0 of 289 CPT I, 20 of 292 3-MCC, and 7 of 234 MMA/PA/MCD. Also included in the 3133 were the additional tests for which our center did not have a presumptive positive: MSUD (1 of 140), 0 of 125 Tyrosine, 2,4 Di (0 of 21), LCHAD/TFP (0 of 5), MADD/MCAD (10 of 280), VLCAD (0 of 13), M/SCHAD (0 of 19), BKT (0 of 13), GA-1 (1 of 40), IBCD (0 of 128), IVA/2-MBCD (1 of 148), HMG/3-MCC (20 of 292), MA (1 of 4), and MMA/PA/MCD (6 of 230) Initial high false positive rates may necessitate changes in cutoff levels. High true positives lead us to re-examine the natural history of a disease. Published data show that a positive screen may not lead to a symptomatic patient. For families, this lack of knowledge about a disease or a false positive result causes undue stress and interfere with the development of attachment to the newborn. For the referral/treatment sites, most states (NY State included) do not reimburse centers for the costs involved in follow-up and confirmatory testing. As the move to expand NBS and equalize NBS across the country continues, efforts must be made to minimize the impact of false positives on families, and provide assistance to referral sites to defray follow-up costs, while everyone benefits from the knowledge gained from expanded NBS.

COE3, a Member of the Collier/Olf1/EBF Family of Transcription Factors, Is Silenced by Promoter Hypermethylation and Induces Apoptosis in Human Malignant Brain Tumors. *W.T. Liu[1], A. Lo[2], P. Lai[1], P.B. Chen[1], M.Y. Lin[1], M. Hsiao[1]* 1) Genomic Research Center, Academia Sinica, Taipei, Taiwan; 2) Graduate Institute of Pathology, College of Medicine, National Taiwan University, Taipei, Taiwan.

Aberrant methylation of promoter CpG islands is known to inhibit transcriptional initiation and cause permanent silencing of tumor suppressor genes, being one of the major mechanisms in tumorigenesis. In this report we investigated the epigenetic and genotypic status of COE3 gene, a member of the COE (Collier/Olf1/EBF) family of transcription factors that was found to be involved in prenatal glial cell development, in human glioma cell lines and clinical samples. Our results showed that the all glioma cell lines (12/12) and a significant percentage of clinical samples especially in stage III and IV gliomas with either reduced or lack of COE3 expression. These results strongly correlated with the methylation status of COE3 promoter region analyzed by methylation-specific PCR and bisulfite sequencing. DNA PCR analysis of COE3 exon showed no deleltions were found in glioma cell lines and clinical samples. Genomic sequencing analysis revealed one mutation in exon 15 of SF126 glioma cell line. Moreover, overexpression of wild-type COE3 gene in non-expressing glioma cell lines suppressed tumor growth via induction of apoptosis by flow cytometry and TUNEL analysis. Taken together, our results indicated that down-regulation of COE3 gene may be associated with promoter hypermethylation in malignant human glioma. Functional expression of COE3 induced apoptosis in glioma cell lines.

Association of *POU2F1* genetic polymorphisms with type 2 diabetes in Hong Kong Chinese. *V.K.L. Lam, J.S.K. Ho, W.Y. So, R.C.W. Ma, J.C.N. Chan, M.C.Y. Ng* Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong, China.

POU2F1 is a ubiquitously expressed transcription factor of the POU family of homeo domain proteins. It is involved in numerous cellular transcriptional regulations including the basal and induced expression of C-reactive protein, in which the latter is a marker of inflammation implicated in the pathogenesis of type 2 diabetes (T2D). This study aimed to investigate for the association of *POU2F1* gene, located at 1q22-23, with T2D in a Chinese population previously showing linkage at chromosome 1q for T2D.

We studied 164 families (645 members) ascertained through a proband with T2D, as well as 410 random T2D patients and 370 healthy controls. Three common SNPs from *POU2F1* (rs12139104, rs10918682 and rs3767436) were genotyped by mass spectrophotometry. Family-based association test by FBAT revealed significant association of rs3767436 with both T2D and obesity ($p < 0.05$). Moreover, rs10918682 was associated with T2D in an independent sample of unrelated cases and controls ($p < 0.01$). Haplotype analyses did not reveal more significance than single SNP analyses. Our results suggest a possible role of *POU2F1* variation on susceptibility to T2D.

Genome-wide Association Studies of Type 2 Diabetes in Mexican Americans. *M.G. Hayes[1], K. Miyake[1], C.L. Hanis[2], G.I. Bell[1], N.J. Cox[1]* 1) Medicine, Univ. Chicago, Chicago, IL; 2) Univ. Texas Health Sciences Center, Houston, TX.

We have undertaken a genome-wide association study using the Affymetrix 100K platform to localize susceptibility genes for type 2 diabetes (T2D) in Mexican Americans from Starr County, TX. Power for our study was increased by choosing the affected sibling with youngest age at diagnosis of T2D from sibships included in previous linkage studies as the case samples (350 for first-stage screen). A random sample of Mexican Americans from Starr County comprises the control sample (350 for first-stage screen). We present results from a data freeze including 287 cases and 316 controls. Using the Affymetrix DM allele-calling algorithm, per marker call rates averaged >93%, with more than 80% of markers having call rates >90%. With the DM algorithm calls, we observed a substantial number of SNPs with departures from Hardy Weinberg equilibrium (HWE) at any threshold of significance, largely attributable to SNPs with excess homozygosity, consistent with non-random missing data (heterozygotes more likely to have missing data). Using an improved allele calling algorithm (GEL) both increased the call rate and reduced non-random missing data. We observed many more significant allelic associations than expected genome wide as empirically assessed by permutation [specifically 3 below a p of $1x10^{-5}$ (1 expected), 38 below a p of $1x10^{-4}$ (15 expected), and 246 below a p of $1x10^{-3}$ (129 expected)]. Our three best signals, those with allelic association $p<1x10^{-5}$, call rates >93%, and HW departure p-values >0.001, occur on chromosomes 4q, 12q, and 20q. We are conducting admixture mapping in these same samples, and will contrast regions of interest across these methods in follow up studies in an additional 700 case and 700 random samples ascertained in the same way from Starr County. Our preliminary analyses of this genome-wide association study suggest it will successfully highlight regions of interest for follow-up in a larger case-control set. Moreover, it may reveal novel genes and pathways to further elucidate the etiology of T2D as well as new avenues for both the treatment and prevention of this complex disease.

LKB1 Tumor Suppressor Gene is Frequently Inactovated in Human Lung Cancers. *T.C. Lai[1], M.S. Huang[2], H.M. Huang[2], M. Hsiao[1]* 1) Genomics Research Center, Acdemia Sinica, Taipei, Taiwan; 2) Department of Internal Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan.

Background: Lung cancer is the second leading cause of cancer-related death in Taiwan in 2003. Discovering the new molecular targets may provide new methods on the treatments of this devastating disease. LKB1, a serine/threonine kinase, is a new tumor suppressor gene with spontaneous mutations and/or deletions found in human cancers. Some reports have recently demonstrated that LKB1 inactivating mutations are appeared in lung adenocarcinomas of sporadic origin, including primary tumors and lung cancer cell lines. Aims: In this study, we investigated LKB1 gene mutations and/or deletions frequencies in 110 Taiwan patients with non-small cell lung carcinomas (NSCLC) and seven lung cancer cell lines. Methods: Mutations and/or deletions were screened by polymerase chain reaction (PCR), sequencing and coamplification. In addition, LKB1 expressions were observed by immunohistochemical analysis (IHC). Results: Five out of 110 (4.5%) patients were found with LKB1 gene exon 8 deletions and 2 out of them were combined with exon 7 deletions. An identical mutation at codon 354 (Phe to Leu) were found in 4 out of 65 (6.2%) patients. Interestingly, a germ-line mutation was found in one patient. Various point mutations were also found in three cell lines. In addition, reduced expressions of LKB1 were observed in cell lines and clinical samples. Our data suggested that LKB1 gene may be involved in the tumorgenesis of NSCLC.

A new test for hardy-weinberg disequilibrium using genotypes. *A. Murphy*[1], *C. Lange*[1,2], *S. Weiss*[2] 1) Dept Biostatistics, Harvard Sch Public Health, Boston, MA; 2) Channing Laboratory, Brigham and Women's Hospital, Boston, MA.

We introduce a new test statistic for Hardy-Weinberg disequilibrium testing that uses only genotype frequencies in affected subjects, which may be applied to both two-sided and one-sided alternative hypotheses. This test statistic does not require specification of the allele frequency of the disease susceptibility allele, which may be over- or under-estimated (relative to the general population) in a completely ascertained sample. We investigate the behavior of the one-sided version of the test statistic under the alternative hypothesis. Additionally, the power of the test statistic is demonstrated via simulation studies, and we show that both the one-sided and two-sided tests generally have improved power in comparison to the standard testing methodology for detecting deviations from Hardy-Weinberg equilibrium in case-only designs. Additionally, we demonstrate the applicability of the new genotype-based test statistic in a candidate gene analysis for asthma.

Utility of acylcarnitine analysis by LC-MS for follow-up of abnormal C5OH on MS/MS screening. *S.E. McCandless[1], M. Stoll[4], P.E. Minkler[4], S. Yang[4], C.L. Hoppel[2,3,4]* 1) Genetics; 2) Pharmacology; 3) Medicine; 4) and Center for Mitochondrial Disease, Case Western Reserve University, Cleveland, OH.

Deficiency of 3-methylcrotonyl-CoA carboxylase (3-MCC) is the most common organic academia (OA) identified by expanded newborn screening programs. The primary marker identified by MS/MS for this disorder is a 5-carbon OH-acylcarnitine (C5OH). Several clinically relevant isomers of C5OH may be seen in OAs, including 3-MCC, beta-ketothiolase (BKT), and 3HMG-CoA lyase deficiencies. Our lab uses HPLC/MS to distinguish isomeric acylcarnitines that can't be resolved by MS/MS, thus allowing diagnosis using a single sample. We use standardized solutions of 3-methylcrotonyl-, tigloyl- and 3-OH-isovaleryl-carnitine to confirm chromatographic characteristics and generate standard curves for each compound to allow precise quantification from biological samples. **Results:** In 308 plasma samples there was a bimodal distribution of 3-OH-isovalerylcarnitine with 98% of samples below the median 0.02 mol/L (99th %ile 1.13). Several adults with known 3-MCC deficiency had concentrations below the median value during times of plasma carnitine deficiency. 3-methylcrotonylcarnitine was present only in small quantities (N=438, mean 0.002 mol/L, median 0, range 0 - 0.13), even in individuals known to have 3-MCC deficiency. Small, but measurable quantities of tigloylcarnitine were found in many plasma (N=938, mean 0.024 mol/L, 99th %ile 0.47) and urine samples (N=571, mean 2.26 mol/g creatinine, 99th %ile 19.98), with the highest concentrations found in a patient with BKT deficiency. Furthermore, like other short chain acylcarnitines, 3-methylcrotonyl-, tigloyl-, and OH-isovaleryl-carnitines are often found in the plasma and urine of patients with propionic and methylmalonic acidemias treated with carnitine, with overlap in the range seen in individuals with 3-MCC deficiency. **Conclusion:** The ability to distinguish 3-OH-isovaleryl- from 3-Me-butyryl-carnitine, and tigloyl- from 3- Me-crotonyl-carnitine by LC-MS allows distinction of several disorders of branched chain OA metabolism. Carnitine deficiency, or treatment with carnitine for other conditions, can make the interpretation more difficult.

Flip-flop associations: confirmation or spurious findings? *P.I. Lin[1, 2], J.M. Vance[2], M.A. Pericak-Vance[2], E.R. Martin[2]* 1) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; 2) Center for Human Genetics, Duke University, Durham, NC.

Multiple studies in several different disorders have reported significant association with the same genetic locus, but with opposite risk alleles. Do such flip-flop associations indicate a confirmation of association or evidence for spurious association? We hypothesized that flip-flop associations may be attributable to multi-locus effects. Theoretical modeling shows that the direction of allelic association may flip when the target risk allele is inversely correlated with another risk allele at another locus, or positively associated with a protective allele at another locus. The likelihood of flip-flop associations depends on allele frequency and inter-locus correlation. This phenomenon is only seen when these two loci act in concert to influence risk of disease. We then considered these findings in review of previous reports. We found the population variation in linkage disequilibrium across the Catechol-O-Methyltransferase (COMT) gene may lead to flip-flop associations for a COMT gene polymorphism with disease risk under a putative disease model. Additionally, we re-examined the association between the glyceraldehyde-3-phosphate dehydrogenase (GAPD) gene and late-onset Alzheimer disease (LOAD). The variation in correlation between a GAPD gene polymorphism and the Apolipoprotein E 4 allele (a confirmed risk factor for LOAD) might result in flip-flop associations of this GAPD gene polymorphism with LOAD in different populations. In summary, opposite effects of the same allele may be found in populations characterized by different patterns of inter-locus correlations. A genuine risk allele may appear to be a relatively protective allele (and vice versa) when a multi-locus effect is not taken into consideration. Caution needs to be exercised when interpreting "flip-flop" associations.

Exploring cryptic genomic aberrations responsible for multiple congenital anomaly with mental retardation using in-house CGH-arrays. *S. Hayashi*[1,2,3], *S. Shozo*[1,2,3], *I. Imoto*[1,2], *J. Inazawa*[1,2] 1) Dept. of Molecular Cytogenetics, Medical Resarch Institute, Tokyo Med. and Dent. Univ, Tokyo, Japan; 2) Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency, Kawaguchi, Japan; 3) Dept. of Pediatrics and Developmental Biology, Graduate Medical School, Tokyo Med. and Dent. Univ, Tokyo, Japan.

We have constructed five different types of CGH-arrays as follows, (1) Whole genome array containing 4523 BAC/PACs throughout human genome (WG-array), (2) Cancer array-800, which harbors 800 different BAC/PACs containing cancer-related genes, (3) 1p36-contig array spanning about 20 Mb at 1p36 with 220 BAC/PACs, (4) Genome disorder array which contains BAC/PACs covering loci responsible for known genomic disorders for their diagnoses, and (5) X-tiling array containing 1003 BAC/PACs throughout X-chromosome other than peudoautosomal regions. Using those arrays we investigated genomic aberrations which would cause unknown multiple congenital anomalies (MCA) and/or mental retardations (MR). A total of 127 cases were analyzed and copy number aberrations (CNA) could be detected in 56 cases. In one case of them, using WG-array we detected 1Mb duplication at Xq22.3 in a girl with MCA and MR. More details were investigated by X-tiling array, FISH and real-time PCR, revealing that the duplication disrupted the IL1RAPL2 gene, which was a candidate gene for MR. This report is probably the first case in which IL1RAPL2 is likely to be involved in MCA with MR. In the other case, 5Mb hemizygous deletion was detected at Xp11.4-11.3 in a girl with severe MR and microcephaly. X-tiling array and FISH confirmed that the deletion disrupted the CASK gene which was associated with neural development in fetal brain. Real-time RT-PCR revealed that the gene expression was reduced in lymphoblastoid cell line from the patient. It may explain her anomaly of central nerves and the severe MR. We also examined parents of each proband in order to assess whether the aberrations were CNP or not. In conclusion, CGH-array was a powerful technique to detect genomic aberrations, which become a good clue to explore cryptic genomic aberrations responsible for unknown MCA with MR.

A conceptual model for developing standardized phenomic measurement scales. *N.J. Markward* Pennington Biomedical Research Center, Baton Rouge, LA.

The Human Phenome Project (HPP) has been conceptualized as an international collaboration of individuals and organizations devoted to the characterization of the phenome--the complete phenotypic representation of the human species. HPP would hypothetically entail the creation of integrated phenomic databases, the standardization of phenotypic definitions and measurement, and the development of novel analytical methods for scaling and interpreting genomic and phenomic data. An additional goal of HPP, specifically, and post-genomic research, generally, might be the construction of a tried and true phenometric system, thereby granting phenomicists the same inferential stability, social connectivity, and cross-contextual predictability afforded to physicists and engineers by the International System of Units (Le Système International d'Unités). Such a framework would be useful for 1) establishing consensus-based guidelines that align the biomedical research community around agreed-upon reference standard metrics for each facet of the phenome; 2) developing genomic surveys of SNP subsets that best map each individuals genomic profile to the error-bounded region of the appropriate phenomic scale; and 3) supporting researchers, hospital networks, and managed care organizations in applying and adapting disease-specific metrics and derived genomic surveys to improve the quality of patient care at the point-of-contact. With these ideas in mind, this research project 1) outlines the theory, mathematics, and applied process that would guide the development of standardized phenomic measurement systems; 2) demonstrates how this analytical framework can be implemented to construct and validate intra-laboratory (specifically objective) phenomic measurement scales from heterogeneous (demographic, anthropometric, clinical, laboratory, psychometric, and genomic) data; and 3) documents the process by which inter-laboratory (generally objective) phenomic reference standards can be developed and maintained by cross-calibrating, linking, and monitoring researcher-, company-, and institute-specific phenomic scales that purport to measure the same or similar health and disease constructs.

In vivo promotor analysis of bioluminescent MC4R transgenic mice. *R. Kesterson[1], C. Lamar[1], W. Wang[2], K. Zinn[3]*
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Obesity is a multifactoral disease currently considered to be of epidemic proportions. Advances in our understanding of energy homeostasis have identified a number of genes linking peripheral signals to CNS neuroendocrine pathways, including melanocortin peptides and their cognate G protein-coupled receptors (e.g. Melanocortin-4 Receptor; MC4R). MC4R is weakly expressed throughout the CNS, including in putative satiety centers of the hypothalamus and brain stem. Genetic ablation in mice has shown that loss of one functional MC4R allele results in obesity. In linkage studies of obese cohorts, loss of function as well as hypomorphic alleles account for approximately 5% of morbidly obese patients. Unfortunately, little is known about how MC4R gene expression is regulated in vivo. We created a series of MC4R promoter-luciferase reporter gene transgenic mice to identify key regions of the promoter involved in proper temporal, spatial, and regulated expression. Bioluminescence imaging (IVIS-100 system, Xenogen) showed that transgenic mice with constructs containing 3300 bp of 5'-flanking sequence (3300MC4Luc) reproducibly displayed CNS preferential expression of the transgene (e.g. hypothalamus and brain stem); 430 bp of 5'-flanking sequence was sufficient to drive hypothalamic expression. In vivo bioluminescence imaging of mice with a heterologous promoter construct containing a highly conserved 32 bp element recapitulated a similar CNS expression profile. We report the first model system to study the regulated expression for the MC4R gene promoter in vivo.

Exclusion of the GOUT1 candidate region on chromosome 4q25 in a large Indian family with an autosomal dominant inherited hyperuricemia and gout. *R. Meda[1], S.K. Nath[2], J.V. Solanki[3], U.C. Patel[3], R. Memon[1], U. Ratnamala[1], U. Radhakrishna[1]* 1) Molecular Genetics Laboratory, Green Cross Blood Bank & Gen, Ahmedabad, India; 2) Arthritis and Immunology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, USA; 3) Department of Animal Genetics & Breeding, Veterinary College, Gujarat Agriculture University, Anand, India.

Gout is an arthritic condition (inflammation of the joints) characterized by the deposition of monosodium urate crystals in the joints or soft tissue. It affects more men than women mostly between 30-50 years of age. It is usually associated with chronic hyperuricemia, a long-lasting abnormally high concentration of uric acid in the blood. The incidence of gout in men and women is 13.6/1000 and 6.4/1000 respectively. Several factors cause hyperuricemia and gout including nutritional, drugs and metabolic/endocrine causes. In addition, genetic factors also contribute to the pathogenesis. The gene responsible for autosomal dominant (GOUT1; MIM 138900) has been mapped to chromosome 4q25 (Am. J. Hum. Genet. 75: 498-503, 2004), but a mutation-causing gene is yet to be identified. We have studied a large five-generation Indian hyperuricemia and gout family with an autosomal dominant mode of inheritance and full penetrance. The pedigree consists of 49 individuals with 16 affected (eight males and eight females) and the age distribution of these affected is from 32-58 years. There are no other associated anomalies present in this family. We have genotyped this family for all polymorphic microsatellite markers that flank the disease loci at chromosome 4q25. These markers were selected since they were used for the original linkage analysis for Gout1 in native Taiwanese families. Analyzing 27 individuals, all markers yielded significant negative (<-2.0) at = 0. Thus GOUT1 can be excluded as the candidate region responsible for hyperuricemia and gout in this family. We are planning to perform genome-wide linkage analysis in this family to identify the responsible locus. Email: u_c_rao@hotmail.com.

Examining the Multiple Dye-Swap Design for Efficient and Effective Microarray Studies. *T.K. Kim* Biostatistics, University of Washington-Seattle, Seattle, WA.

For 2-group comparison microarray experiments with biological replicates, Kerr (Biometrics, 2003) showed that a multiple dye-swap strategy is a very efficient, practical design. For more than two groups, the design extends naturally, but there is more than one generalization when there are four or more groups. We explore different options for the extension of the multiple dye-swap design for 3, 4, 5, and more groups and evaluate these options in terms of their efficiency for making pairwise group comparisons.

Detection and characterization of supernumerary marker chromosomes by array CGH. *S.A. Horner[1], S.G. Sulpizio[2], R.M. Lloyd[2], B.A. Bejjani[1,2], L.G. Shaffer[1,2], B.C. Ballif[2]* 1) Health Research and Education Center, Washington State University, Spokane, WA; 2) Signature Genomic Laboratories, LLC, Spokane, WA.

Small supernumerary marker chromosomes are found in ~0.04% of live births. However, the frequency of marker chromosomes is nearly ten times as high (0.41%) in individuals with mental retardation (Liehr et al., 2004). By definition, the characterization of small marker chromosomes can not be done unambiguously by conventional chromosome banding techniques. Furthermore, marker chromosomes are often found in only a small percentage of the cells which can make them difficult to detect without screening large numbers of cells. Array-based comparative genomic hybridization (array-CGH) is a powerful platform which offers high resolution analysis using genomic DNA extracted from uncultured peripheral blood. We have constructed a high-density microarray using 974 FISH-mapped BAC clones covering ~5 Mb of the most proximal unique sequence adjacent to the centromere on all 43 unique pericentromeric regions of the human genome (excluding the acrocentric short arms). This array was used to characterize the chromosomal content of 11 marker chromosomes found in routine diagnostic specimens. The enhanced coverage of this array over the pericentromeric regions not only uncovered the chromosomal origin of each marker but also distinguished between the involvement of the short arm and/or the long arm of each chromosome, and uncovered complex rearrangements or multiple markers in a single individual. Furthermore, the expanded coverage of the pericentromeric regions was adequate to refine the breakpoints in over half (56%) of the markers. However, 44% of the markers contained 5 Mb of unique sequence suggesting that additional coverage in the pericentromeric regions may be even more valuable in characterizing these rearrangements. Finally, most of these marker chromosomes were mosaic suggesting that array CGH is a powerful tool for the detection and characterization of low-level mosaicism in a clinical diagnostic setting.

Osteopoikilosis, short stature and mental retardation as key features of a new microdeletion syndrome on 12q14.

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We have identified a microdeletion of chromosomal band 12q14 in two unrelated patients with mild mental retardation, osteopoikilosis and short stature. Using array-CGH the size and boundaries of both deletions were determined. Both microdeletions are ~6 Mb in size with a 5.3 Mb common deleted region. This region encompasses 28 known genes, including LEMD3 which was previously shown to be the causal gene for osteopoikilosis. The observation of two unrelated patients with a similar microdeletion and sharing a common phenotype, suggests that this might represent a new chromosomal microdeletion syndrome. Reporting additional patients with deletions in this chromosomal region will allow more accurate genotype-phenotype studies. In particular, the identification of a new autosomal gene for short stature within this region could provide us with more insights in the pathways controlling linear growth.

Study of CFTR gene mutations in Iranian CF patients. *F. Mirzajani[1], R. Mirfakhraie[1], M. Amiri[2], M. Jalalirad[1], H. Kianifar[3], M. Rafiei[4], E. Talachian[5], M. Houshmand[1]* 1) Medical Genetics, NIGEB, Tehran,Iran; 2) Khatam university, Tehran, Iran; 3) Mashhad Medical University, Mashhad, Iran; 4) Tabriz Medical University, Tabriz, Iran; 5) Iran Medical University, Tehran, Iran.

Numerous mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene have been found to impair CFTR activity to different extent, causing CF. This disorder exhibits considerable allelic heterogeneity in different populations and ethnic groups. Due to very high heterogenousity of Iranian population identification of mutations specific to the Iranian population would be helpful in designing an appropriate CF molecular diagnosis. 85 blood samples from unrelated CF families were collected from different provinces of Iran. DNA samples were screened for the most common mutations including delF508, G542X, W1282X, and N1303K using ARMS-PCR. Exons 4, 7, 9, 10, 11, 13, 20, and 21 were screened using SSCP-Sequencing. DelF508 mutation covered only 18%; of the mutated alleles, followed by W1282X(11%), G542X(7%) and N1303K(2.5%) respectively. SSCP-Sequencing also revealed the occurrence of some rare mutations including R117H, R347H, A120T, S549R, 1677delTA and 2183AAG. Moreover M470V mutation was found in high number of Iranian CF patients.

Midkine as a prognostic marker and therapeutic target in osteosarcoma. *H. Maehara[1], T. Kaname[2,3], K. Yanagi[2], I. Ohwan[1], K. Naritomi[2,3], F. Kanaya[1]* 1) Department of Orthopedics, University of the Ryukyus, Nishihara, Okinawa, Japan; 2) Department of Medical Genetics, University of the Ryukyus, Nishihara, Okinawa, Japan; 3) Solution Oriented Research for Science and Technology (SORST), Japan Science and Technology, Kawaguchi, Japan.

A heparin-binding growth factor, midkine and its truncated form are involved in generation and progression of various tumors. It is, however, the relationship between midkine and osteosarcoma is unclear yet. We show midkine is overexpressed in osteosarcoma patients and its intensity correlated with their prognosis ($p<0.05$). No truncated form of midkine was observed in any osteosarcoma cells, which may suggest that there is no association between intronic mutation of midkine gene and tumorigenesis of osteosarcoma. Treatment with functional antibodies against midkine has inhibited cell growth between approximately 20% and 60% in osteosarcoma cell lines, 9N2, 3N1, Saos2 and Nos1 in vitro. Reducing of midkine expression in those cells by RNAi gave the same results. Our findings suggest that midkine will be used as a prognostic marker and will be one of the strong candidate for therapeutic target for osetosarcomas.

Molecular diagnostics for Autosomal Recessive Polycystic Kidney Disease (ARPKD). *M. Losekoot, C.R. Haarloo, S.J. White, M.H. Breuning, D.J.M. Peters* Center for Human & Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands.

ARPKD is a severe form of polycystic kidney disease characterized by enlarged kidneys and congenital hepatic fibrosis. It is caused by mutations in the Polycystic Kidney and Hepatic Disease 1 (PKHD1)-gene, which consists of 86 exons that are variably assembled into a number of alternatively spliced transcripts. Mutation analysis was performed by direct sequencing of the 67 exons encompassing the largest open reading frame. So far, in 55 families the following mutations were found: 12 nonsense, 20 deletions/insertions, 8 splice site mutations, and 47 missense mutations. To classify missense variants we combined evolutionary conservation, using the human, chimpanzee, dog, mouse, chicken and frog Pkhd1 sequences, with the Grantham score for chemical differences. Thirty-nine missense mutations were considered pathogenic and 8 were classified as rare, probably or possibly pathogenic variants. In 39 patients two mutations were found while in 6 patients only one mutation was found, leading to a mutation detection ratio of 76%. The analysis of amino acid conservation as well as applying the Grantham score for chemical differences allowed us to determine the pathogeneity for almost all new missense variants and thus proved to be useful tools to classify missense variants. In addition to sequence analysis, MLPA was used to identify multiple exon deletions. We selected 9 probes equally spread over the gene but did not find deletions, indicating that large deletions in the PKHD1 gene are not a frequent cause of ARPKD.

Identification of the gene responsible for defect in hair development of the hairpoor mouse derived from ENU mutagenesis. *J. Kim[1], E. Kim[2], D. Cha[2], C. Song[2], J. Yoon[3], S. Kim[1]* 1) Biomedical Sciences, Catholic University of Korea, Seoul, Korea; 2) Korea Institute of Toxicology, Yusong-gu, Daejon 305-343, Korea; 3) Department of Biochemistry and Protein Network Research Center, Yonsei University, Seoul 120-749, Korea.

The hairpoor mouse strain (hrutr) was established from a pheno-deviant which was generated by ENU mutagenesis. The single locus determined the phenotype of total hair loss in the homozygotes, while the heterozygotes showed intermediate phenotype such as less than about 30% hair compared to that of the normal littermates. Thus, the hrutr phenotype was inherited as single locus in a semidominant mode. To identify the gene responsible for the hairpoor phenotype, the positional candidate gene approach was applied. The genetic linkage analysis was performed using F2 mice produced from the mating between the offspring of the BALB/c mutant and the C57BL/6 normal mice. We examined the genotype of 50 mice, 25 homozygote and 25 littermate wild type mice by PCR-SSLP using the STRs. The hrutr locus was found to be linked to mouse chromosome 14 and the order of the markers was D14Mit142-D14Mit203-D14Mit34-hrutr-D14Mit228-D14Mit107. D14Mit34 (40.0 cM) showed the complete linkage to the hrutr locus. The mutation analysis by PCR followed by sequencing of the strongest candidate gene in the region, the hr gene, revealed the single nucleotide change, T403A, at exon 1. Currently, the analysis is under way to delineate its effects on hr gene at the molecular level.

Analysis of Inquiries to a Federal Public Education and Health Care Professional Resource on Genetic and Rare Diseases. *J. Lewis[1], D. Lea[2], H. Hyatt-Knorr[3]* 1) Dept Health, LockheedMartinAspenSystemsCorp, Rockville, MD; 2) National Human Genome Research Institute (NHGRI), Bethesda, MD; 3) Office of Rare Diseases (ORD), Bethesda, MD.

For more than 4 years, the Genetic and Rare Diseases Information Center (GARD) has provided information and reliable resources on genetic and rare diseases. A rare disease is considered to have a prevalence of fewer than 200,000 affected individuals in the United States. Together, the more than 6,000 rare diseases affect approximately 25 million Americans. Information about many of these conditions can take hours to locate and may be hard to understand; this difficulty can, in turn, add to the stress of a genetic or rare disease diagnosis for a patient, a patients family, and the health care provider.

To help ease the frustration of this process, the National Human Genome Research Institute (NHGRI) and the Office of Rare Diseases (ORD), National Institutes of Health, launched GARD to provide free and timely access to accurate, reliable information about genetic and rare diseases in English and Spanish.

Data have been collected for the 14,500+ inquiries on more than 3,500 diseases handled by GARD to date. Of the people who provided information about themselves (86%), inquiries came from patients (28%), their families (42%), health professionals (10%), and the general public (20%). Approximately 92%; of GARD users request information about a specific disease. The diseases for which GARD has received the most information requests are porphyria and trimethylaminuria.

Being aware of the types of information people are seeking when they contact GARD will help genetics professionals stay abreast of their clients information needs. Other stakeholders in genetics (researchers, allied health professionals, and others) might also find it helpful to know how they can use GARD to assist them in connecting people to information and resources about genetic and rare diseases.

Oral-facial-digital syndrome type I. Case report. *D.M. Mendoza-Ugalde[1], G. García-Sánchez[1], C.F. Martínez-Cruz[2,3], A. García-Huerta[1], M.R. Baez-Reyes[4], M. Díaz-García[1]* 1) Instituto Nacional de Rehabilitación.Genética; 2) Instituto Nacional de Perinatología.Departamento de Seguimiento Pediatrico. Comunicación Humana; 3) Instituto Mexicano del Seguro Social.Hospital General de Zona 53.Pediatría; 4) Instituto Nacional de Perinatología. Genética. E mail:guillegs@yahoo.com.mx.

Oral-facial-digital syndrome type 1 (OFD1) is characterised by an X linked dominant mode of inheritance with lethality in males. The clinical features include facial dysmorphism with oral, tooth, digits and distal abnormalities, sometimes associated with polycystic kidney disease and central nervous system malformations. The gene responsible for this syndrome is OFD1 in the Xp22 region and sometimes its diagnosis can be difficult because there is an overlap with other types of oral-facial-digital syndromes. We report a 5 years old female patient with clinical characteristics compatible with an OFD1 syndrome which presented cleft lip (between the medial third and the internal third), cleft palate, dental caries, hypoplastic nostril, ears with bilateral low implantation, hypoplastic helix and antihelix, redundant skin on elbows and appendix in right elbow, hypotrophy of shoulders and arms, in right hand with syndactylya between the third and fourth fingers and absence of nail in the second finger, right hand with agenesis of distal phalange of the third finger, right foot with syndactylya between the third and fourth toes and absence of nail in the fourth toes, systolic blowing in aortic focus. Audiogram showed a mild conductive hearing loss due to otitis media. Analysis of the familial pedigree shows this case like a sporadic type.

Jervell and Lange-Nielsen syndrome. *C.F. Martínez-Cruz[1,2], M. Marquez-Murillo[3], G. Garcia-Sanchez[4], D.M. Mendoza-Ugalde[1]* 1) Instituto Nacional de Perinatología. Departamento de Seguimiento Pediatrico. Comunicacion Humana. Mexico, D.F; 2) Instituto Mexicano del Seguro Social.HGZ 53. Pediatria. Mexico, D.F; 3) Instituto Nacional de Cardiologia. Electrocardiografia. Mexico, D.F; 4) Instituto Nacional de Rehabilitacion. Genetica. Mexico, D.F.E. mail: guillegs@yahoo.com.mx.

The Jervell and Lange-Nielsen syndrome (J-LN), the long-QT syndrome (LQTS) variant associated with deafness and caused by homozygous or compound heterozygous mutations on the KCNQ1 or on the KCNE1 genes encoding the I(Ks) The patients can present episodes of syncope and undergo sudden death. Prevalence 1:200 000 individuals. The intention of this work is to present a Mexican family with JLNS of which two affected brothers (male and female) of 3 and 4 years of age respectively, consanguineous matting children of prime brothers; they present congenital deafness. The physical exam was negative. The pedigree showed two relatives with deafmutism who presented sudden death after emotional problem; by the previous things we suspected JLNS. EKG type Holtter was made demonstrating long QTc (>499 mseg) in both brothers. Conclusion: In patients wit clinical diagnosis the study of molecular genetics must be made and in positive cases early therapy with beta-blockers and/or implanted cardioverter/defibrillators must be considered.

Cell Cycle in Adult Mesenchymal Stem Cells. *R. Izadpanah[1], F. Tsien[3], C. Kriedt[1], B. Bunnell[1,2]* 1) Division of Gene Therapy, Tulane National Primate Center, Tulane University Health Sciences Center, Covington, LA; 2) Center of Gene Therapy, Tulane University Health Sciences Center, Tulane University, New Orleans, LA; 3) Department of Genetics, LSU Health Sciences Center, New Orleans, LA.

Gene Therapy holds great promise for treating many human degenerative diseases such as lysosomal disorders and neural degeneration. Stem cells are undifferentiated cells with the ability to proliferate, duplicate, produce a large number of differentiated progeny, and regenerate tissue after injury. The therapeutic efficacy of stem cells largely relies on their ability to replicate. Therefore, strategies to manipulate stem cells require an understanding of their cell cycle control. The result of this study demonstrated that the cell cycle kinetics of adult stem cells is similar to the general cell cycle regulation in mammalian cells. Cell cycle in mesenchymal stem cells (MSCs) isolated from two distinct tissues, bone marrow and adipose tissue were evaluated in these studies. MSCs derived from human and nonhuman primate tissue sources were compared. MSC cultures were established up to 30 passages for human adipose tissue and monkey bone marrow MSCs. Human bone marrow and monkey adipose tissue cultured up to 20 passages. Comparison the human and Rhesus karyotypes showed many similarities in G-banding pattern were observed, with most of the chromosomal differences between monkeys and humans apparently resulting from inversions, duplications, and translocations. Human bone marrow and adipose tissue derived MSCs maintained the diploid karyotype (46 chromosomes) up to passage 20 and 30 respectively. Rhesus chromosomes transformed from the normal diploid chromosome number of 42 at passage 1 to tetraploid (84 chromosomes) with increased cell passage, beginning at passage 20. Cell cycle analysis of MSCs by flowcytometry revealed a large population of MSCs in S phase in human MSCs at passage 20 and higher, while there was a significant aneuploidy in rhesus MSCs at passages 20 and higher. These results indicate that the long-term culture of MSCs results significant changes in cell cycle kinetics. This suggests that it is imperative to track the cell cycle kinetics in MSCs prior to their clinical application.

IL-6 promoter polymorphisms and quantitative traits related to the metabolic syndrome in KORA S4. *T. Illig[1], H. Grallert[1], C. Huth[1], M. Kolz[1], C. Meisinger[1], C. Herder[2], K. Strassburger[3], G. Giani[3], H.-E. Wichmann[1], J. Adamski[1], W. Rathmann[3]* 1) Institute of Epidemiology, GSF- Research Center for Environment and Health, Munich, Germany; 2) German Diabetes Clinic, German Diabetes Center, Leibniz Institute at the Heinrich-Heine-University, Düsseldorf, Germany;; 3) Institute of Biometrics and Epidemiology, German Diabetes Center, Leibniz Institute at the Heinrich-Heine-University, Düsseldorf, Germany,.

Interleukin-6 (IL-6) is a pleiotropic cytokine which is linked to age related metabolic disturbances such as the metabolic syndrome or type 2 diabetes. Polymorphisms located in the promoter region of IL-6 have been reported to be involved in the regulation of IL-6 transcription. This study investigates whether IL-6 promoter variants -174 G/C and -573 G/C are associated with quantitative traits related to the metabolic syndrome (International Diabetes Federation criteria) in a population of normoglycemic subjects (n=878) from the latest KORA survey (KORA S4). Genotyping was performed using MALDI-TOF MS. Besides lower height (p=0.01) the -174 CC genotype was independently associated with lower waist (p=0.002) and hip (p=0.01) circumferences in men. Furthermore, the -174 CC genotype was associated with BMI (p=0.004) when adjusted for waist and hip circumference. The present study does not suggest associations with further components of the metabolic syndrome. The association with height seems to be the central factor indicating an influence of IL-6 on growth through impaired bone metabolism. However, the complex relationships need further investigation.

Different evolutionary fates of recently integrated human and chimpanzee LINE-1 retrotransposons. *K. Han[1], J. Lee[1], R. Cordaux[1], J. Wang[2], D.J. Hedges[1], P. Liang[2], M.A. Batzer[1]* 1) Department of Biological Sciences, Biological Computation and Visualization Center, Center for BioModular Multi-Scale Systems, Louisiana State University, 202 Life Sciences Building, Baton Rouge, LA 70803, USA; 2) Department of Cancer Genetics, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263, USA.

The long interspersed element-1 (LINE-1 or L1) is a highly successful retrotransposon in mammals. L1 elements have continued to actively propagate subsequent to the human-chimpanzee divergence, ~6 million years ago, resulting in species-specific inserts. Here, we report a detailed characterization of chimpanzee-specific L1 subfamily diversity and a comparison with their human-specific counterparts. Our results indicate that L1 elements have experienced different evolutionary fates in humans and chimpanzees within the past ~6 million years. Although the species-specific L1 copy numbers are on the same order in both species (1,200-2,000 copies), the number of retrotransposition-competent elements appears to be much higher in the human genome than in the chimpanzee genome. Also, while human L1 subfamilies belong to the same lineage, we identified two lineages of recently integrated L1 subfamilies in the chimpanzee genome. The two lineages seem to have coexisted for several million years, but only one shows evidence of expansion within the past three million years. These differential evolutionary paths may be the result of random variation, or the product of competition between L1 subfamily lineages. Our results suggest that the coexistence of several L1 subfamily lineages within a species may be resolved in a very short evolutionary period of time, perhaps in just a few million years. Therefore, the chimpanzee genome constitutes an excellent model in which to analyze the evolutionary dynamics of L1 retrotransposons.

Genetic contribution and characteristics of founders of Irish origin in the population of Quebec (Canada). *M. Letendre, L. Houde, H. Vézina, M. Tremblay* University of Quebec at Chicoutimi, Chicoutimi, Quebec, Canada.

This study is part of a research project on the formation and the structuration of the Quebec (Canada) population. European settlement in Quebec began in the early 17th century, with the arrival of the first French pioneers. After the British Conquest in 1760, immigrants from the British Isles began to settle in some parts of the Quebec territory. Several of these migrants, or their descendants, have intermixed with the French catholic population of Quebec. Three mutations identified in the Quebec population, R408W[H1] and I65T for phenylketonuria & W138X for cystinosis, are believed to have been introduced by Irish immigrants. The purpose of this study is to identify and characterize the immigrant founders of Irish origin in the Quebec population and to estimate and compare their genetic contribution to Quebecs total and regional gene pools. Genealogical data was retrieved from the BALSAC-RETRO database, to form a set of 2223 ascending genealogies covering the entire Quebec territory. These genealogies go back to the early 17th century and contain information on places and dates of marriage of the 155000 ancestors that were identified. On average, the genealogical depth is over 9 generations, with many branches reaching 16 or 17 generations. Characterization of immigrant founders and measurement of their genetic contribution has been performed using various procedures developed by the GRIG and the BALSAC research teams. Results indicate that nearly 21% of the 2223 subjects have at least one ancestor of Irish origin and the genetic contribution of the 203 Irish founders identified in the genealogies constitute 1% of the samples total gene pool. A majority of these Irish founders settled in Quebec in the 19th century. Children of founders from the 1815-1860 period tended to marry more frequently within the Irish group. Accordingly, subjects with Irish ancestors have a higher kinship coefficient than the rest of the subjects. Results also show that Irish founders who settled in Quebec during the French Regime (1608-1760) have a proportionally higher genetic contribution than founders who immigrated after this period.

Identification of a SNP haplotype of the PON1 gene associated with acute rejection in kidney allografts using a custom SNP chip. *W.S. Oetting[1], S. Basu[2], M.J. Brott[1], A.J. Matas[3]* 1) Dept. Medicine, Univ. Minnesota, Minneapolis, MN; 2) Dept. Biostatistics; 3) Dept. Surgery.

Studies attempting to associate specific DNA variants to transplant outcome have been restricted to a select set of candidate DNA polymorphisms. We have designed a custom chip that allows for the analysis of 3,590 single nucleotide polymorphisms (SNPs), many of which are thought to be functional variants within biologically relevant genes. We have also included haptag SNPs in candidate genes with no obvious functional variant. We have used this chip to analyze individuals with and without acute rejection (AR) to identify predisposing patterns of SNPs. **Methods:** Blood was obtained with informed consent and DNA isolated from 91 kidney allograft recipients, 46 of whom had AR within 6 months of transplant, and 45 of whom did not have any detectable AR after at least 8 years post-transplant (NoAR). All received Ab induction and CNI with either MMF or sirolimus. SNPs were genotyped using a chip based system (Affymetrix) for SNP detection and genotyping. SNPs were selected within genes associated with multiple metabolic pathways including immunity, cell signaling, cell cycle, ADME related genes and cell growth and proliferation. **Results:** Of the 10 most significant variants, 4 were within the paroxonase gene (PON1) including a nonsynonymous SNP (rs 662; p.192R/Q; c.575A/G). A total of 15 SNPs within the PON1 gene were analyzed and 4 yielded a P value less than 0.008. Logistic regression analysis associated one haplotype with AR ($p = 0.0277$) and a second haplotype with NoAR ($p = 0.0369$), after multiple comparison adjustment. This haplotype was constructed using the four SNPs that showed significant association with AR. **Conclusion:** We have created a chip that allows us to analyze multiple SNPs to help us identify patterns of DNA variants, at a substantially less cost than analyzing each SNP individually. Our analysis has identified a haplotype within the PON1 gene as being associated with AR. This haplotype contains a potentially functional SNP and may be the causative variant. If this association is verified in a larger sample it could be used to individualize immunosuppression.

***NALP1*, a key regulator of the innate immune system, is a novel major gene for multiple autoimmune/autoinflammatory disease.** *Y. Jin[1], C. Mailloux[1], D. Bennett[2], C. Dinarello[3], P. Fain[1,3], R. Spritz[1]* 1) Hum Med Genet Prog, Univ Colorado Hlth Sci Ctr, Aurora, CO; 2) Div Basic Med Sci, St. George's, Univ London, UK; 3) Dept Med, Univ Colorado Hlth Sci Ctr, Aurora, CO.

Autoimmune/autoinflammatory diseases involve multiple genetic risk factors and environmental triggers. We previously defined epidemiological association among a specific group of autoimmune/autoinflammatory diseases (generalized vitiligo, autoimmune thyroid disease, adult-onset autoimmune diabetes mellitus, rheumatoid arthritis, psoriasis, pernicious anemia, systemic lupus erythematosus, Addisons disease), and by genetic linkage mapped a susceptibility locus for vitiligo and associated autoimmune/autoinflammatory disease to chromosome 17p, co-localizing with a lupus/vitiligo susceptibility locus, *SLEV1*. By high-density SNP genotyping across the linkage region and pedigree-based association analysis in 114 multiplex families, we defined a preliminary high-risk SNP haplotype that spans the proximal coding region and extended promoter of *NALP1*. By DNA resequencing across 76 kb in 15 individuals heterozygous or homozygous for the high-risk haplotype, we identified a large number of additional SNPs, and by pedigree-based association analysis defined a more specific high-risk haplotype that includes a *NALP1* missense substitution. Highest risk, both for vitiligo alone and for a broad spectrum of autoimmune/autoinflammatory diseases, is associated with homozygosity for the high-risk haplotype, consistent with segregation analyses indicating that vitiligo results from homozygosity at multiple recessive loci. *NALP1* encodes a key regulator of the innate immune system, playing a central role in initiation of both inflammation and cellular apoptosis. Functional analyses of peripheral blood monocytes from patients with the high-risk *NALP1* haplotype show a defect of apoptosis compared to controls lacking this haplotype. Our findings indicate that *NALP1* is a broad-spectrum autoimmune/autoinflammatory disease susceptibility gene. NALP1 and the innate immune system may provide targets for both genetic susceptibility testing and treatments to reduce risk for this group of autoimmune/autoinflammatory diseases.

Identification and characterization of novel polymorphic LINE-1 insertions through comparison of two human genome sequence assemblies. *M.K. Konkel[1], J. Wang[2], P. Liang[2], M.A. Batzer[1]* 1) Dept. of Biological Sciences, Biological Compution and Vizualization Center, Center for BioModular Multi-Scale Systems, Louisiana State University, Baton Rouge, LA; 2) Department of Cancer Genetics, Roswell Park Cancer Institute, Buffalo, NY.

Mobile elements represent a relatively new class of markers for the study of human evolution. Long interspersed elements (LINEs) belong to a group of retrotransposons comprising approximately 21% of the human genome. Young LINE-1 (L1) elements that have integrated recently into the human genome can be polymorphic for insertion presence/absence in different human populations at particular chromosomal locations. To identify putative novel L1 insertion polymorphisms, we computationally compared two draft assemblies of the whole human genome (Public and Celera Human Genome assemblies). We identified a total of 148 potential polymorphic L1 insertion loci, among which 73 were candidates for novel polymorphic loci. Based on additional analyses we selected 34 loci for further experimental studies. PCR-based assays and DNA sequence analysis were performed for 34 loci in 80 unrelated individuals from four diverse human populations: African-American, Asian, Caucasian, and South American. All but two of the selected loci were confirmed as polymorphic in our human population panel. Approximately 47% of the analyzed loci integrated into other repetitive elements, most commonly older L1s. One of the insertions was accompanied by a BC200 sequence. Collectively, these mobile elements represent a valuable source of genomic polymorphism for the study of human populations. Our results also suggest that the exhaustive identification of L1 insertion polymorphisms is far from complete, and new whole genome sequences are valuable sources for finding novel retrotransposon insertion polymorphisms.

Mucopolysaccharidosis IVA: Characterization of novel mutations causing attenuated phenotype. *A.M. Montaño[1,2], K. Sukegawa[2], Z. Kato[2], R. Carrozzo[3], P. Di Natale[4], E. Christensen[5], T. Orii[2], N. Kondo[2], S. Tomatsu[1]* 1) Department of Pediatrics, Saint Louis University, Saint Louis, MO,USA; 2) Department of Pediatrics, Gifu University, Gifu, Japan; 3) Servizio di Genetica Medica, Istituto Scientifico Ospedale San Raffaele, Milan, Italy; 4) Department of Biochemistry and Medical Biotechnologies, University of Naples Federico II, Naples, Italy; 5) Department of Clinical Genetics, The Juliane Marie Center, Copenhagen University Hospital, Denmark.

Mucopolysaccharidosis IVA (MPS IVA, Morquio A disease), is an autosomal recessive lysosomal storage disorder caused by the deficiency of N-acetylgalactosamine-6-sulfate sulfatase (GALNS) leading to generalized skeletal dysplasia in patients while not affecting their intelligence. Molecular characterization of MPS IVA has resulted in the identification of 148 unique mutations in the GALNS gene. The clinical manifestations of this disease include severe and attenuated forms. We report three families with different ethnical backgrounds, from which seven patients exhibit attenuated phenotypes. We identified the presence of the mutations of p.F167V, p.R253W, and p.P484S as the cause for the attenuated phenotypes with a residual activity of 7.5%, 9.1%, and 10.1%, respectively, by stably transfecting the mutants into pCXN. Although the reported p.N204K mutation showed a lower residual enzyme activity (1.2%) after in vitro mutagenesis, it is still responsible for an attenuated phenotype. Tertiary structure analysis and kinetic studies revealed that the stability and affinity towards the substrate were very different among the mutants studied. This study reveals that mutant proteins leading to attenuated phenotypes are heterogeneous in the property and the location of the mutation.

Association of APOE with Obstructive Sleep Apnea (OSA) in Children. *M. KALRA[1], R. KAUSHAL[2], P. PAL[2], R. DEKA[2], K. FITZ[1], R. CHAKRABORTY[2]* 1) Division of Pulmonary Medicine, Cincinnati Children's, Cincinnati, Ohio; 2) Department of Environmental Health, University of Cincinnati , Cincinnati, Ohio.

Obstructive sleep apnea (OSA) is a major cause of morbidity in children and both morbidity and mortality in adults. APOE variants have been reported to be associated with increased risk of adult OSA. The goal of this study was to test this association in a pediatric population. All Caucasian children diagnosed with OSA at Cincinnati Childrens Sleep Center between January and April 2006 were recruited as cases and ethnicity matched controls were selected from the population-based Princeton School District Study. The 5 SNPs for the APOE gene that were selected using SNP browser version 3.5 included rs157580, rs2075650, rs8106922, rs405509, rs7412. Genotyping was performed using the SNPlex (Applied Biosystems) high-throughput genotyping platform. OSA was determined based on apnea hypopnea index >1 as measured by overnight polysomnogram; cases were then compared to population-based controls. Hardy-Weinberg (HWE) test of genotype frequencies and haplotype inference was performed by Helix Tree version 3.0. Significant associations at allelic and haplotypic level were ascertained using the permutation test of r x c contingency tables with 10,000 replications. The mean age of the 60 OSA cases was 14.0 years (S.D. 3.8), 62% were males, and mean BMI was 27.0 (S.D. 11.6). The mean age of the 106 controls was 14.6 years (S.D. 2.6), 62 % were males, and mean BMI was 23.6 (S.D. 7.9). The genotype frequencies of all SNPs were in HWE. Allele frequencies of the SNP rs157580 were significantly different in cases versus controls (44% vs. 32% respectively; p=0.03). Haplotype analysis using 5 SNPs revealed a significant difference in the groups (overall p=<0.0001). The haplotype GAACC was found in 16% of cases but was completely absent in controls (p=<0.0001). There was no difference for allele frequencies or haplotypes between the groups divided by BMI. This is the first report of association of APOE with OSA in children, supporting the role of APOE in the pathogenesis of pediatric OSA independent of obesity.

Clinical phenotype and autozygosity mapping of phenotypic diarrhoea of infancy. *J.L. Hartley[1,2], P. Gissen[1,2], B. Dawood[3], S. Watson[3], R. Pollitt[4], W.H. Kahr[5], D. Chitayat[6], D.A. Kelly[2], C.A. Johnson[1]* 1) Section of Medical and Molecular Genetics, Division of Reproductive & Child Health, University of Birmingham Medical School, Birmingham B15 2TT, U.K; 2) The Liver Unit, Princess of Wales Childrens Hospital, Steelhouse Lane, Birmingham B4 6NH, U.K; 3) Centre for Cardiovascular sciences, Institute of Biomedical Research, University of Birmingham Medical School, Birmingham B15 2TT, U.K; 4) Clinical Chemistry Department, The Childrens Hospital, Sheffield, S10 2TH, U.K; 5) Department of Paediatrics, Division of Haematology/Oncology, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, M5G 1X8, Canada; 6) Department of Paediatrics, Division of Clinical and Metabolic Genetics, The Hospital for Sick Children, University of Toronto, 555 University Avenue, Toronto, Ontario, M5G 1X8, Canada.

Phenotypic diarrhoea of infancy (PDI; also known as tricho-hepato-enteric syndrome, MIM 222470), an autosomal recessive disorder with multisystemic involvement is characterized by intractable diarrhoea, dysmorphism, hepatic dysfunction and brittle hair. Our cohort of consanguineous (n=6) and non-consanguineous (n=4) families provide the first comparative description of the PDI phenotype in a large cohort. Dysmorphic features included broad forehead, hypertelorism and broad flat nasal root. Hair was brittle, with trichorrhexis nodosa and a characteristic amino acid composition. Severe secretory and osmotic diarrhoea presented in the first few months of life. Some cases developed liver cirrhosis in the neonatal period. Decreased immunoglobulins, with IgG the being most severely affected. Platelet electron microscopy revealed intermittently enlarged platelets, decreased or abnormal platelet alpha granules and abnormal surface-connected canalicular system. Studies showed a defective release of granule contents. To our knowledge, the platelet defects are unique features of this disorder. A genome-wide linkage search using the strategy of autozygosity mapping in consanguineous families is being used to identifying the causative disease gene. The identification of the PDI gene will enable genetic testing, prognostication and identification of therapeutic targets.

Linkage and association study of *MYO9B* variants in Finnish and Hungarian populations supports the role of *CELIAC4* locus (19p13) in celiac disease. *L. Koskinen[1], K. Viiri[2], I. Korponay-Szabo[3], K. Mustalahti[2], K. Kurppa[2], K. Kaukinen[2], C. Wijmenga[4], J. Kere[1,5], M. Mäki[2], J. Partanen[6], P. Holopainen[1]* 1) University of Helsinki, Finland; 2) University of Tampere and Tampere University Hospital, Finland; 3) Heim Pal Childrens Hospital, Budapest and University of Debrecen, Hungary; 4) University Medical Center Utrecht, the Netherlands; 5) Karolinska Institute, Huddinge, Sweden; 6) Finnish Red Cross Blood Service, Helsinki, Finland.

Celiac disease (CD) is an autoimmune intestinal inflammation triggered by dietary gluten in genetically susceptible individuals. Recently, myosin IXB (*MYO9B*) gene in 19p13 (*CELIAC4*) was associated with CD in Dutch population with highest relative risk in homozygous carriers of the found risk haplotype. Three *MYO9B* SNPs tagging the Dutch risk haplotype were genotyped from altogether 472 Finnish and Hungarian CD pedigrees including 933 patients and 563 healthy family members. The *MYO9B* markers showed linkage to CD in the combined material (NPL 2.57, p=0.005) which was mainly due to Hungarian pedigrees alone (NPL 2.65, p=0.003). Neither the Dutch risk haplotype nor any other haplotype or allele showed transmission tendency to affected offspring in our pedigrees. Electrophoretic mobility shift assay (EMSA) showed binding of nuclear proteins to the probe spanning the highest risk SNP, which is a putative transcription factor binding site, but the binding did not differ between the two alleles. The observed linkage at 19p13 supports the role of *CELIAC4* locus in CD susceptibility. However, the lack of significant association in our materials indicates that presence of the primary risk haplotype or gene elsewhere in the region can not be excluded. The EMSA results imply that the risk allele in SNP rs2305764 does not affect protein binding of the gene and may not be crucially involved in gene regulation. In concordance with recent findings in UK and Swedish-Norwegian populations, this may be due to population differences, lower relative risk than previously estimated, or possibly a type I error in the Dutch study. Further studies in *MYO9B* gene and neighboring genes will be required.

Genome-wide detection of human copy number variations using high density DNA oligonucleotide arrays. *S. Ishikawa[1], K. Fitch[2], D. Komura[1], J. Hung[2], F. Shen[2], W. Chen[2], R. Mei[2], J. Zhang[2], G. Liu[2], K. Nishimura[1], H. Nakamura[1], S. Ihara[1], M.H. Shapero[2], K.W. Jones[2], H. Aburatani[1], Genome Structural Variation Consortium* 1) Genome Science & Dependable and High-performance Computing, Research Center for Advanced Science and Technology, University of Tokyo; 2) Affymetrix, Inc., Santa Clara, CA.

In the recent years following completion of the human genome sequence, new progress in unraveling the complexities of the genomes architecture has revealed a remarkable degree of structural variation present among normal individuals. In particular, copy number variations (CNVs) appears to contribute more to the nucleotide diversity than single nucleotide polymorphisms (SNPs) within the human genome. Additionly, the contribution of CNVs to human disease susceptibility may be greater than previously expected, although a complete understanding of the phenotypic consequences of CNVs is incomplete. As the part of the Genome Structural Variation Consortium project, we report here a comprehensive view of CNVs among 270 HapMap samples using the Affymetrix GeneChip Human Mapping 500K Early Access (500K EA) arrays. Using a novel approach which combines an improved version of the Genomic Imbalance Map (GIM) algorithm and an adapted SW-ARRAY procedure, we introduced large scale data processing for all the pair-wise signal comparisons among 270 samples to precisely define CNV boundaries and accurately estimate CNV copy number. With median probe spacing of 2.3Kbp on the 500K EA array, 1,203 unique CNVs were identified, ranging in size from 103 bps to 106 bps. Independent testing of a subset of CNVs by quantitative PCR and mass spectrometry demonstrated a greater than 95% verification rate. To increase the coverage in more complex genomic regions, arrays containing quantitative probes which reside on the more than 1.3M fragments which can be interrogated with the 500K NspI biochemistry were made. The use of high resolution oligonucleotide arrays allow precise boundary information to be extracted, thereby enabling a accurate analysis of the relationship between CNVs and other genomic features.

Simultaneous detection of common alpha-thalassemia point mutations and deletions by reverse-hybridization. *C. Oberkanins[1], H. Najmabadi[2], H.-Y. Law[3], W. Krugluger[4], E. Baysal[5], V. Viprakasit[6], S. Pissard[7], A. Taher[8], A. Al-Ali[9], H. Puehringer[1]* 1) ViennaLab Labordiagnostika GmbH., Vienna, Austria; 2) Genetics Research Center, The Social Welfare and Rehabilitation Sciences University, Tehran, Iran; 3) Genetics Service, KK Women's and Children's Hospital, Singapore; 4) Department of Clinical Chemistry, Rudolfstiftung Hospital, Vienna, Austria; 5) Genetics Department, Al Wasl Hospital, Dubai, UAE; 6) Dept. Paediatrics, Siriraj Hospital, Mahidol University, Bangkok, Thailand; 7) Lab de Genetique et de Biochimie, Hopital Henri Mondor, Creteil, France; 8) Department of Internal Medicine, American University of Beirut Medical Center, Beirut, Lebanon; 9) Department of Clinical Biochemistry, College of Medicine, King Faisal University, Dammam, Saudi Arabia.

Alpha-thalassemia is observed in high frequencies throughout Southeast Asia, India, the Middle East, parts of Africa and the Mediterranean area. It is characterized by the reduced synthesis or absence of alpha-globin chains due to mutations affecting one or both genes. The clinical phenotype varies from asymptomatic to lethal (Hb Barts hydrops fetalis) according to the number of impaired alpha-globin genes. We have developed a reverse-hybridization assay (Alpha-Globin StripAssay) for the rapid and simultaneous detection of 21 alpha-globin mutations: 2 single gene deletions (-3.7; -4.2), 5 double gene deletions (MED; SEA; THAI; FIL; -20.5 kb), anti-3.7 triplication, 2 point mutations in the alpha 1 gene (cd 14 TGG-TAG; cd 59 Hb Adana) and 11 point mutations in alpha 2 (init cd T>C; cd 19 -G; IVS1 5nt del; cd 59 G>A; cd 125 Hb Quong Sze; cd 142: Hb Constant Spring, Hb Icaria, Hb Pakse, Hb Koya Dora; poly A1 AAA-AAG; poly A2 AAA-GAA). The test is based on multiplex DNA amplification and hybridization to teststrips presenting a parallel array of oligonucleotide probes specific for each mutant and wild-type allele. The procedure is simple and convenient, and requires very small amounts of samples, which is of particular importance for prenatal diagnosis. The new assay has been carefully validated on pre-typed reference and routine diagnostic samples in a multi-center study.

Novel 22q11 deletions not mediated by Low Copy Repeats involving the Cat Eye Syndrome region. *G.R. Jalali[1], J.A. Vorstman[1], T.H. Shaikh[1], A.M. Hacker[1], D.M. McGinn[1], E.H. Zackai[1], A.E. Urban[2], B.S. Emanuel[1]* 1) Div.of Human Genet., Children's Hosp. Phila., Phila, PA; 2) Yale Univ. New Haven, CT.

The majority of deletions responsible for DiGeorge/Velocardiofacial syndromes (DGS/VCFS) are mediated by four copies of low copy repeats (LCRs) in 22q11. The LCRs are large stretches (100-450 kb) of sequence that share 96% sequence identity. They predispose 22q11 to errors in recombination leading to microdeletions and microduplications. Copy number changes within 22q11 where at least one end-point is not within an LCR are rare. We report two patients with essential features of the DGS/VCFS phenotype with novel deletions in the 22q11 region, initially detected by fluorescence in situ hybridization (FISH). Proband 1 (p1) is a 19 year old male and proband 2 (p2) is a 7 year old male. Both patients have congenital cardiac defects; a valvular pulmonary stenosis (p1) and a double aortic arch (p2). P1 has a submucosal cleft palate. P2 has no anatomic abnormalities of the pharyngeal cavity, but he did have nasal regurgitation of food as an infant. Both patients show typical facial features of DGS/VCFS, had recurrent middle ear infections and speech delay. In addition, p1 has a disorder in the anxiety spectrum and in p2 the diagnosis of autism was made. We have used a combination of FISH, Multiplex Ligation-dependent Probe Amplification (MLPA) and high density, oligonucleotide microarrays to map the deletion breakpoints in both cases. Remarkably, in both cases breakpoints are outside the LCRs. The proximal breakpoints of both deletions are centromeric to the common proximal endpoint of the 3 Mb typically deleted region (TDR) in DGS/VCFS, extending into the Cat Eye Syndrome (CES) region. The distal breakpoints also do not localize to LCRs and are proximal to the distal endpoint of the TDR. Further, in p1 the TBX1 gene is not deleted. Additional analysis is currently on-going to characterize the deletion end-points at the nucleotide level. The variant deletions associated with DGS/VCFS reported here underscore the need for improved detection methods such as high density microarrays and MLPA in the detection and diagnosis of genomic disorders resulting from deletions of 22q11.

PCSK9 variants are associated with reduced serum low-density lipoprotein cholesterol levels in childhood and adulthood. The Bogalusa Heart Study. *M. Hallman[1], S.R. Srinivasan[2], W. Chen[2], E. Boerwinkle[1], G.S. Berenson[2]* 1) Human Genetics Ctr, Univ. of Texas Health Science Center, Houston, TX; 2) Tulane Center for Cardiovascular Health, Tulane Univ. School of Public Health and Tropical Medicine, New Orleans LA.

Specific mutations in proprotein convertase, subtilisin-kexin type 9 (PCSK9) have been associated with reduced plasma low-density lipoprotein cholesterol (LDL-C) and lower coronary heart disease risk. These mutations may produce lifelong reduction in LDL-C, but data in younger individuals are lacking. We related two PCSK9 nonsense variants (Y142X and C679X) and one missense mutation (R46L) with serum LDL-C in 478 African Americans and 1086 whites examined 3-8 times (meanSD: 5.21.3) in the Bogalusa Heart Study at 4-38 years of age. Frequency of the L46 allele was 0.01670.0032 in whites (27 carriers) and 0.00150.0015 in African Americans (1 carrier). The frequency of the X142 allele was 0.00620.0031 in African Americans (5 carriers) and 0.00070.0007 in whites (1 carrier), while that of the X679 allele was 0.00950.0038 in African Americans (7 carriers) and 0.00070.0007 in whites (1 carrier). In whites, LDL-C was significantly lower in L46 carriers (78.921.8 mg/dl) than non-carriers (89.724.9 mg/dl; p=0.035) at examination 1 (mean age: 9.43.2 yrs). In African Americans, LDL-C was significantly lower in X142 carriers (66.69.6 mg/dl) than non-carriers (91.623.7 mg/dl; p=0.015) at examination 1 (mean age: 9.03.0 yrs), but did not differ significantly between X679 carriers (84.913.9 mg/dl) and non-carriers (91.023.8 mg/dl; p=0.467), although African Americans carrying *either* X142 or X679 had significantly lower LDL-C than non-carriers (77.315.1 vs 91.423.9 mg/dl; p=0.037). Multilevel models for repeated measures showed statistically significant associations between lower LDL-C at all ages and the L46 allele in whites, the X142 allele in African Americans, and the combined X142 and X679 alleles in African Americans. In combination with evidence from older cohorts, these findings show that these PCSK9 variants are associated with significantly lower LDL-C levels across most of the human lifespan.

Discovery and Profiling of microRNAs that Regulate Cancer. *G.A. Owens, C.H. Kim, J. Baer, X. Wu, E. R., D.J. Munroe* SAIC-NCI Frederick, Frederick, MD.

MicroRNAs are a class of small (~22 nt long) non-coding RNAs that regulate gene expression by translational repression or transcriptional gene silencing. The total number of known human miRNAs is rising above 800 which is >3% of all human genes. Since miRNAs regulate developmental processes and gene expression, any misregulation of miRNAs may lead to cancer. Furthermore, novel miRNAs may be activated in cancer cells. In order to discover novel microRNAs involved in cancer, we are cloning and sequencing microRNAs in cancer cell lines and tissues. In parallel, we are hybridizing purified-miRNAs to Affymetrix tiling arrays in order to identify novel microRNAs . In addition, we have developed a highly sensitive microarray of miRNAs for analyzing the miRNA expression in cancer cells. The microarray of miRNAs will allow us to identify, in a high-throughput manner, the regulatory miRNAs which are activated or inactivated in cancer. These miRNAs can serve as targets for drug therapy or as molecular diagnostic for cancer prognosis and progression. This project has been funded in whole or in part with Federal Funds from the National Cancer Institute, National Institutes of Health, under Contract No. N01-C0-12400.

The Benefit of MLPA in TSC 1 and 2 Analysis. *T.A. Maher[1], A. Milunsky[1,2], M. Ito[1,2], J. Yang[1], J.M. Milunsky[1,2,3]* 1) Center for Human Genetics, Boston University School Medicine, Boston, MA; 2) Department of Pediatrics, Boston University School of Medicine, Boston, MA; 3) Department of Genetics and Genomics, Boston University School of Medicine, Boston, MA.

Tuberous Sclerosis Complex (TSC) is an autosomal dominant disorder that manifests with highly variable symptoms and signs that include mental retardation, epilepsy, skin lesions, and hamartomas in multiple organ systems including heart, brain and kidneys. Fetal cardiac rhabdomyomas have almost invariably been correlated with a subsequent diagnosis of TSC. Sequencing analysis of TSC 1 and 2 enables detection of ~80% of all mutations, which are located within exons or their intron boundaries. Southern blot is able to detect large deletions of part or the entire gene, but is laborious, costly and may miss smaller exonic copy number changes. Multiplex Ligation-dependant Probe Amplification (MLPA) is now a standard technology in the molecular laboratory to detect copy number changes in targeted genes. We examined a cohort of 104 patients submitted for TSC analysis by full gene sequencing and MLPA analysis. This cohort included 8 prenatal samples submitted due to the detection of fetal rhabdomyomas on prenatal ultrasounds. 45/104 had TSC2 mutations detected by sequencing. 21/104 had TSC1 mutations detected by sequencing. MLPA revealed 5 partial TSC2 gene deletions and 1 partial TSC1 gene deletion. MLPA increased the detection of TSC mutations by ~6% in our cohort. MLPA is less expensive, quicker and more precise than Southern blot in the characterization of TSC deletions and is a recommended standard part of TSC clinical molecular diagnosis.

High through-put targeting induced mutations using Applied Biosystems 3730 series capillary electrophoresis system. *L. Joe[1], B. Finkelnburg[2], D. Baker[3], L. MacPherson[3], J. Clarke[3]* 1) Genetic Analysis R&D, Applied Biosystems, Foster City, CA; 2) Applera Deutschland GmbH, Darmstadt, Germany; 3) John Innes Centre Genome Laboratory, Norwich, United Kingdom.

Targeting induced mutations in genomes is a strategy used in reverse genetic studies to identify series of chemically induced point mutations in specific genes. The detection of point mutations exploits the ability of the CEL1 endonuclease to cleave genomic DNA at mis-matched heteroduplexes. Polyacrylamide slab-gel electrophoresis systems are traditionally used to detect the cleavage products. These methods are labour intensive and not easily automated. We have developed the use of Applied Biosystems 3730 series capillary electrophoresis system for cleavage product detection and sizing up to 1200 bp range in a 5-color dye-set. Following optimization of the CEL1 cleavage reaction, clean-up and capillary run conditions point mutations could be identified in populations with up to 12 fold pooling. For high through-put fragment analysis the AFLP Analysis Method in Applied Biosystems GeneMapper Software v4.0 was optimized for effective peak detection and enables accurate cleavage product sizing from 1Kb target regions. We present our progress in this method in a range of organisms and discuss strategies for the development of an automated processing system as well as alternative methods for high through-put detection of induced mutations.

Strong evidence for complex gene-gene interactions in multiple sclerosis using Random Forest analyses. *E. Madden, P.P. Ramsay, G. Artim, L.F. Barcellos* Division of Epidemiology, School of Public Health, University of California, Berkeley, CA.

Despite strong evidence for polygenic inheritance in multiple sclerosis (MS), only the major histocompatibility complex (MHC) on ch. 6p21 has consistently demonstrated linkage and association with risk for disease. It appears that the HLA-DRB1*15 association largely explains this linkage signal; however, the exact mechanism(s) by which MHC gene(s) operate in MS are not known. Genome screens have been performed in MS and indicate that non-MHC genes with strong individual effects in MS are not involved. Novel non-parametric approaches such as tree-based classification methods that accommodate large marker datasets and allow for genetic heterogeneity and interactions among variables are useful for MS studies. Recent theoretical work has also shown that the prediction accuracy of classification trees is significantly improved by building a collection of trees, or Random Forests. We analyzed whole genome screen SNP data (total 4,506 SNPs) for 830 ASPs from 730 multicase MS families using a Random Forest approach to identify evidence for loci potentially interacting with the MHC region. Genetic sharing (mean IBD) at other regions of the genome (1 cM map intervals), and both country and gender distribution for each MS ASP (total of 3,631 predictors) were used to predict sharing at HLA-DRB1. At least five non-MHC chromosomal regions were identified by Random Forests as predictive of HLA-DRB1 sharing status in MS ASPs: these included chromosomes 2, 12, 14, 18 and 19. Our results suggest that multiple non-MHC loci are influencing HLA-associated susceptibility for MS, and that in the context of large genetic studies where unknown interactions may exist, an initial screen using Random Forests is a powerful approach that can significantly reduce the number of variables for further study. The authors would like to acknowledge the International MS Genetics Consortium, the Nordic MS Genetics Group and Australian MS Genetics Group for providing pedigree and SNP data for these analyses.

Whole Genome Association Studies at the NCBI. *M.D. Mailman, Y. Jin, Z. Wang, R. Bagoutdinov, L. Phan, J.D. Beck, A. Graeff, K. Sirotkin, D.R. Maglott, S.T. Sherry, J. Ostell* NCBI, NLM, NIH, Bethesda, MD.

The National Center for Biotechnology Information (NCBI) has created a web resource to store and browse phenotypic information from clinical, longitudinal, and case/control studies from many sources and link them with high-throughput genotype information. All users have unrestricted access to browse variable descriptions, questionnaires and supporting documents, statistical summaries of phenotypes and allele frequencies, as well as all metadata from all studies. In addition, users may apply for controlled access accounts to obtain genotype and phenotype data from de-identified individuals in the studies, consonant with consents and permissions appropriate to each study. Clinical and epidemiological data in various formats are all converted to a common data model and stored in the new database. The best information about the meaning of data are found in associated questionnaires, protocols, and supporting documents. These are converted into a standard XML format, and specific questions and sections are linked to the appropriate phenotype measurement columns. Users can search and browse document text to locate the most thorough explanations of data columns that may be of interest, then navigate easily to summary information about that measure, identify studies relevant to a research goal, and be directed to the group with authority to grant access to more detailed data. Controlled access requests to individual data are evaluated in a distributed manner by the institution responsible for each study. Access permissions are then set in the central database with a single account per user. As much as possible, these data will be integrated with other NCBI resources such as RefSeq, Probe, dbSNP, OMIM, PubMed, and a variety of other genome resources. For some studies, such as GAIN, we will also calculate and make available some simple associations. Phenotype and genotype data managed by these methods will include the Genetic Association Information Network (GAIN); The Genes and Environment Initiative (GEI); The Framingham Heart Study; the NINDS Parkinsonism, stroke, and ALS studies; and the National Eye Institute macular degeneration study, among others.

System development of biological markers for the diagnosis and cure of cerebrovascular disease. *Y.J. Kim[1], H. Choi[1], H. Kang[1], T. Son[2], S. Hwang[2], A. Han[2], K.O. Choi[1]* 1) Functional Genome Res. Center, KRIBB, Daejeon, Daejeon, Korea; 2) Korea Bioresource Information Center, KRIBB, Daejeon, Daejeon, Korea.

Complex disorders like stroke or cancers have two or more genetic loci and environmental factors contribute to the diseases. To find the candidate genes related to a complex disease or trait and to show their relationship is necessary to reveal its mechanism. The objective of this study is to provide a systematic approach to analyze the complex effect of genes and to represent the frame of their relations associated with specific diseases. We developed gene annotation database on the association of HGNC to biological databases such as UCSC, GO, KEGG, HPRD, etc. Etiology of stroke was classified and accordingly tens of genes directly related to and hundreds of genes to the etiology of stroke were identified. Those genes were annotated to have information of transcription, translation, structural function, and relatedness to diseases. The networked stroke gene maps describing gene-gene interaction and biological pathway were also developed. We found that a PPI (protein-protein interaction) network associated with stroke follows the power-law degree distribution as other biological networks do. This stroke network can be used as a frame of the systematic genomic research for other complex diseases.

Paternal History of Linguistically diverse East African Populations. *J. Hirbo[1], S. Omar[2], M. Ibrahim[3], S. Tishkoff[1]*
1) Department of Biology, University Maryland, College Park, MD; 2) Kenya Medical Research Institute, Centre for Biotechnology Research and Development (CBRD) Nairobi, Kenya; 3) Department of Molecular Biology, Institute of Endemic Diseases, University of Khartoum, Khartoum, Sudan.

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, Nilosaharan, Niger Congo, and Khoisan) are found in the region. As part of a large study of population genetic diversity of East and North Eastern Africa, we genotyped the following Y chromosome markers (analyzed in a hierarchical manner to construct haplotypes) in a total of ~1200 male individuals from ~ 40 Tanzanian, Sudanese, and Kenyan populations: 46 SNPs, one Alu and one LINE insertion polymorphism. To make inference about age estimates and migration pattern, we further genotyped all the individuals for 12 Y microsatellites commonly used in population genetic studies. We compared our results with published results of Y chromosome 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 lineages in Africa, suggesting that they may have been an ancient source of dispersion throughout Africa. Additionally, we find evidence for historical geneflow between East Africa and the Middle East. We also ascertained the effect of the Bantu-expansion on the peopling of East Africa, as well as the genetic signature of recent migration of Cushitic-speaking groups originating from Ethiopia throughout East Africa. We compare results of this study to patterns observed in the same populations for mtDNA and autosomal genetic diversity to check for congruity.

Novel mutation in *GJA8* linked with autosomal dominant congenital cataract in an Indian family. *Vanita. Kumar[1], H.C. Hennies[2], D. Singh[3], P. Nürnberg[2], K. Sperling[4], J.R. Singh[1]* 1) Centre for Genetic Disorders, Guru Nanak Dev University, Punjab, Punjab, India; 2) Cologne Center for Genomics and Institute for Genetics, University of Cologne, Germany; 3) Dr Daljit Singh Eye Hospital, Amritsar, India; 4) Institute of Human Genetics, Charite Humboldt-University, Berlin, Germany.

Purpose: To identify the genetic defect in an autosomal dominant congenital cataract family having 15 members in 3-generations affected with bilateral congenital cataract that gave the appearance of full moon with Y-sutural opacities. **Methods:** Detailed family history and clinical data were collected. A genome-wide scan by 2-point linkage analysis using nearly 400 microsatellite markers in combination with multipoint lod score and haplotype analysis was carried out. Mutation screening was performed in the candidate gene by bidirectional sequencing of amplified products. **Results:** Significantly positive lod scores greater than 3.0 at =0.0 indicative of linkage were obtained with markers at 1q21. Haplotype analysis placed the cataract locus to a 14.1 cM region between D1S221 and D1S498, in close proximity to gene for the gap junction channel protein connexin50 (GJA8). Mutation screening in GJA8 identified a novel G>C transversion at nucleotide position c.235. This nucleotide change resulted in the substitution of highly conserved Valine by Leucine at codon 79 (V79L). The observed change segregated only in affected individuals and was neither seen in any unaffected member of this family nor in 90 unrelated control subjects tested by sequence analysis of GJA8. **Conclusions:** The present study describes the mapping of a locus for autosomal dominant congenital cataract at 1q21 and identifies a previously unreported mutation in GJA8. These findings thus expand the mutation spectrum of GJA8.

Partial evidence of association between epidermal growth factor A61G polymorphism and the age at onset in male schizophrenia. *K.Y. Lee[1], E.J. Joo[3], Y.M. Ahn[1,2], S.H. Kim[2], Y.S. Kim[1,2]* 1) Department of Neuropsychiatry, Seoul National University Hospital, Seoul, Korea, MD; 2) Department of Psychiatry and Behavioral Science, Seoul National University College of Medicine, Seoul, Korea, MD; 3) Eulji University School of Medicine, Department of Neuropsychiatry, Daegeon, Korea, MD.

Epidermal growth factor (EGF) is well known to enhance development of dopaminergic neuron as well as regulate the various neuronal cells. From animal studies and postmortem studies, dysfunction of EGF signals has been demonstrated to risk factor for schizophrenia, suggesting important candidate gene. Recently, several association studies including ages at disease onset with EGF A61G, functional polymorphism, have been performed but yielded controversial results. Thus, we investigated whether this polymorphism on EGF gene has a role for predisposition of schizophrenia and effects on the ages at onset. Total 190 of patients with schizophrenia and 347 healthy controls were included in this study. The 3 different points of age at onsets, 1) age at first occurrence of positive psychotic symptoms, 2) age at first medication, 3) age at first hospitalization as schizophrenia, were assessed. In this study, we did not find the differences in allele and genotype frequencies between patients with schizophrenia and controls. In turn, we also did not find any associations between this polymorphism and the ages at onset across stratified points in general but patients with AA genotype showed significant gender difference in the ages at onset of all stratified points although significant impact of interactions with gender by genotype to age at onset was not obtained. When we re-analyses the G allele distribution between clinical subsets with 20 years as the cutoff of ages at onset, patients with early onset schizophrenia showed more common AA homozygote than patients with adult-onset schizophrenia in male. In conclusion, though we could not support an association of this polymorphism and schizophrenia, AA genotype in EGF gene might have a disease modifying role differentially according to gender.

Prospective study of warfarin dosage requirements based on CYP2C9 and VKORC1 genotypes. *M. Lee[1], M.H. Weng[2], L.S. Lu[1], H.P. Juang[1], T.H. Lee[2], F.M. Sun[1], J.Y. Wu[1], Y.T. Chen[1]* 1) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) Chang-Gung Memorial Hospital, Linko, Taiwan.

Warfarin is a widely prescribed anticoagulant for the prevention of thromboembolic diseases. However, warfarin treatment is problematic because the dose requirement for warfarin is highly variable, both inter-individually and inter-ethnically. Therefore, a patients INR needed to be monitored regularly to prevent serious complication. We recently reported a novel functional VKORC1(vitamin K epoxide reductase complex, subunit 1) promoter polymorphism which was highly associated with warfarin dosage requirement. This promoter polymorphism, in conjunction with CYP2C9 variants, could underscores the inter-individual and inter-ethnic differences in warfarin sensitivity. In this prospective study, we aimed to predict an individuals warfarin dosage requirement by determining the genotypes of the VKORC1 promoter -1639 A>G polymorphism and CYP2C9 variants. Patients were prescribed appropriate warfarin dosage based on the genotypes and were then asked to returned once a week for monitoring for minimum of 5 weeks. Patients plasma was banked to determine vitamin K status during the course of treatment and INR was also measured to ensure the INR falls in the desired range of 2-3. Warfarin Doses were adjusted if the INR do not fall in this range. Age, BMI, medication and dietary intake were also recorded. 80 Han Chinese patients have been recruited from the cardiology clinics. By using this pharmacogenetic dosing, patients with VKORC1 and CYP2C9 variants reached stable, therapeutic doses quicker than those without pharmacogenetic dosing. Without taking account of patients age, sex, BMI, other medications and dietary history, 70% of the patients maintenance dose did not deviate from the predicted dose. 23% of the patients maintenance dose was approximately 1 mg higher than predicted and the rest was over-estimated by 1 mg. The high sensitivity of this pharmacogenetic dosing demonstrated feasibility of this strategy for a safer, less cumbersome and more effective warfarin treatment.

Genetic variation in upstream stimulatory factor 1 (*USF1*) - a key transcriptional regulator of cardiovascular genes - associates with the severity of coronary atherosclerosis and risk of sudden cardiac death. *K. Kristiansson[1], E. Ilveskoski[2], T. Lehtimäki[2], L. Peltonen[1,3], M. Perola[1,3], P.J. Karhunen[2]* 1) Dept. Molecular Medicine, NPH Inst, Helsinki, Finland; 2) School of Medicine, Univ. Tampere, Finland; 3) Dept. of Medical Genetics, Univ. Helsinki, Finland.

Atherosclerosis is a complex disease with multigenic background and various phenotypes. Upstream Stimulatory Factor 1 (USF1) is known to regulate the transcription of more than 40 cardiovascular genes involved in lipid metabolism, hemostasis, inflammation and endothelial function. Genetic variation in the *USF1* locus has been recently associated with abnormalities in lipid metabolism characterizing familial combined hyperlipidemia (FCHL). Although FCHL dramatically increases the risk for early myocardial infarct, the effects of variants of *USF1* gene on coronary and aortic atherosclerosis have not been studied. We analyzed the association of genetic variation across the *USF1* gene locus to the severity of morphometrically measured coronary and aortic atherosclerosis and risk of sudden prehospital cardiac death (SCD) among two independent autopsy series of middle-aged (33 - 70 years) males (The Helsinki Sudden Death Study, n=700). Due to the high linkage disequilibrium on the *USF1* locus on chromosome 1q23, six haplotype tagging SNPs (htSNPs) captured the common genetic variation of the gene. We found that the htSNP *rs2516839* showed the strongest association to the phenotypes studied with the common allele (T) of the SNP harboring a 2 -fold risk of SCD when compared to non-carriers of the allele (OR 2.00, 95 % CI 1.16-3.44, p=0.01). Among study subjects with coronary atherosclerosis, the T-allele of *rs2516839* was also associated with larger total plaque area in abdominal aorta (p=0.003), as well as with larger fibrous (p=0.002), complicated (p=0.02) and calcified (p=0.01) plaque areas in the coronary artery. The data emerging from the vessel-wall analyses of the study sample collected from the genetically homogenous Finnish population would imply that the allelic variant(s) of the *USF1* gene significantly contribute to the risk of atherosclerosis and SCD.

High prevalence of autosomal dominant spinocerebellar ataxia linked to 16q22.1 (16q-ADCA) in the Japanese families with autosomal dominant cerebellar ataxia. *Y. Ichikawa, S. Tsuji, J. Goto* Neurology, The University of Tokyo, Tokyo, Japan.

Autosomal dominant cerebellar ataxias (ADCA) are heterogeneous neurodegenerative diseases characterized by progressive cerebellar ataxia occasionally accompanied with other features, such as extrapyramidal signs, pyramidal signs and ophathalmoplegia. A single nucleotide substitution (-16C>T) in the 5 UTR of the puratrophin-1 gene has recently been identified as the putative mutation in patients of families linked to chromosome 16q22.1 (16q-ADCA). The clinical features of the patients with 16q-ADCA have been shown to be characterized by slowly progressive cerebellar ataxia occasionally accompanied by hearing impairment. (Ishikawa et al., Am J Hum Genet; 2005). To investigate molecular epidemiology of 16q-ADCA in Japanese AD-SCA, we examined the -16C>T substitution in the 5 UTR of the puratrophin-1 gene by denatured high performance liquid chromatography (DHPLC) on the 87 Japanese ADCA families, 61 sporadic cases and 51 with undetermined mode of inheritance cases. The possibilities for spinocerebellar ataxia types (SCA) 1, 2, 6, 7, 8, 12, 17, Machado Joseph disease/SCA3, and dentatorubral-pallidoluysian atrophy (DRPLA) have been excluded. Of the 87 ADCA families, 24 families (27.6%), and 2 of the 51 with undetermined mode of inheritance (3.9%) carried the -16C>T substitution. Their clinical features are available for the 25 families from the medical information. The average age at onset was 56.1 years (40 to 68 years), all patients showed ataxic gait or dysarthria as their initial symptoms, 13 patients (46.4%) had hyper tendon reflexes, and only one patient (3.7%) suffered from hearing impairment. The present study demonstrates that 16q-ADCA comprises a substantial proportion among the ADCA families without mutations in the previously identified genes. The present study further demonstrates a strong founder effect in the Japanese 16q-ADCA families.

Thyroid phenotype associated with sensorineural hearing loss and enlarged vestibular aqueducts. *A.C. Madeo[1], S.P. Pryor[1], Y. Yang[1], C.C. Brewer[1], C.K. Zalewski[1], J.A. Butman[1], K.S. Arnos[2], W.E. Nance[3], J. Thomsen[4], J.C. Reynolds[1], A.J. Griffith[1]* 1) National Institutes of Health, Bethesda, MD; 2) Gallaudet University, Washington, DC; 3) Medical College of Virginia, Richmond, VA; 4) Pediatric ENT of Atlanta, GA.

SLC26A4 mutations can be associated with sensorineural hearing loss and an enlarged vestibular aqueduct (EVA) in combination with goiter (Pendred syndrome: PS), or with nonsyndromic EVA (NSEVA). The PS goiter has a delayed onset and is incompletely penetrant, so the perchlorate discharge test (PDT) has emerged as a sensitive test to detect the underlying iodine organification defect. We previously reported a genotype-phenotype correlation in 39 EVA subjects (J Med Genet 42:159-165, 2005): sequence analysis of PCR-amplified *SLC26A4* exons and flanking intronic sequences revealed biallelic *SLC26A4* mutations in subjects with PS, whereas NSEVA was associated with one or zero detectable *SLC26A4* mutations.

Here we present *SLC26A4* genotypic and phenotypic results for an expanded cohort of 80 EVA subjects (43 F, 37 M; $1^{7/12}$-59 y, avg $11^{11/12}$ y). Evaluations included a history and physical examination, thyroid ultrasonography, serologic tests of thyroid function and anti-thyroid antibodies, *SLC26A4* mutation analysis and, in some cases, a PDT (n=40). Eleven subjects (9 F, 2 M; $13^{8/12}$-59 y, avg 35 y) had a goiter: seven of these had two mutant alleles. Clinical findings in the four goitrous subjects with no *SLC26A4* mutations were inconsistent with PS as the etiology of goiter. Our results confirm the correlation of PS with two mutant alleles of *SLC26A4*, and NSEVA with one or zero mutant alleles ($p<=.001$). This correlation is predicated upon detection of goiter phenocopies and other thyroid conditions that confound interpretation of the PDT. We present the ultrasonographic findings, thyroid serologic test results, and PDT conditions and interpretation that detect the thyroid gland abnormality associated with biallelic *SLC26A4* mutations with high sensitivity and specificity. These results should facilitate evaluation and management of the thyroid gland in patients with EVA.

Colorectal Cancer Risks in Relatives of Young Onset Cases: Greater in Sibs Than in Parents. *S.K. Nigon[1], L.A. Boardman[1], B.W. Morlan[1], K.G. Rabe[1], G.M. Petersen[1], J. Goldberg[3], S. Gallinger[2], N.M. Lindor[1]* 1) Mayo Clinic College of Medicine, Rochester, MN USA; 2) Mount Sinai Hospital, University of Toronto, Toronto CANADA; 3) Tufts University, Boston MA.

Background: Several single-gene Mendelian disorders have been discovered that account for some of the familial aggregation detected in large population studies of CRC. Mutations in the DNA Mismatch Repair (MMR)genes cause HNPCC-Lynch Syndrome, the most common of the recognized CRC-predisposition syndromes. It is unclear how fully this recognized syndrome accounts for observed familial aggregation particularly among first degree relatives of younger onset CRC patients. **Aim**: To assess familial risk, we studied cases with CRC diagnosed under age 50 years identified through Minnesota Cancer Surveillance System (MCSS) and Mayo Clinic, Rochester, MN. CRC patients were excluded if there was evidence of DNA MMR deficiency by tumor microsatellite instability. A total of 278 probands (131 from MCSS; 147 from Mayo Clinic) were included in this study. Data on a total of 1862 relatives was collected, of whom 68 had had CRC, and an additional 165 had had primary cancers of other types. Standardized incidence ratios (SIR) were estimated by comparing to the Surveillance Epidemiology and End Results (SEER) database. *MYH* gene mutations were tested in probands with affected siblings. **Results**: Compared to SEER data, relatives of young onset CRC probands had increased risks for developing CRC. The SIR for CRC was increased among first degree relatives (SIR=1.65; 95% C.I. =1.29-2.07), and this risk was greater for siblings (SIR = 2.67; 95% C.I=1.50-4.41) than for parents (SIR= 1.5; 95% C.I. =1.14-1.94). No mutations were found in the *MYH* gene in the probands who had siblings diagnosed with CRC. **Conclusions**: While the risk of CRC among relatives of young onset probands is significantly higher than the general population, and was elevated in parents, the risk was much greater among siblings. This increased sibling risk was not accounted for by *MYH* gene mutations. *This work was supported in part by the National Cancer Institute of the National Institute of Health under RFA #CA-95-011*..

A tree based approach to modelling disease associations in the MHC, reveals evidence of class II-independent type 1 diabetes susceptibility loci. *J.M.M. Howson, S. Nejentsev, N.M. Walker, S. Field, J.S. Szeszko, H.E. Stevens, D.G. Clayton, J.A. Todd* JDRF/WT Diabetes and Inflammation laboratory, Cambridge Institute for Medical Research, Cambridge University, Cambridge, United Kingdom.

The MHC class II loci, *HLA-DRB1* and *HLA-DQB1*, are well known to be involved in the etiology of type 1 diabetes (T1D). Not all the linkage in the region however, is thought to be accounted for by these loci alone. Over the past 10 years, the hunt has intensified to find other MHC loci that also contribute to T1D susceptibility. A number of positive and negative associations have been found with *HLA-A, -B, -C* from the class I region, *MICA, BTNL2* and *HLA-DPB1*. However many of these studies suffered from small sample sizes and resort to subgroup analysis due to statistical difficulties in the modelling of the class II effects (100s of genotypes). This is why no non-class II loci from the region are generally accepted to be associated with T1D in a specific and convincing way. Here we describe the use of recursive partitioning to the modelling of the confounding effects of *HLA-DRB1* and *HLA-DQB1* caused by linkage disequilibrium between their alleles and non-class II test loci. The grouping method does not rely on risk estimates. However the splitting criteria are based on categorizing individuals as cases and controls according to genotype, so can be considered a risk-based method. Both phased and unphased genotypes were considered. We applied recursive partitioning to 458 UK and 365 USA families typed at 82 microsatellites and SNPs from across the MHC. We obtained evidence for two non-class II loci associated with T1D. The results were replicated in an independent sample set of 1500 British cases and 1500 controls, which further strengthens the candidacy of these immune response genes in T1D susceptibility.

Genome-wide association study of acute post-surgical pain in humans. *H. Kim[1], H. Lee[1], J. Brahim[2], J. Rowan[3], S. Wahl[2], R.A. Dionne[1]* 1) NINR/NIH, Bethesda, MD; 2) NIDCR/NIH, Bethesda, MD; 3) Dept. of Nursing, NIH, Bethesda, MD.

Candidate gene studies on the basis of biological hypotheses have been performed to identify relevant genetic variation in complex traits such as pain. However, the complicated mesh of contributing factors and the thousands of molecules involved in different pain phenotypes makes it difficult to detect responsible genetic variations for an individuals unique susceptibility to pain or response to analgesics. It is unlikely that one common variation in a single gene acts dominantly on pain. The contribution of each gene seems to be subtle on multiple pain pathways, making its signal difficult to detect. Therefore, most of genetic associations reported with pain phenotypes so far are weak and debatable. To overcome the limitations of candidate gene association studies, we have genotyped 500,768 single nucleotide polymorphisms from the whole genome in humans who underwent standardized surgical removal of impacted third molars. Based on maximum post-operative pain ratings with a 100 mm visual analogue scale (VAS) anchored to no pain as 0 and most pain imaginable as 100, pain sensitive patients (N=28, VAS = 83.3 8.3, range 72-99, 'severe' pain) and pain insensitive patients (N=31, VAS = 41.4 5.3, range 30-49, 'slight' to 'moderate' pain) from a total of 112 European American patients were analyzed with Helix Tree. There were no significant differences between groups in gender, age, surgical difficulty and adjunctive drugs. The analysis revealed that 7 SNPs show associations with maximum post-operative pain after local anesthetics offset at the level of $p < 10^{-6}$. Four of them are uncharacterized, two of them are located in untranslated region (LOC283867 and SIPA1L1) and one is located in an intron (CDKAL1). Further characterization of these 7 regions with dense genotyping may identify genetic loci that contribute to interindividual variability in pain due to tissue injury and the acute inflammation responses following minor surgery.

Gene-Gene Interaction Associated with Drug Response in Unipolar Major Depression Disorder. *A.A. Motsinger[1], K. Haman[1], M.K. Hahn[2], A. Steele[3], B. English[3], H. Fentress[3], R. Meyers[3], L. Hazelwood[3], M. Mazei[3], E. Sanders-Bush[3], R.D. Blakely[2], M.D. Ritchie[1], R. Shelton[3]* 1) Center for Human Genetics Research, Vanderbilt Univerity, Nashville, TN; 2) Department of Psychiatry, Vanderbilt Univerity, Nashville, TN; 3) Department of Pharmacology, Vanderbilt Univerity, Nashville, TN.

Unipolar major depression disorder (MDD) is a complex disorder that affects nearly 10 million people each year in America. Fortunately, the disorder is often treatable. Several antidepressants are currently available. However, not all patients respond and many factors are assumed to contribute to this. We hypothesize that genetic variants may be associated with treatment response. In the current study, we ascertained genetic data on a group of 50 subjects (mean age 42.8, 39% male) with MDD. Response was assessed using the Hamilton Rating Scale for Depression (HAM-D). Individuals were treated with serotonergic antidepressants and response was measured at 16 weeks. Of the subjects ascertained, 18 had no response to medication and 32 had a significant response or remitted. We genotyped 14 single nucleotide polymorphisms (SNPs) in 8 genes hypothesized to be important in the pharmacogenomics of depression. To detect genetic variations associated with drug response we used Multifactor Dimensionality Reduction (MDR), a powerful statistical approach used to detect gene-gene interactions with or without main effects. We detected an interesting interaction between SNPs 5HT2A A_1438G and MAOA VNTR longshort variant that predicted response with 73.33% ($p<0.055$) accuracy. This model is marginally significant and is likely to reach highly significant levels with an increased sample size. These findings highlight the importance of looking for gene-gene interactions in the study of complex phenotypes, like drug response. Such associations hold the promise of individualized medicine, where appropriate treatment regimens are tailored to individuals genetic profile.

JAG1 mutations in patients affected by atypical Alagille syndrome. *G. Massazza[1], D. Marchetti[1], P. Stroppa[2], L. Melzi[2], A.R. Lincesso[1], A. Sonzogni[3], G. Torre[2], M. Iascone[1]* 1) Lab. Genetica Molecolare; 2) USC Pediatria; 3) USC Anatomia Patologica, Ospedali Riuniti, Bergamo, Italy.

Alagille syndrome (AGS) is a dominantly inherited multisistem disorder involving the liver, heart, eyes, face and skeleton, caused by mutations in JAG1, located on chromosome 20p12. In approximately 15% of the AGS patients, the liver disease progresses to cirrhosis and liver failure, necessitating liver transplantation. AGS shows highly variable penetrance, and diagnosis in mildly affected patients can be difficult. Here we report the genetic characterisation of 34 patients (mean age 8 months, range 50 days-30years): 13 patients with a strong clinical evidence of AGS while 21 with unexplained cholestasis and not complete features of AGS. All 26 exons and flanking intronic regions of JAG1 gene were analysed by SSCP or dHPLC. The abnormal samples were re-amplified and sequenced. JAG1 mutations were identified in 11 of 13 AGS patients and no clear pathogenic variation was found in patients affected by unexplained cholestasis. Eight mutations were nonsense/frameshift while the remaining 3 were missense. Five mutations were parental inherited. JAG1 is the only gene currently known to be associated with AGS. The diagnosis of AGS is primarily based on clinical findings and the majority of individuals who meet clinical criteria presents JAG1 mutations. Molecular test is not routinely recommended in mildly affected or atypical cases, except for relatives of a clinical diagnosed AGS patient. No genotype-phenotype correlation exists between clinical manifestations of AGS and JAG1 mutations. However, our data seems to suggest a correlation between the severity of hepatic manifestation and type of mutation because the 3 patients with missense mutation have a mild liver disease while the remaining eight patients underwent liver transplantation.

R1514Q substitution in Lrrk2 is not a pathogenic Parkinson disease (PD) mutation. *W.C. Nichols[1,5], D.K. Marek[1], M.W. Pauciulo[1], N. Pankratz[2], C.A. Halter[2], A. Rudolph[3], C.W. Shults[4,6], J. Wojcieszek[2], T. Foroud[2], The Parkinson Study Group - PROGENI Investigators* 1) Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 2) Indiana Univ. Medical Center, Indianapolis, IN; 3) Univ. of Rochester, Rochester, NY; 4) Univ. of California, San Diego, San Diego, CA; 5) Univ. of Cincinnati College of Medicine, Cincinnati, OH; 6) VA San Diego Healthcare System, San Diego, CA.

PD is the second most common neurodegenerative disorder. Clinical features include resting tremor, rigidity, bradykinesia, and postural instability. The fifth, most recently identified PD gene is leucine-rich repeat kinase 2 (*LRRK2*). Reports of pathogenicity the substitution of arginine 1514 with glutamine (R1514Q) have been conflicting. In our ongoing effort to identify additional PD susceptibility genes, we have recruited 954 PD patients from 429 families through 59 Parkinson Study Group sites located throughout North America. Sixteen of 954 (1.8%) PD patients from 12 different families were shown to be heterozygous carriers of the R1514Q variant. Of the 28 PD patients in these 12 families, 12 of them do not carry the R1514Q variant. No significant difference between the R1514Q carrier group (16) and the non-carrier group (938) was detected for age of disease onset, disease duration, MMSE score, Blessed Functional Activity Scale, Hoehn and Yahr, and ethnicity. In addition, two of 92 (2.2%) control subjects were found to be heterozygous for R1514Q. Taken together, our data suggest that R1514Q is a non-pathogenic Lrrk2 variant that does not contribute to the development of PD. Due to their low frequency, penetrance estimates for most *LRRK2* mutations are not available. However, we believe data regarding pathogenicity, penetrance, and variability in age of onset for putative mutations are necessary for proper genetic counseling of patients undergoing genetic testing. This is especially critical for at risk individuals undergoing presymptomatic testing. We urge caution and due diligence in implementation of *LRRK2* genetic testing to ensure that the patients best interests are realized, and the risks of misinterpretation and potential harm are minimized.

The IKBL protein inhibits activation of gene expression by NF kappa B. *A. K. Mankan[1,2], J. Daly[1,2], E. Caraher[1,2], D. Kelleher[1,2], R. McManus[1,2]* 1) Department of Clinical Medicine, Trinity College Dublin, Dublin,Ireland; 2) Institute of Molecular Medicine, Dublin Molecular Medicine Centre, Trinity Centre for Health Sciences, Trinity College Dublin, Dublin, Ireland, 8.

The Inhibitor of NF Kappa B like (IKBL) gene encodes a protein homologous to the IKB family. A number of different polymorphisms in the gene have been reported to be associated with diseases such as Rheumatoid Arthritis, Diabetes Mellitus, Celiac Disease, Crohns Disease and Myocardial Infarction. One of these polymorphisms, in the E-box element of the IKBL promoter region, differentially binds to the E-box protein, E47, and the ubiquitously expressed transcription factor, USF1.

So far the exact function of IKBL protein has not been reported. Using a GFP tagged IKBL vector we have demonstrated, by both EMSA and Luciferase Assays, that over-expression of IKBL in HCT-116 cell lines inhibits the activity of NF kappa B.

We have further demonstrated, using an IL-8 promoter Luciferase construct that IKBL regulates the expression of the pro-inflammatory cytokine IL-8. We have shown that localisation of IKBL is limited to the nucleus in HCT-116 cells, but that there is no apparent interaction between IKBL and the p65 and p50 subunits of NF kappa B following stimulation of these cells with IL-1beta. Finally dissection of the protein shows NF kappa B inhibition to be dependent on the presence of the IKBL ankyrin repeats.

Expression of IKBL increases in cells stimulated with Dexamethasone and decreases with IL-1 Beta stimulation. This pattern also reflects the anti-inflammatory nature of the protein.

Our study suggests that IKBL is a member of the novel inhibitors of NF kappa B such as MAIL that are located in the nucleus and may inhibit the activity of NF kappa B by regulating the activity of other proteins that bind to NF kappa B within the nucleus.

Association of the IL18 gene with celiac disease in the Irish population. *R. McManus[1], K. Brophy[1], A. Ryan[1], C. Feighery[1], C. O'Morain[1], N. Kennedy[2], R. McLoughlin[1], F. Stevens[3], D. Kelleher[1]* 1) DMMC and University of Dublin, Trinity College, Ireland; 2) Dept. Medicine, University of Dublin, Trinity College, Ireland; 3) NUI, Galway, Ireland.

IL18 is a proinflammatory cytokine which promotes development of the Th1 lymphocyte response. IL18 is known to play an important role in inflammatory, autoimmune and infectious diseases. The aim of this study was to investigate any genetic association between IL18 and celiac disease in the Irish population. We genotyped 395 celiac disease patients and 354 controls for 4 SNPs within IL18. SNPs were chosen to allow a haplotype tagging approach based on previously defined haplotypes consisting of 27 SNPs as characterised in a European population. SNPs were also chosen based on potential effects on protein structure or expression. Haplotypes were constructed computationally and compared between groups. Three SNPs in IL18 (IL18_S2-137_rs187238, IL18_S3_rs5744241 and IL18_S4-607rs1946518) showed a significant association with disease either at allele frequency, genotype frequency or carrier status level prior to correction for multiple testing. IL18-137 was significantly associated with disease at the genotype level (p=0.0380). IL18_S3 was significantly associated with disease when allele frequencies (p=0.0385) and carrier status (G allele; p=0.0490) were examined. IL18_S4-607 also showed a significant association at the allele frequency level (p=0.0100), the genotype level (p=0.0265), and at the allele carrier status level. (G allele; p=0.0159). Total gene haplotype analysis was carried out using all four SNPs. A significant association was observed for haplotype rs1946518T, rs187238G, rs2043055A, rs5744241A (p=0.0379). Analysis of the IL18 promoter SNPs (S4-607 and S2-137), which have been associated with altered IL18 expression, revealed a significant association for two promoter haplotypes (haplotype -607 G, -137 G) (p=0.0153), (haplotype -607 T, -137 G) (p = 0.0053). We have shown that IL18 is significantly associated with disease status at the level of haplotype and functional SNP allele and genotype distribution suggesting that IL18 is involved in the pathogenesis of celiac disease.

Computing power for association studies using DNA pooling with incorporation of genetic model parameters. *F. Ji[1], C. Haynes[1], N.R. Mendell[2], S.J. Finch[2], D. Gordon[3]* 1) Lab of Statistical Genetics, Rockefeller Univ, New York, NY; 2) Department of Applied Math and Statistics, Stony Brook University, Stony Brook, NY; 3) Rutgers University, Piscataway, Piscataway, NJ.

Power calculations for association methods using DNA pooling to date have focused primarily on the effects of machine-error, replicate size and the size of the pool. We extend previous work on these calculations by incorporating genetic model parameters as part of the design. We use the non-centrality parameter (NCP) for one way Analysis of Variance test to compute analytic power for genetic association tests with DNA pooling data on cases and controls. Parameters involved in the power calculation are disease allele frequency, SNP marker allele frequency of the marker SNP in coupling with the disease locus, disease prevalence, genotype relative risk, sample size, genetic model, number of replicates, number of individuals per pool, and proportion of total error variance due to machine error. Several significance levels are considered, including stringent significance levels (due to the increasing popularity of 100K and 500K SNP chip data). We examine the effects of these parameters on analytic power using a 2^k factorial design given two settings of each parameter. We perform regression analysis to examine what parameters most significantly affect power. The top four most significant parameters affecting power for association are: (1) genotype relative risk (F statistic1,466=840), (2) genetic model (F statistic1,466=549), (3) sample size (F statistic1,466=282), and (4) the interaction term between disease and SNP allele frequencies (F statistic1,466=97). Of relatively minor importance are the design parameters: number of the replicates of the pool (F statistic1,466=30), proportion of total variance due to machine error (F statistic1,466=6), and the number of individuals per pool (F statistic1,466=4). The optimal number of individuals per pool and the number of replicates of the pool is given as a function of machine error and the genetic error model parameters.

Association study of cerebrospinal fluid amyloid levels with late-onset Alzheimers disease associated polymorphisms. *J.S.K. Kauwe[1], A.M. Fagan[2], M.L. Spinner[2], A.R. Shah[2], K. Mayo[1], D.M. Holtzman[2,3], A. Grupe[4], A. Goate[1,2,3]* 1) Dept Psychiatry, Washington Univ, St Louis, MO; 2) Dept Neurology, Washington Univ, St Louis, MO; 3) Alzheimers Disease Research Center, St. Louis, MO; 4) Celera Diagnostics, Alameda, CA.

Amyloid (A) peptides are normal products of -amyloid precursor protein processing and can be detected in cerebrospinal fluid (CSF). It has been suggested that the aggregation of amyloid peptide into insoluble plaques in the brain is a central feature of Alzheimers disease (AD) pathology. Reduced levels of CSF A42 in AD CSF are one of the most consistent findings in the AD biomarkers field. In addition, low levels of CSF A42 have recently been shown to correlate with the presence of brain amyloid. We hypothesize that the use of CSF A levels as an endophenotype will lead to the identification of novel genetic risk factors for AD. To test this hypothesis we have genotyped 12 SNPs from strong candidate genes in 120 individuals for whom we have CSF A40 and A42 measurements. We genotyped 12 SNPs in these samples. Included in this group of SNPs are 10 SNPs, which are significantly associated with AD in the meta-analysis of published data provided at Alzgene.org and 2 SNPs that have shown association with late onset AD in multiple datasets from our own studies. CSF A levels were adjusted for age, disease severity (based on CDR), and presence or absence of the APOE4 allele by regression. Due to the large effect of gender on the levels of both A40 and A42, the genetic analyses were stratified by gender (Males, N=50; Females, N=70). Genotypes at these SNPs were tested for association with levels of CSF A40, A42, as well as the ratio of A42/A40 using ANOVA. In males an uncorrected p-value of 0.004 was observed between rs4877365 (DAPK1) and the ratio of A42/A40. In females rs440446 (APOE) showed significant association with A40 levels (uncorrected p-value = 0.008). These findings suggest that these polymorphisms may affect risk for AD through an A related mechanism. We are currently genotyping these SNPs in additional samples to increase our power to detect association with CSF A levels.

Molecular dissection of NF1-related disease features in segmental NF1 patients. *O. Maertens[1], S. De Schepper[2], J. Vandesompele[1], S. Janssens[1], F. Speleman[1], E. Legius[3], L. Messiaen[1, 4]* 1) Center for Medical Genetics, Ghent University, Ghent, Belgium; 2) Department of Dermatology, Ghent University, Ghent, Belgium; 3) Department of Human Genetics, Catholic University Leuven, Leuven, Belgium; 4) Department of Genetics, University of Alabama at Birmingham, Birmingham, United States.

Within segmental neurofibromatosis type 1 (NF1) different clinical subtypes emerge. Analysis of these phenotypes may provide insight into the cell types and mutational mechanisms involved in the development of particular NF1-related disease features. For this purpose three segmental NF1 patients with different clinical manifestations were investigated at the molecular level.

The first patient presented with several café-au-lait macules (CALMs) within a pigmented background. MLPA analysis revealed an *NF1* microdeletion exclusively present in the melanocytes. A second alteration in the wild-type *NF1* allele was only detected in the melanocytes of the CALM. The second patient had several neurofibromas. Mutation screening on cultured Schwann cells derived from two neurofibromas revealed an identical mutation in addition to two tumor specific alterations. Real-time qPCR demonstrated low percentages of the first hit in fibroblasts derived from both neurofibromas. The third patient had CALMs and several neurofibromas located on the hand within a hyperpigmented background. Analysis of neurofibroma derived Schwann cells and melanocytes derived from the hyperpigmentation lesions revealed an identical *NF1* mutation in all samples. Moreover, LOH was detected in the melanocytes. Real-time qPCR revealed low percentages of the intragenic *NF1* mutation in fibroblasts derived from both the neurofibroma and hyperpigmented area. Fibroblasts derived from the peripheral CALM were negative.

These data confirm the tumorigenic properties of Schwann cells in neurofibroma development and demonstrate for the first time that bi-allelic *NF1* inactivation in melanocytes might be an important trigger for NF1-related CALM development. Interestingly, both disease features arise even within a background containing predominantly $NF1^{+/+}$ cells.

Understanding genetic heterogeneity in kidney disease among African American patients. *M. Orloff[1], J. Sedor[2,3], C. Winkler[4], J. Schelling[2,3], J. Kopp[5], S. Iyengar[1]* 1) Epidemiology & Biostatistics, Case Western Reserve Univ, Cleveland, OH; 2) Department of Medicine, Case Western Reserve Univ, Cleveland, OH; 3) Kidney Disease Research Center, MetroHealth System, Cleveland, OH; 4) Laboratory of Genomic Diversity, NCI, Frederick, MD; 5) Kidney Disease Section, NIDDK, NIH, Bethesda, MD.

Focal Segmental Glomerulosclerosis (FSGS), a common kidney disorder, often progresses to renal failure; is caused by diverse etiologies, and has significant genetic heterogeneity with each disease allele having modest effect. To test if the joint effect of variations in Wilms tumor telomeric neighbor 1 (WIT1), Wilms tumor (WT1), Wilms tumor interacting protein (WTIP), a WT1 transcriptional repressor, and Wilms tumor associating protein (WTAP) genes mediate susceptibility to idiopathic FSGS, 19 SNPs in 499 (Cases=218, Controls=281) African American (AA) subjects, in a multicenter study, were genotyped. WT1 controls differentiation of podocytes, a critical determinant of the filtration barrier; WTIP and WTAP directly interact with WT1 and regulates its function. After adjusting for population stratification, single locus and haplotype analyses showed rs6508, a nonsynonymous SNP in WIT1, and 5region SNPs in the WT1 promoter, associated with FSGS (p-value= 0.001). SNPs in the WTIP LIM domain (p-values= 0.025 and 0.048) and at the 5 end of the WTAP (p-values= 0.012 and 0.047) genes also were associated with FSGS. Cladistic and hierarchical cluster analyses of each of the four genes revealed significant associations of haplotype groups and interaction effects of diplotypes, which either share a recent common ancestor or have similar haplotype profiles. Significant interactions between WIT1, WT1, WTAP and HIV-1 status were also observed (p-value= 0.001). Interestingly, HIV-1 status and gender were additional factors contributing to the heterogeneity influencing the FSGS phenotype. Using models, we show that the genetic variants of four genes from a single pathway interact with gender and HIV-1 status to influence FSGS. Multiple risk alleles in WT1 and genes encoding proteins that regulate its function may combine to cause FSGS in AAs..

A novel method for rapid carrier screening of Fragile X syndrome. *D. Huang[1], Y. Li[1], J. Rooke[2], S. Potts[1], W. Sun[1], M. McGinniss[1], C. Strom[1]* 1) Quest Diagnostics Inc., San Juan Capist, CA; 2) U.S. Genomics, Woburn, MA.

Fragile X syndrome (FX) is the most common cause of inherited mental retardation, seen approximately 1 in 4,000 males and 1 in 8,000 females. The mutation responsible for FX involves the expansion of CGG repeats in the FMR-1 gene on the X chromosome. Pilot studies have revealed carrier frequencies 1:70, 1:113 and 1:259 in British, Israeli and French Canadian populations respectively. Since FX is an X-linked disorder, population- based carrier screening would have a higher yield than already implemented programs for Cystic Fibrosis, Tay Sachs Disease and Canavan Disease. Current FX carrier testing involves a combination of PCR and Southern blot analysis in order to detect all possible carrier range repeat sizes. This procedure is not suitable for high throughput automated testing that would be required for population-based carrier screening. We developed a novel method of automated, rapid detection of all FX carriers. Genomic DNA is prepared in 96 well microtiter plates format on an automated DNA purification robot and then digested with restriction enzymes yielding fragments containing the CGG repeats. DNA is fractionated by capillary electrophoresis, and fractions are collected corresponding to expected sizes for normal, premutation and expanded alleles. Each fraction is further restriction-digested to release the 3 flanking sequence from the repeats, and a multiplex fluorescent PCR is then performed to detect the presence of the flanking sequence in each fraction. In addition, restriction fragments with sizes reflective of each collected fraction are co-amplified in the PCR reaction to serve as internal controls for both the capillary fractionation as well as the PCR process. This assay detected 24 known female carriers and 41 normals with no false positives or false negatives. This series contained 5 carriers with premutations and 19 affected patients with expanded allele ranging from 250 to 1450 repeat. The results are completely concordant to the results of Southern blotting. This method is robust, automated, high throughput and accurate enough to enable population based carrier screening for FX.

USF1 and coronary heart disease; Allelic imbalance of a transcription factor results in differential regulation of lipid metabolism- and immune genes. *J. Naukkarinen[1,2], V. Lyssenko[3], L. Groop[3,4], M-R. Taskinen[4], L. Peltonen[1,2,5]*
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We recently reported the association in Finnish families of allelic variants of USF1 with FCHL, a common dyslipidemia predisposing to cardiovascular disease (CVD) (Pajukanta et al, 2004). This association with CVD was also replicated in a large prospective follow-up study of a Finnish population sample (Komulainen et al, 2006). We here defined for the first time, allele-specific expression differences of this low-abundance transcript USF1 in subject fat-biopsies by monitoring for allelic imbalance in subjects heterozygous for the best associating SNP, located in the 3-UTR of the gene. Quantitative sequencing of genomic- and cDNA revealed an average of ~20% lower expression of the risk-allele of USF1 in 10 out of 13 samples tested. In a set of 47 expression arrays of subject fat-biopsies, differential expression of numerous USF1 target genes was evident -among them many genes of lipid metabolism and immune-response. Interestingly, several genes in the 1q locus, flanking USF1 also showed allele-dependent expression, most notably the neighboring F11R gene. This suggests the possibility of a strong cis-regulatory element controlling the expression of regional genes. These findings are currently being confirmed in muscle tissue biopsies -another relevant target tissue for dyslipidemia and the development of CVD. In summary, common non-coding polymorphisms of the the USF1 transcription factor, with risk-alleles decreasing its expression, result in differential expression of numerous target genes and predispose carriers of risk alleles to dyslipidemia and coronary heart disease.

A Hybrid Bayesian Method for Detecting Multiple Causal Variants from Genome-Wide Association Studies. *C.J. Hoggart[1], T.G. Clarke[1], M. De Iorio[1], J.C. Whitaker[2], D.J. Balding[1]* 1) Department of Epidemiolgy and Public Health, Imperial College, London; 2) Epidemiolgy and Public Health, London School of Hygiene and Tropial Medicine.

Testing one SNP at a time does not fully realise the potential of genome-wide association studies to identify multiple causal variants of small effect, which is a plausible scenario for many complex diseases. Moreover, many simulation studies assume a single causal variant and so more complex realities are ignored. Analysing large numbers of variants simultaneously is now becoming feasible, thanks to developments in Bayesian stochastic search methods. We combine Bayesian shrinkage methods together with a local stochastic model search to identify complex interactions, both local and distal. Our approach can analyse up to 10,000 SNPs simultaneously, and leads to multiple potential disease models each with an associated probability. We illustrate its power in comparison with a range of alternatve methods, in simulations that incorporate multiple causal loci, acting singly or in interacting pairs, among 4,000 SNPs in a 20Mb region. We argue that, implemented in a two-stage procedure, our hybrid Bayesian analysis can provide a powerful solution to the problem of extracting maximal information from genome-wide association studies.

Identification of der(1;19)(q10;p10) in 5 oligodendrogliomas suggests mechanism of concurrent 1p and 19q loss characteristic of this tumor. *L. Morsberger, K. Murphy, P. Burger, C. Griffin* Dept of Pathology, Johns Hopkins University, Baltimore, MD.

Deletions of portions of chromosomes 1p and 19q are closely associated with the oligodendroglioma (OG) histological phenotype. In most cases, 1p and 19q are co-deleted, often involving the entire chromosomal arm, yet the mechanism of dual loss is unexplained and the few reported metaphase cytogenetic analyses of OG have not elucidated this. We have analyzed 5 cases of OG (WHO grade III) in which metaphase cytogenetics suggests a possible mechanism for concurrent 1p and 19q loss. The G-banded metaphases of 4 OGs were near diploid and included a derivative chromosome consisting of what appears to be the whole arms of 1q and 19p joined at the centromere forming a der(1;19)(q10;p10). A fifth case was near tetraploid and contained 2 or more copies of the derivative chromosome. Metaphase FISH with 1p, 1q, 19p, and 19q probes (Vysis/Abbott Molecular) confirmed loss of 19q and 1p and that the derivative chromosome was composed of 1q and 19p material in 3 cases; in 2 cases with few metaphases, FISH was limited to the 19p/19q probe set where we found loss of 19q, and presence of 19p material on the derivative chromosome. In all cases, interphase FISH showed net loss of 1p and 19q in 77-92% of cells. Microsatellite studies were consistent with 1p and 19q loss in 4 cases and are pending in 1. To our knowledge this is the first report of metaphase cytogenetic analysis of OG identifying a derivative chromosome comprised of 1q and 19p. We hypothesize that the following occurs in OG: formation of a balanced whole-arm translocation between chromosomes 1 and 19, forming two derivative chromosomes, one composed of 1q and 19p, the other composed of 1p and 19q. Subsequent loss of the der(1;19)(p10;q10) would then result in the simultaneous 1p and 19q loss observed in OG, with retention of the der(1;19)(q10;p10) as observed in these 5 OGs.

Multivariate distance based methods for testing and accommodating population substructure. *C.M. Nievergelt, J.R. Kelsoe, C. Shimizu, J.C. Burns, N.J. Schork* UCSD, La Jolla, CA.

It is well known that ignoring cryptic population structure, which arises from mechanisms such as genetic drift, admixture, and assortative mating, can have negative impacts on association studies by generating spurious results and/or mask the effect of truly disease-associated loci. Anecdotal information about population history often exists, but the precise effects of events such as migration or mating patterns on an individuals gene pool requires a more detailed empirical assessment. Self-identified ethnicity contains some information, but is often not detailed enough, especially in admixed populations such as African and Mexican Americans. Sophisticated model-based methods to assess individual ancestry have been developed and successfully applied (e.g., maximum likelihood and bayesian methods). They usually require 1) prior knowledge of the populations ancestry, 2) DNA from ancestral populations, and 3) availability of specific, ancestry informative markers (AIMs). Study subjects are typically genotyped on 50 - 100 AIMs. High costs associated with the collection and phenotyping of study subjects in combination with decreasing genotyping costs have resulted in the availability of hundreds or thousands of genetic markers (e.g., microsatellites, SNPs) genotyped for specific candidate genes or in whole genome linkage and association studies, of which only very few will be associated with disease. Here we describe intuitive distance based methods to test and accommodate population substructure that make use of these available genotypes. We applied these methods to large cohorts genotyped on several hundred markers, such as a collection of mostly European American multi-generational bipolar families, and multi-ethnic studies such as the Family Blood Pressure Program including unrelated hypertensives, and a Kawasaki disease candidate gene association study. Our results show evidence for significant substructure and we consider methods to incorporate this information into association studies. Our procedures are powerful and comparable to model-based methods, make use of any available genotype data, and can be applied retrospectively to existing datasets.

Attitudes Regarding Genetics and Genetic Testing: A Pilot Community Survey. *V. Henrich[1], C. Christianson[1], K.P. Powell[1], S.E. Hahn[2], D. Spoon[1], D. Bartz[1], S. Blanton[2], P. Lietz[3], J. Vance[2], M. Pericak-Vance[2]* 1) Univ North Carolina Greensboro; 2) Duke University, Durham, NC; 3) Moses Cone Health System, Greensboro, NC.

The Guilford Genomic Medicine Initiative is developing a survey to ascertain the general populations knowledge and attitudes regarding genetic testing. Survey questions were developed from community focus group transcripts. As part of the development process a pilot survey was administered to 301 college students. Respondents correctly answered questions about the importance of family history (93%), age at diagnosis (83%), and race (82%) as disease risk determinants. Most knew that gene-environment interactions underlie disease risk (88%), and that breast cancer can be inherited from both sides of the family (89%). The vast majority (91%) thought everyone with a family history of a disease would benefit from genetic testing. Over half (60%) thought genetic testing was appropriate for everyone with breast cancer. Almost half (46%) knew that pharmacogenetic tests exist. Three factors were extracted using a principal component analysis of the surveys attitude assessment portion. Responses in this section suggested that most students are comfortable with genetic testing (87%) and think it should be done (86%). The majority (90%) would have a genetic test if they had a family history of cancer. Most students are not worried about discrimination (79%) or privacy issues (75%). A majority (89%) knew that North Carolina laws prevent discrimination by employers, and 64% believe the government will protect them from discrimination. Over half (60%) of respondents think insurance companies will use genetic tests to determine health insurance rates. Conclusions: Respondents are generally knowledgeable about the benefits of family history, but education in the area of genetic testing is warranted. Attitudes towards testing are generally favorable. Students apparently are confident that government agencies will protect them from discrimination based on genetic testing, but are concerned about insurance discrimination. Based on the pilot, a community wide survey will be conducted in summer 2006.

Effects of carbonic anhydrase 8 deficiency on cerebellar gene expression profiles in the wdl mouse. *Y. Jiao[1], J. Yan[2], F. Jiao[1], H. Tu[1], J. Stuart[2], L.R. Donahue[3], W.G. Beamer[3], X. Li[4], B. Roe[5], M.S. LeDoux[6], W. Gu[1]* 1) Orthopaedic Surgery, Univ Tennessee HSC, Memphis, TN; 2) Department of Medicine, University of Tennessee Health Science Center, Memphis, TN; 3) The Jackson Laboratory, Bar Harbor, ME; 4) Functional Genomics Facility, University of Chicago, Chicago, IL; 5) Department of Chemistry and Biochemistry, University of Oklahoma, Norman, Ok; 6) Departments of Neurology and Anatomy & Neurobiology, University of Tennessee Health Science Center, Memphis, TN.

The waddles (*wdl*) mouse is a unique animal model that exhibits ataxia and appendicular dystonia without pathological abnormalities of either the central or peripheral nervous systems. Recently, we detected a 19 bp deletion in exon 8 of the carbonic anhydrase-related protein VIII gene (Car8) within the wdl locus on mouse Chr 4 from wdl mice by high-throughput temperature gradient heteroduplex analysis (Jiao et al., Genetics. 2005 Nov;171(3):1239-46). Although regarded as a member of the carbonic anhydrase gene family, the encoded protein (CAR8) has no reported anhydrous enzymatic activity. However, CAR8 has recently been reported as an inositol triphosphate receptor 1-binding protein expressed at high levels in cerebellar Purkinje cells. Our study shows that, in +/+ mice, CAR8 is abundantly expressed in cerebellar Purkinje cells as well as several other cell groups. Compatible with nonsense-mediated decay of mutant transcripts, CAR8 is virtually absent in wdl mice. To examine the molecular aberrations contributing to motor dysfunction in wdl mice, cerebellar gene expression profiles were examined and key genes were confirmed by qPCR. Our data indicate that genes involved in signaling, cell division, zinc-ion binding, synapse integrity and plasticity are down-regulated in wdl mice. Gene expression profiling also detects several up-regulated genes which encode proteins that function in the Golgi apparatus, suggesting that CAR8 deficiency has an important impact on synapse vesicle formation and transport.

Chondrodysplasia Punctata in Infants of Mothers With Autoimmune Diseases. *V. Kirkland[1], U.T. Sundaram[1], G. Brookshire[2], M. Nino[3], M. Bober[4], N. Braverman[5]* 1) Dept Human Genetics, Virginia Commonwealth University, Richmond, VA; 2) Children's Medical Ctr, Dallas, TX; 3) NIH/NIDA, Baltimore, MD; 4) DuPont Hosp for Children, Wilmington, DE; 5) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins Med Ctr, Baltimore, MD.

Chondrodysplasia punctata (CDP) is a skeletal dysplasia characterized by punctate calcific stippling in multiple epiphyses. It can be inherited in an autosomal or X-linked, dominant or recessive manner. In addition, there are phenocopies consequent to maternal Warfarin exposure and other vitamin K deficiency states in pregnancy. There are ~10 case reports that link maternal SLE and a fetus with CDP; the clinical picture resembles that seen in CDP caused by gene changes or maternal Warfarin use. We present two additional cases associated with non-SLE maternal autoimmune diseases. The first is a female infant of a mother with Sjogrens disease. Prenatal ultrasound at 23 weeks showed a 3 week delay in growth with shortened femurs and humeri, depressed nasal bridge and punctate calcifications involving numerous epiphyses. The infant was delivered prematurely. Examination showed a depressed nasal bridge with small upturned nares, absent skin ichthyosis, small hands with brachytelephalangy, stippled epiphyses and rhizomelia. She developed pneumonia and expired shortly. The second is a 2 yo male born to a mother with an unclassified autoimmune renal disease, Graves disease and rheumatoid arthritis. Postnatally, the infant was noted to have generalized CDP, rhizomelia and brachytelephalangy. He also had hearing loss, hypospadius and hypothyroidism. Testing on both infants excluded most known etiologies for CDP and included normal RBC plasmalogens, plasma 8(9)-cholestenol levels, karyotype analysis and for case 2, mutation analysis of Arylsulfatase E, a vitamin K dependent enzyme. Eye exams were normal. These cases highlight the possibility that a factor common to several autoimmune diseases relates to CDP. Comparison of maternal autoantibodies amongst these cases may provide clues to the underlying mechanism of CDP. Knowledge about this association will help management of pregnancies with maternal autoimmune diseases.

A reliable, quantitative method for GSH and GSSG determination using tandem mass spectrometry. *A.K. Niemi*[1], *G.M. Enns*[1], *T. Kwan*[3], *K.R. Atkuri*[2,1], *T.M. Cowan*[3,1] 1) Dept Pediatrics, Stanford University, Stanford, CA; 2) Dept Genetics, Stanford University, Stanford, CA; 3) Dept Pathology, Stanford University, Stanford, CA.

Glutathione (-glutamylcysteinylglycine, GSH) is important in the defense against oxidative stress, as occurs with mitochondrial disorders and organic acidemias. Decreased GSH may play a role in the pathophysiology of these and other disorders, although this has been difficult to demonstrate in practice because of its inherent instability and propensity to autooxidize to GSSG. We have developed a rapid, reliable method for measuring GSH and GSSG in whole blood using tandem mass spectrometry (MS/MS) that has widespread clinical applicability. Blood is mixed in a 1:5 ratio with sulfosalicylic acid, N-ethylmaleimide and EDTA in a single preparative step. After centrifugation, supernates are mixed with stable-isotope standards of GSH and GSSH, and are analyzed without column separation by MS/MS. Results are linear(r^2>0.9999) over the ranges of physiologic normal for both analytes, with intra- and inter-assay CVs < 3% for GSH, and 14% and 27% respectively for GSSG. GSH and GSSG are stable in refrigerated whole blood for 3 days, and in deprotienized supernatants stored at -80C C for at least 3 months. This method was used to evaluate 19 metabolic patients (mitochondrial myopathies, organic acidemias, cystinosis and G6PD deficiency) and 22 normal controls. GSH and GSSG levels in the metabolic patients, the majority of whom were clinically well, did not differ from normal controls (GSH normal mean, SD = 1115, 187; GSSH normal mean, SD = 1.6, 1.3). The patient with G6PD deficiency, evaluated during an acute crisis, had significantly decreased GSH (95.2M)) and mildly increased GSSG (4.1M). Results compare favorably to those using Hi-D fluorescence activated cell sorting (FACS), previously reported for evaluating markers of oxidative stress in metabolic patients. These studies will be extended to the broader evaluation of metabolic patients, as well as to more common conditions (e.g., diabetes, neurodegenerative disorders) for which oxidative stress may play an important role in pathogenesis.

Y Chromosome and Mitochondrial DNA Analysis of First Settlers of the Chesapeake Bay's Hoopers Islands to Investigate the Presence of Native American Lineages. *H. McCammon, U.A. Perego, J. Ekins, K. Ritchie, N. Myres, R. Hughes, N. Angerhofer, I. Masannat, S.R. Woodward* Sorenson Molecular Genealogy Foundation, SLC, UT.

The first European(-made) map of the Chesapeake Bay dates to 1608 and included three islands known as Hoopers Islands (Upper Hoopers Island, Middle Hoopers Island and Lower Hoopers Island). The first colonial families (Creighton, Gibson, Hooper, Meekins, Parker, Phillips, Price, Ruark, Tolley, Travers, Wallace, and Woodland) began settling on the island in 1659, when the Chesapeake Bay became an important fur-trading center. Oral histories surviving to this day purport early admixture with the islands' natives. Genetic analysis can support the legitimacy of such historical accounts as they are reflected in the extant genetic composition of the descendants of Hoopers Islands' first settlers. The present analysis correlates genetic data to oral and written genealogical records to estimate the degree of Native American admixture present in today's descendants. 43 Y chromosome STR loci and mitochondrial DNA sequences from HVR1 and HVR2 were analyzed from a dataset of over 100 descendants of the first settlers and compared to known Native American haplogroups. The combined Y chromosome and mitochondrial DNA analysis revealed a complex genetic history of Hoopers Islands.

Formalization of matching strategies for duplicated Y chromosome STR loci. *L.A.D. Hutchison[1,2], N.M. Myres[2], K. Ritchie[2], R. Hughes[2], N. Angferhofer[2], J. Ekins[2], B.A. Berger[1], S.R. Woodward[1]* 1) Computational Biology Lab, CSA, MIT, Cambridge, MA; 2) Sorenson Molecular Genealogy Foundataion, Salt Lake City, UT.

Two different alleles are typically observed at duplicated Y chromosome STR loci such as DYS385 and DYS459. Current cost-effective genotyping techniques do not yield information about the originating locus homolog for each allele of a duplicated-locus genotype. Though several of these loci are in common use, the literature employs a variety of different approaches for counting matches and mismatches at these loci. It has also been debated as to whether a duplicated locus should be treated as one locus or two, and a formal analysis of the relative merits of different approaches has not previously been performed. In this research we analyze the error rate of various matching strategies for duplicated-locus genotypes, and compare the observed number of matches between two genotypes to the actual number of matches that would be expected if locus copies were distinguishable. We are able to measure the error rate of various matching strategies by pairing non-duplicated Y chromosome STR loci to produce a pseudo-duplicated genotype consisting of alleles of known origin. We show that there is a dramatic difference (an order of magnitude) in the error rate of various matching strategies that on the surface all seem somewhat plausible. We formalize a new matching strategy that delineates exactly when a duplicated-locus match or mismatch may be unambiguously treated as two loci, and when it cannot be treated as two different loci without possible spurious matching. The error rate of the new matching strategy in predicting actual number of matches from observed number of matches is measured and shown to be significantly lower than the alternatives. We suggest how to further lower the error rate, and discuss the extension of this technique to the related problem of the matching of autosomal loci. This work should enable improved accuracy of genotype matching in several fields such as forensics, genetic genealogy and population genetics.

IGF1 and IGFBP3 gene polymorphisms are associated with plasma levels and prostate cancer risk in African Americans. *R.A. Kittles[1], C. Grenade[1], C. Bonilla[1], E.R. Santos[1], W. Hernandez[1], C. Ahaghotu[2]* 1) Ohio State Univ, Columbus, OH; 2) Howard Univ, Washington, DC.

IGF1 is a strong inhibitor of apoptosis and mediates the effects of growth hormone. The interaction between IGF1 and its receptor is predominantly regulated and directed to its target tissues by IGFBP3. In addition to regulating and protecting IGF1 from proteolytic degradation, IGFBP3 can mediate apoptosis independent of IGF1. Previous studies have linked IGF1 and IGFBP3 plasma levels to cancer risk. Here, we investigate the relationship between a C/T SNP (rs7965399) and dinucleotide repeat $(CA)_n$ within the 5 region of the IGF1 gene, the -202 A/C SNP in the IGFB3 gene, and corresponding plasma levels and prostate cancer risk (Pca) in 767 African Americans (AAs) enrolled in a clinic-based case-control study at Howard University Hospital. Total IGF1 and IGFBP3 levels were measured using ELISA and the polymorphisms were typed in all Pca cases (n=401) and healthy age and ethnicity matched controls (n=366). Multiple linear regression and multivariable unconditional logistic regression were used to test for associations between genotypes and circulating IGFs (including molar ratio of IGF1/IGFBP3) and Pca risk, respectively. No relationship was observed for plasma IGF levels and Pca risk. We did observe that the IGBP3 -202 C allele was strongly correlated with decreased IGFBP3 plasma levels (3,532ng/ml versus 3,106ng/ml; P=0.008). This result is in strong agreement with a previous study examining -202A/C and IGFBP3 levels. More importantly, we observed a two-fold increase in Pca risk for individuals homozygous for the IGBP3 -202 C allele (OR=2.1; 95%CI=1.2-4.3). Contrary to previous findings in European Americans, no association with Pca risk was observed for IGF1 $(CA)_n$ repeats or the IGF1 rs7965399 SNP in AAs. This suggests that different genetic and environmental factors may influence IGF1 plasma levels and Pca risk across populations and reveals the need to fully explore IGF1 and IGFBP3 genetic variation across diverse populations. Our study adds further clarity and support to previous findings implicating plasma IGFBP3 levels and IGFBP3 -202A/C in prostate carcinogenesis.

Genetic and neural basis of Williams syndrome in a six-member family. *J.R. Korenberg[1], X.-N. Chen[1], U. Bellugi[2], P.S. Eis[6], A.M. Galaburda[3], D.L. Mills[4], A.L. Reiss[5], T.A. Richmond[6], R.R. Selzer[6]* 1) Depts Medical Genetics/Pediatrics, Cedars-Sinai Med Cntr/UCLA, Los Angeles, CA; 2) Salk Institute Laboratory for Cognitive Neuroscience, La Jolla, CA; 3) Dept Neurology, Harvard Medical School, Boston, MA; 4) Dept Psychology, Emory University, Atlanta, GA; 5) Stanford Psychiatry Neuroimaging Laboratory, Dept Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, CA; 6) NimbleGen Systems, Inc, Madison, WI.

Williams syndrome (WS) is a compelling model for understanding the genetic origins of human cognition and behavior. To focus hypotheses on the genetic contributions to brain development and adult function of single genes or clusters in the WS region, we used a multidisciplinary approach to characterize the genes deleted in a large cohort of persons with WS. We identified a small subset of persons, each with a smaller, unique deletion, and previously showed that part of the variability of WS visual-spatial processing correlated with deletion of the genes encoding GTF2I and GTF2IRD1. Further, parental origin of the deletion contributed to the variation in gene expression. We now report the analysis of a six-member family with a subset of WS features associated with a smaller deletion. We used multicolor fluorescence in situ hybridization (FISH) with a BAC array, combined with somatic cell hybrids and quantitative RTPCR, to show a 500 kb deletion including the genes ABHD11, CLDN3, CLDN4, WBSCR27, WBSCR28, ELN, LIMK1, WBSCR1, LAT2, and RFC2 in all six family members. Further, we mapped the breakpoints of this deletion at exon-level resolution using oligonucleotide array CGH [Selzer et al. (2005) Genes Chromosomes Cancer 44:305] with a probe tiling-path design of Chr 7 (isothermal probes with Tm = 76C; median probe spacing of 135 bp). We combined these data with sequence-specific haplotype analyses to show the contribution of each of the maternal chromosomes 7 to different siblings. Combining genetic and cognitive data strongly support the hypothesis that the genes deleted in this family contribute more subtlety than GTF2IRD1 or GTF2I to the typical cognitive and physical features of WS.

Deletion or duplication of multiple exons account for a significant percentage of mutant *GLDC* alleles in glycine encephalopathy. *B. Kuchinka[1], J. Toone[2], J. Mitchell[3], R. Martin[4], K. Martin[4], D. Applegarth[2], B. Casey[1,2]* 1) Children's Hospital, Vancouver, British Columbia; 2) University of British Columbia, Vancouver, British Columbia; 3) McGill University, Montreal, Quebec; 4) Washington University, St. Louis, Missouri.

Glycine encephalopathy, also known as non-ketotic hyperglycinemia (NKH), results from impaired glycine metabolism and is characterized biochemically by an increased ratio of CSF:plasma glycine. Hepatic enzyme analysis, though the diagnostic gold standard, requires a large biopsy, and prenatal diagnosis utilizing CVS has both false positives and false negatives. We have developed a DNA-based assay for this disorder that encompasses direct resequencing, deletion/duplication detection utilizing multiplex ligation-probe amplification (MLPA), and allele tracking with polymorphic repeats. Of 24 cases analyzed to date, we identified 3 with *AMT* mutations and 18 with *GLDC* mutations. The *GLDC* nonsense mutation R424X appeared in three probands of different ethnic and geographic backgrounds, suggesting the possibility of a mutation hotspot. Five multi-exon deletion alleles were identified as well as the first reported multi-exon duplication within *GLDC*. These six alleles account for approximately 25% of the unique *GLDC* mutations. One of the deletions was shown to have occurred *de novo*, an event initially suggested by non-mendelian inheritance of polymorphic alleles and one that may be unexpectedly common. The combination of direct resequencing, deletion/duplication detection by MLPA, and allele tracking will provide a valuable alternative or complement to standard confirmatory or prenatal enzyme diagnosis.

Direct isolation of short sequence length polymorphisms in 3' flanking sequences of Alu repeats for microarray analysis. *J.J. Jonsson[1,2], H.G. Thormar[1,2], B. Gudmundsson[3], G.H. Gunnarsson[2,3], M.H. Halldorsson[4], Y. Vigfusson[4], H. Thorgeirsson[4]* 1) Dept Genetics/Molecular Med, Landspitali-Univ Hosp, Reykjavik, Iceland; 2) Dept Biochem/Mol Biol, Faculty of Medicine, Univ of Iceland, Reykjavik, Iceland; 3) BioCule Inc., Reykjavik, Iceland; 4) Dept Computer Science, Univ of Iceland, Reykjavik, Iceland.

Approximately 1.2 million Alu repeats are distributed over the human genome. A fraction of them contains short sequence length polymorphisms (SSLPs) in their adenine-rich 3' flanking (Alu3F) sequences. We developed a method for direct isolation of Alu3F-SSLPs based on two-dimensional conformation-dependent gel electrophoresis (2D-CDE) (Gunnarsson et al, Nucleic Acid Res 2004:e23). The Alu3F sequences were selectively amplified with complex PCR (Alu3F-PCR) on DNA samples from ten individuals. The amplicons were pooled, denatured and renatured. Mismatched heteroduplexes were isolated from perfectly matched homo- and heteroduplexes using 2D-CDE and cloned. Up to 80% of sample Alu3F sequences isolated with this approach contained SSLP. We designed a microarray platform for large scale genetic studies of SSLPs in Alu3F sequences. We wrote a computer program CATTAGAT for virtual PCR reactions on the entire human genome.The program is accessible for use on www.genome.cs.hi.is and available under the terms of the GNU General Public License. This program simulates complex PCR reactions and extracts sequences between primer binding sites with or without mismatch and adaptors at restriction sites. Length restrictions can be specified. The program predicted that Alu3F-PCR amplifies 200 thousand sequences. Further software was created allowing automatic microarray probe design based on sequences extracted using GATTAGAT. These probe sequences have been used to construct oligomer microarrays (NimbleGene) to select optimized probes corresponding to Alu3F-SSLP in the human genome.

An Ethnic-Specific Polymorphism in Glutamate Cysteine Ligase Affects Cellular Survival Following Oxidant Injury. *T.M. Le[1], F.E. Barr[1], A.S. Willis[2], M.L. Summar[3]* 1) Division of Pediatric Critical Care Medicine, Vanderbilt Children's Hospital, Nashville, TN; 2) Department of Genetics, Johns Hopkins University, Baltimore, MD; 3) Division of Medical Genetics, Vanderbilt University, Nashville, TN.

Background: Polymorphisms within the gene encoding the catalytic subunit (GCLC) may affect an individual's ability to produce glutathione and respond to oxidant injury. Previously, we have identified 11 polymorphisms in the GCLC gene and demonstrated that some of these polymorphisms are associated with an increased incidence of post-operative pulmonary hypertension as well as increased markers of oxidant injury. Of note, only one of these polymorphisms was a nonsynonymous polymorphism (1384T), and this polymorphism was only found in individuals of African descent.

Objective: To determine the effects of the 1384T polymorphism on the viability of mammalian cells subjected to oxidant injury.

Design/Methods: Wild-type GCLC enzyme and the enzyme encoded by the 1384T polymorphism were expressed and used in an *in vitro* assay to produce -glutamylcysteine (-GC), a glutathione intermediate. Wild-type and 1384T GCLC enzymes were also overexpressed in MRC5 cells. These cells were exposed to H_2O_2 and cell viability was subsequently determined using Trypan blue staining.

Results: In an *in vitro* assay, the 1384T GCLC enzyme produced less -GC as compared to the wild-type enzyme. In addition, MRC5 cells overexpressing the 1384T variant enzyme had increased susceptibility to oxidant injury as compared to wild-type.

Conclusions: Mammalian cells that overexpress a nonsynonymous, ethnic-specific polymorphism in the GCLC gene have increased susceptibility to oxidant injury as compared to cells overexpressing the wild-type gene. This ethnic-specific polymorphism may predispose certain segments of the population of African descent to rapid glutathione depletion and increased cellular injury following oxidative stress.

Gene-Gene Interaction Models Identified for Alzheimer Disease using MDR-PDT. *L.W. Hahn[1], T.L. Edwards[1], J.R. Gilbert[2], M.A. Pericak-Vance[2], E.R. Martin[2], M.D. Ritchie[1]* 1) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 2) Center for Human Genetics, Duke University, Durham, NC.

Alzheimer disease (AD) is the most common form of progressive dementia in the elderly. It is a neurodegenerative disorder characterized by the neuropathologic findings of intracellular neurofibrillary tangles and extracellular amyloid plaques that accumulate in vulnerable brain regions. In this study, we examined Alzheimers susceptibility in 738 Caucasian families in 10 candidate genes using 48 SNPs. All cases met the National Institute of Neurological Disorders and Stroke/Alzheimer Disease and Related Disorders Association (NINDS/ADRA) criteria for probable or definite AD, and 82% of the families had a positive family history. The Multifactor Dimensionality Reduction Pedigree Disequilibrium Test (MDR-PDT) was used to search for single locus through four-locus gene-gene interaction models associated with AD. MDR-PDT is a novel computational approach for the detection of gene-gene interactions in pedigree data. MDR-PDT was performed in the full dataset, and in the dataset with the known risk locus Apolipoprotein E (APOE) removed so that it did not obscure less potent associations. Significant two-locus and three-locus interaction models were identified in the dataset without APOE. MDR-PDT detected a two-locus model between individual markers in the genes leucine rich repeat transmembrane 3 (LRRTM3) and angiotensinogen converting enzyme (ACE), $p = 0.009$. In addition, a three-locus model was detected which included the same markers in these genes and also a marker in the alpha 2 macroglobulin (A2M) gene, $p = 0.005$. These findings suggest a multilocus model among candidate genes influencing Alzheimer disease risk, as well as demonstrate the utility of exploring interactions in family data using MDR-PDT.

Improving Regulation with Genetic Information: The Case of Particulate Matter and Asthmatics. *C. Kramer*[1, 2], *A. Cullen*[1, 2], *E. Faustman*[1, 2] 1) University of Washington, Seattle, WA; 2) Center for the Study & Improvement of Regulation.

Government efforts to improve public health increasingly acknowledge the potential of genetic information as they set research strategies and policy to elicit the best possible science. Government agencies fund, develop, and use human health risk assessment, epidemiological, and toxicological studies to set regulatory standards on pollutants. These standards aim to protect the most susceptible citizens from harmful exposures. For example, we consider the case of asthmatics and exposure to particulate matter (PM). The federal Clean Air Act requires the Environmental Protection Agency to set National Ambient Air Quality Standards for criteria pollutants at levels to protect sensitive subpopulations from adverse health effects with an adequate margin of safety. Asthmatics, an identified subpopulation, may carry multiple genetic susceptibilities to disease onset and progression that are exacerbated by exposure to pollutants regulated under the CAA, such as PM. In an effort to evaluate and facilitate maximizing the potential of incorporating genetic information into health science used in regulatory standard setting, we reviewed government documents used in PM standard setting along side current developments in research on genetics and asthma. Given that asthma is a complex genetic disease with varied environmental and social influences, we developed a guiding paradigm to encourage health science on asthma genetics most relevant to supporting PM standard setting (or other air pollutants). We establish and use criteria specific to regulation to identify IL-13 as an exemplary candidate gene, or biomarker for susceptibility to asthma. We then illustrate within a decision analytic framework how association studies on the -1111 C/T promoter polymorphism and future scientific studies may improve decision making in PM standard setting. Our analysis identifies substantial research gaps in associating genetic information with asthma, PM exposure and related health effects. Consequently, we recommend a coordinated research strategy to assess effective regulatory protection of susceptible subpopulations, including those genetically predisposed to asthma.

Frequency of variants in 184 genes involved with metabolism and transport using a novel analytical platform. *R. Hockett[1], T. Daly[1], P. Hardenbol[2], X. Miao[2], C. Bruckner[2], R. Njau[1], N. Bauer[1], C. Dumaual[1], R. Haman[1], N. Lewin-Koh[1], S. Kirkwood[1]* 1) Dept Diag & Exper Medicine, Eli Lilly, Indianapolis, IN; 2) Affymetrix, South San Francisco, CA.

It is estimated that a significant fraction of therapeutic unresponsiveness and drug related adverse events is accounted for by differences in drug disposition. In addition, understanding drug metabolism and transport are increasingly important to the concept of personalized medicine. Currently available assay systems for genotyping patients measure only a fraction of the known drug metabolic enzymes and transporters (DMETs). To solve this problem, we developed a genotyping platform to comprehensively measure ~2,000 polymorphisms in the 184 known DMETs. The system uses Affymetrix Molecular Inversion Probes and Affymetrix Tag arrays. A subset of 29 genes (168 variants), with documented effects on drug disposition, have been validated for clinical use according to regulatory guidelines. Data on >1,000 patients, on all 2,000 variants, will be presented, with emphasis on the frequency of alleles in ethnic subsets. A summary of the comparison of data generated on this system to the literature will be discussed, highlighting the discrepancies uncovered. Finally, the potential applications of this tool will be outlined, including how the frequency of gene variants impacts building a patient database of known genotypes that could be recruited into new clinical trials.

Chromosome 2q37 Deletion Syndrome: Defining Clinical Features. *F. Lacbawan, M. Jones, A. Dutra, V. Rodriguez, S. Chandrasekharappa, E.S. Doherty* National Human Genome Research Institute, Bethesda, MD.

The chromosome 2q37 deletion syndrome is gaining increasing clinical recognition. Here, we describe molecular breakpoint-phenotype correlation for 10 patients (ages 3 to 16 years) with apparently pure chromosome 2q37 deletions. The karyotypes ranged from a cryptic deletion to del(2)(q37.1). The breakpoints ranged in size from 1 Mb to 10 Mb. We compared our clinical findings with more than 25 previously published genotyped cases of comparative size. The clinical gestalt that emerged may be helpful for evaluation and counseling of individuals with a pure terminal 2q37 deletion. The functional outcome was most affected by the presence of major congenital anomalies and clinically diagnosed autism. In our experience, patients with larger deletions tended to have more severe developmental delay and behavioral problems. Incomplete documentation in the literature gives partial support to this finding. Midline defects were not common, and no patient had holoprosencephaly. The AHO-like phenotype comprising short stature, round facies, obesity, and brachymetaphalangy is a useful mnemonic for clinical consideration of the 2q37 deletion syndrome. However, the mnemonic did not hold up well to closer scrutiny. Four patients with brachymetaphalangy had normal stature, and their facies were not always round. The facial dysmorphisms typical of the 2q37 deletion syndrome have been variably described in the past. In our experience, patients had thin, arched brows with deeply set eyes, a flat midface, hypoplastic nares, prominent columella, and a thin vermilion border. Individuals presenting with this facial gestalt and developmental delay should be evaluated for a 2q37 deletion. Molecular breakpoint analysis was performed with informative microsatellite markers. Results of further refinement of the breakpoints using molecular cytogenetic methods: oligonucleotide arrayCGH (Agilent Technologies) and FISH will also be presented.

High-density oligonucleotide array-based genotyping using whole genome amplified DNA. *M. Kibriya, F. Jasmine, I. Andrulis, E. John, J. Chang-Claude, H. Ahsan* Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY.

In large scale genome-wide single nucleotide polymorphism (SNP) association study, the quantity of available high quality genomic DNA (gDNA) is a practical problem. We therefore studied the feasibility of utilizing whole genome amplification (WGA) to overcome this issue. We also compared the conventional dynamic model (DM) and recently available BRLMM statistical algorithms for calling genotypes using high-density oligonucleotide array. We studied a set of 60 gDNA samples run on 60 early access Affymetrix Mendel Nsp Array chip containing 224,940 SNPs/ chip. The overall genotype call rate (meanSD) was significantly higher using BRLMM algorithm compared to DM algorithm (97.3% 1.37 vs. 89.5%3.36%, $p<0.001$). Concordance rate was 99.44%. We compared the genome-wide genotype call concordance using a total of 14 gDNA samples from 3 different centers (4 samples / center and 2 reference samples) and their corresponding 14 WGA samples on the same 224,940 SNPs. We used Repli-g DNA Polymerase for WGA from 25 ng gDNA. Overall, genotype call rate was 97.07% 1.55 for gDNA and 97.77% 0.87 for WGA DNA ($p=0.435$). There was no significant difference in call rates within the gDNA from different centers [96.66%1.04%, 96.161.86 & 97.671.59%, $p=0.405$] as well as within their corresponding WGA samples [98.17%0.41, 97.41%0.71 98.36%0.60, $p=0.109$]. We also did not observe any change in call rates using 60 g or 90 g of amplified PCR product of WGA samples for hybridization. Concordance between genotype calls from gDNA and WGA was 97.64% 1.29 without significant difference between centers [97.50%1.09, 97.001.41, 98.40%1.26, $p=0.32$]. Reproducibility, calculated as concordance in duplicate sample, was 99.45% for gDNA. Detailed characteristics of the small fraction of SNPs producing discordant calls for each of the comparisons will be presented in the meeting. Our study confirms that high density oligonucleotide array-based genotyping can yield reproducible data and WGA DNA products can be effectively used for genome-wide SNP analysis.

Genomic Copy Number Analysis Using High Density SNP Chips and Tiling Arrays. *B. Merriman, Z. Chen, M. Ogdie, S.F. Nelson* Deptartment of Human Genetics, University of California, Los Angeles, Los Angeles, CA.

Identifying genomic deletions or duplications can help localize genes that contribute to genetic disease or tumor progression. Traditional detection methods based on karyotping and FISH analysis have resolution limited to several megabases or larger, while the ongoing advances in DNA microarray technology are enabling rapid improvements in array-based copy number detection. Here we summarize the resolution limits, genomic coverage, and discovery rates from applying the highest density arrays presently available to a large number of human samples. Specifically, we apply Affymetrix 500k SNP chips and Human Genome Tiling arrays to detecting micro-deletions and duplications, with data based on more than 800 genomic samples run on 250k (or full 500k set) arrays, and selected samples run on Tiling arrays for breakpoint mapping. Based on these data, and using custom, highly optimized detection algorithms, we estimate that deletions exceeding 100kb can be detected with > 90% probability genomewide using the full 500k SNP chip set, with average detection resolution of 60kb, and that the Tiling arrays can effectively map deletion breakpoints with ~1 kb resolution. We also show that cell-line induced copy-number artifacts are rare, by comparing analysis of cell-line and blood-derived genomic DNA for several individuals. We conclude that the current high density SNP chips, combined with proper analystical methods, provide a highly effective tool for whole genome copy number analysis down to ~ 100kb detection resolution with high confidence, and that by selecting the best-performing probes from the tiling arrays to create an optimized copy-number detection chip, a single chip providing genomewide wide 510 kb detection resolution is feasible with existing technology.

Applications of Pedigree-Free Identity-By-Descent Mapping to Localizing Disease Genes. *S. Nelson, B. Merriman, Z. Chen, M. Ogdie, J. Stone, S. Strom* Deptartment of Human Genetics, UCLA Medical Center, Los Angeles, CA.

Risk factors for genetic disease can result in a founder effect, wherein many affected people in the population share a risk factor tracing back to a common ancestor, and thus share an interval of DNA inherited identical-by-descent (IBD) from the founder. Traditionally, such intervals are detected by establishing a detailed pedigree connecting affected individuals, and genotyping sufficient numbers of affecteds on a sufficiently dense set of markers to reliably identify the IBD intervals. The pedigree plays a key role, both in providing a priori evidence that such IBD intervals exist, and in providing for their reliable detection by tracing recombination events which occur rarely in the human genome with each generation. High density SNP genotyping(greater than 250K SNPs) now provides sufficient information to infer if large intervals of genomic DNA are inherited IBD between even distantly related affected individuals. This enables the direct comparison of affected individuals to search for shared IBD intervals, as an efficient linkage mapping approach for localizing genetic risk factors. Here we present several applications of the Pedigree-Free IBD mapping approach. First, we demonstrate it in the context of a traditional Case-Control study for ADHD, in which 150 cases and 100 controls are genotyped on, and show that IBD can be clearly detected between unrelated affected individuals, at a rate and scale that clearly exceeds the population background level, and which identifies several candidate loci genome-wide. Second, we investigate pedigree-free IBD detection under a major linkage peak for Autism, based on high density SNP typing of 250 Autism trios, and show that the unrelated affected children have ancestral IBD sharing suggestive of a founder effect. Finally, we show that within a previous successful whole genome SNP association study for a common disease, the locus identified by standard means is also highlighted by IBD between various unrelated affecteds. This latter observation also suggests that pedigree-free IBD mapping can be combined with standard association analysis to reinforce the findings of both approaches.

Association of the ERBB2 W452C Variant with Sporadic Breast Cancer. *H.L. Patney[1], R.E. Ellsworth[1], C.D. Shriver[2]* 1) Clinical Breast Care Project, Windber Research Inst, Windber, PA; 2) Clinical Breast Care Project, Walter Reed Army Medical Center, Washington, DC.

Alterations of the ERBB2 (Her2/neu) gene have been associated with poor prognosis in patients with invasive breast cancer. Aggressive behavior of breast tumors is often associated with large amplifications of the ERBB2 gene region but point mutations in ERBB2 also may be associated with deleterious effects. To determine relationships between a C->T DNA base substitution (W452C) in ERBB2 and breast pathogenesis, we genotyped 225 women (80% Caucasian, 20% African American) with invasive breast cancer and 225 age- and ethnically-matched disease-free controls. The overall minor (T) allele frequency (MAF) was 0.02, in agreement with published values, but was not observed in Caucasians. Two African American women without breast disease were heterozygous for the minor allele, while 16% of African American women with invasive breast cancer were homozygous for the minor (T) allele; these results were verified by double-stranded sequencing. The increase in frequency of the minor allele in African American cases was significant ($P<0.05$), compared to African American controls. None of the patients with the TT genotype were HER2 positive, either by FISH or by IHC analysis. Approximately 60% of the TT homozygotes were pre-menopausal at diagnosis and over half of the patients had stage I disease. These data suggest that, in addition to the traditional model of amplification and over-expression, DNA variants may contribute to breast cancer development in African American women. Determination of the functional changes associated with W452C will increase our understanding of the underlying biology of tumors from African American women and provide new targets for customized breast cancer treatments.

CFTR Mutations Panel of Persian Males with Congenital Bilateral Absence of the Vas Deferens. *R. Radpour[1], H. Gourabi[1], M.A. Sadighi Gilani[2], A. Vosough Dizaj[2], S. Mollamohamadi[3]* 1) Department of Reproductive Genetics, Reproductive Biomedicine Research Center of Royan Institute; 2) Department of Male Infertility, Reproductive Biomedicine Research Center of Royan Institute; 3) Department of Stem Cell, Reproductive Biomedicine Research Center of Royan Institute.

Congenital bilateral absence of the vas deferens (CBAVD) is a frequent cause of obstructive azoospermia and nearly 75% of Patients have at least one detectable CFTR mutation. To study the CFTR gene mutations in Persian CBAVD patients with presumed low cystic fibrosis (CF) frequency, we analyzed 112 Persian CBAVD and 7 CUAVD males from Iran with 52 fertile males as control. All 27 exons and their flanking sequences of CFTR gene was analyzed using a combination of the DGGE or by SSCP and direct DNA sequencing. Forty-six of the 112 patients with CBAVD (41.07 percent) had two mutations in the CFTR gene, 41 of them had the 5T allele (in 11 cases two allele of 5T was detected). Forty-three patients (38.39 percent) had a mutation in one copy of CFTR gene in witch 9 of them had just one 5T allele. In 23 patients (20.53 percent) no CFTR mutations were found. IVS8-5T was observed with TG12 or TG13 haplotypes, on 61 chromosomes thus confirming the association of this variant with CBAVD in Persian patients. The F508del mutation in exon 10 was uncovered on 28 chromosomes. Screening for the IVS8-5T and F508del together led to the identification of more than one-third of alleles. Exon 9 skipping was strongly joined with 5T/5T genotype, the rate of normal CFTR mRNA increased by having IVS8-9T (TG) 9-10 and IVS8-7T (TG) 10-11-12. 5T/M470 genotype was found in 21 patients, 5T/V470 was found in 3 and 5T with heterozygote form of M470V was found in 26 CBAVD patients. We could detect one novel nonsense mutation (K536X) in the NBD1 region and two novel missense mutations (Y122H & T338A) in the M2 and M6 regions of CFTR gene. The conservation of changed nucleotide and amino acid in mutated regions were analyzed by aligning with nine different species. The combination of the 5T allele in one copy of the CFTR gene with a cystic fibrosis mutation in the other copy is the most common cause of CBAVD in Persian population.

Association of Long Polyglutamine Tracts in Exon 1 of the Androgen Receptor Gene with Idiopathic Male Infertility and Impaired Sperm Production in Iranian Population. *M. Rezaee[1], R. Radpour[2], A. Tavasoly[3], A. Saleki[3]* 1) Department of Nanotechnology, Avesina Research Institute, Beheshti University; 2) Department of Reproductive Genetics, Reproductive Biomedicine Research Center of Royan Institute; 3) Department of Urology, Biomedical Research Center of Military University of Medical Sciences.

It is estimated that 10%-20% of patients with male infertility could have reduced androgen receptor function as a result of long polyglutamine tracts. To study the association of CAG trinucleotide repeats in the androgen receptor gene with idiopathic male infertility in Iranian population, we performed a case-control study of 178 Iranian males with idiopathic infertility witch were excluded for androgen receptor mutations and Y chromosome microdeletions and 152 fertile males as control. The length of the CAG repeat segment was evaluated by using PCR-sequencing in exon 1 and PCR-SSCP in exons 2-8. We were able to identify 13 different alleles in the infertile group with a range of 18-32 CAG repeats corresponding to 19-33 glutamine residues. The mean of the AR gene CAG repeat length in the infertile group was 24.13 1.64 (range 18-32), while that of fertile controls was 20.32 0.92 (range 16-25) and the difference was statistically significant (P = 0.001). The highest mean length of the AR gene CAG number in infertile group was observed in oligospermia group (24.21) but there were not significant differences between the Oligospermia and Azoospermia. In addition, the frequency of having a CAG repeat number (21) was 79% in infertile men and 27.1% in fertile controls (P = 0.001), whereas the of having a CAG repeat number (26) was 23.6% in infertile males versus 0% in fertile controls. No mutation was detected in exons 2-8 of androgen receptor gene in infertile patients. Long (26) androgen receptor CAG alleles, which are found in up to 38% of infertile males, are associated with male infertility and defective spermatogenesis in our studied population and the odds ratio for male infertility was higher for patients with 26 CAG repeat.

Associated malformations in cases with oral clefts. *C. Stoll, Y. Alembik, B. Dott, MP. Roth* Genetique Medicale, Faculté de Médecine, Strasbourg, France.

Objective: Infants with oral clefts (OCs) often have other associated congenital defects. The reported incidence and the types of associated malformations vary between different studies. The purpose of this investigation was to assess the prevalence of associated malformations in a geographically defined population.Methods: The prevalences at birth of associated malformations in infants with OCs were collected between 1979 and 2003 on all infantss born in the area covered by the registry of congenital anomalies of Northeastern France in 334,262 consecutive births. Results: Of the 651 cleft infants born during this period, 35.9% had associated malformations. Associated malformations were more frequent in infants who had cleft palate (47.9%) than in infants with cleft lip and palate (34.9%) or infants with cleft lip only (14.4%). Malformations in the central nervous system and in the skeletal system were the most common other anomalies, followed by malformations in the urogenital and cardiovascular systems. Weight, length, and head circumference of children with OCs and multiple associated malformations were lower than in controls, as was the weight of the placenta. Prenatal diagnosis was rarely done by fetal ultrasonographic examination in isolated clefts. However, even in multiple associated malformations, prenatal diagnosis by fetal ultrasonographic examination had a low sensitivity, 43.9%. Conclusion: The overall prevalence of malformations, which was one in more than three infants, emphasizes the need for a thorough investigation of infants with clefts. A routine screening for other malformations especially skeletal, central nervous system, and cardiac defects may need to be considered in infants with clefts, and genetic counseling seems warranted in most of these complicated cases.

Methylenetetrahydrofolate reductase gene polymorphisms in patients with nonalcoholic steatohepatitis (NASH).

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Nonalcoholic fatty liver disease(NAFLD) is the most common cause of abnormal hepatic steatosis in the absence of alcohol worldwide. Nonalcoholic steatohepatitis (NASH) is the most progressive form of NAFLD. The aim of this study was to investigate the role of methylenetetrahydrofolate reductase gene C677T and A1298C polymorphisms (MTHFR) in the etiopatopysiology of NASH. We analysed 57 nonalcoholic steatohepatitis (NASH) patients and 100 healthy controls using a PCR-RFLP method.The diagnosis of the patients was based on liver biopsy. The following results were obtained. We studied 57 NASH patients 26 (45.6%) of whom was women and 31 (54.4%) was men. The healthy controls were 100, and 49 (49.0%)of whom was women and 51(51.0%) was men. The frequency of the 677T allele of the MTHFR gene was 25.44% in the cases and 31.00 % in the controls (Chi2=1.511;df=2;P=0.470).The 1298C allele of the MTHFR gene was 47.37% in the cases and 32.50% in the controls. The frequencies of the MTHFR C677C, C677T and T677T genotypes were 56.1%, 36.8%, and 7.0% in the cases, and 46.0%, 46.0%, 8.0% in the controls respectively. The frequencies of the MTHFR A1298A, A1298C and C1298C genotypes were 22.8%, 59.6%, and 17.5% in the cases and 43.0%, 49.0% and 8.0% in the controls. The distribution of the MTHFR 677T allele was not significant in the NASH patients compared with the healthy controls (Chi2= 1.511;df=2;P=0.470). However the distribution of the MTHFR 1298C allele was statistically significant in NASH patients compared with the healthy controls (Chi2=7.814;df=2;P=0.020). The MTHFR A1298A genotype was protective for NASH(OR=0.392; 95%CI=0.188-0.816; Chi2=6.452; df=1;P=0.011). Although the MTHFR C1298C genotype gave a 2.447 odds ratio, it lacked the power (OR=2.447; 95%CI= 0.906-6.611; Chi2=3.258;df=1;P=0.071). To conclude with, the MTHFR 1298C allele was a genetic risk factor for NASH. Presumably, the individuals carrying this genotype were predisposed to develop NASH. The MTHFR A1298A genotype, on the contrary, provided protection towards NASH in the Turkish population.

Interleukin-18 (IL-18) gene Haplotype and Allergic Dermatitis and Bronchial Asthma. *K. Yanamandra[1], D. Napper[1], P. Boggs[1], H. Chen[1], S. Ursin[1], S. Bahna[1], J.A. Bocchini Jr.[1], R. Dhanireddy[2]* 1) Dept Pediatrics, LSU Medical Ctr, Shreveport, LA; 2) Dept Pediatrics, Neonatology Section, Univ Tennessee Health Sciences Center, Memphis, TN 38163.

Interleukin (IL)-18 is involved in the regulation of TH1- and TH2-mediated immune responses, and is involved in the pathogenesis of TH1 and TH2 chronic inflammatory diseases. Allergic athma is a typical TH2-mediated disease and the serum IL-18 levels have been shown to be elevated during the acute asthmatic exacerbations, and secretion of IL-18 by peripheral mononuclear cells is increased in asthma and atopic dermatitis. Controversially, another study demonstrated that in bronchioalveolar lavage fluid IL-18 levels were reduced in asthma when compared with healthy controls. Also, there was a mention in the literature of reduced expression of IL-18 mRNA levels in atopic dermatitis. Similar conflicting results have been obtained in mouse models of allergic asthma. IL-18 stimulates TH1 or TH2 responses depending on its cytokine milieu. When IL-18 is given alone, it exhibits pro-allergic functions like stimulating IL-4 and histamine release from basophils; however, when it is given in a TH1 milieu i.e. together with IL-12, it prevents the development of allergic reactions. In view of the conflicting results on IL-18 role in inflammation, the present investigation was undertaken to study the association of IL-18 gene loci in the etiology of dermatitits and bronchial asthma. In the present investigation seventy-nine patients who presented with allergic rhinitis, dermatitis and asthma from our Northwest Louisiana region and one hundred and sixty controls were genotyped for the IL-18 gene polymorphisms. Our IL-18 haplotype data revealed no significant difference in the mutant haplotype frequency between patients and controls (0.21 vs. 0.26). Based on our data, we conclude that the IL-18 gene marker haplotype may not play a significant role in the etiology of asthma. This was the first study on the role of IL-18 gene haplotype in the etiology of asthma or dermatitis in the African American community. Data and statistics on the individual genotypes of IL-18 gene will be presented.

The First Genome-wide Scan to search for Genes predisposing to Coronary Artery Disease using 500,000 Single Nucleotide Polymorphism (SNP) marker set. *A. Stewart, R. McPherson, L. Vo, Y. Wang, J. Rutberg, G. Ewart, G. Wells, K. Williams, N. Kavaslar, H. Doelle, S. Hebert, T. Naing, R. Roberts* University of Ottawa Heart Institute, Ottawa, Ontario, Canada.

Background: Coronary artery disease (CAD) remains the number one killer in the western world and world wide by 2010. Genetics account for over 50 percent of the risk for CAD. Genetic screening and early prevention in individuals identified at increased risk could dramatically reduce CAD. Thus, the necessity to identify genes predisposing to CAD. Genes identified by the candidate gene approach have not been replicated due in part to inadequate sample size. Genome wide scan association studies have been limited by the use of thousands of markers rather than the hundreds of thousands required and hundreds of individuals rather than the thousands required (Science 2005;307:1072). Equally important is the need to replicate positive findings in an independent population. To detect a Minor Allele Frequency of 5 percent, an odds ratio for risk of 1.3, with 90 percent; power, we estimate 14,000 (9,000 affected and 5,000 control) subjects are required (Nature,2005:6;109). Methods: The Affymetrix 500,000 marker set provides a marker every 6,000 base pairs to genotype 1,000 premature CAD cases and 1,000 normals followed by replication in 8,000 cases and 4,000 controls. The phenotype is confirmed or excluded on the basis of coronary arteriograms by catheterization or multi slice CT. Results: We are averaging 4 million genotypes per day with 123 million genotypes analyzed for 99 controls and 164 cases. On average each DNA sample analyzed provided accurate and interpretable genotypes for 94 percent of the 500,000 SNPs. Where allele frequencies for cases and controls are in Hardy-Weinberg equilibrium (Chi squared, $p>0.05$), over 500 SNPs showing a difference in allele frequency ($P=0.001$) between cases and controls have been identified. Conclusions: This is the first whole genome scan for CAD genes utilizing a marker every 6,000 base pairs. Several haplotypes potentially associated with CAD have been identified.

Analysis of gene dosage of -synuclein gene in multisystem atrophy. *T. Tsunemi, K. Ishikawa, H. Jun, H. Mizusawa*
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Recently increasing gene dosage was reported to cause Parkinsons disease or Alzheimers disease. The genetic association between multisystem atrophy (MSA) and -synuclein have been reported. MSA is pathologically characterized by glial cytoplasmic inclusions which ?-synuclein is strongly immunoreactive for anti-synuclein antibodies. Transgenic mice overexpressing human -synuclein in oligodendrocytes showed features similar to human MSA patients. To clarify whether gene dosage of ?-synuclein gene (SNCA) are altered in MSA patients, we assessed gene dosage of SNCA in 30 patients with MSA using a quantitative real time PCR method. After informed consent was obtained, DNA was prepared using standard methods. We set primers and TaqMan probes in each six SNCA exons and then compared copy number of each exon. CACNA1A was used as an endogenous reference. No genomic multiplication (duplication or triplication) was found in either exons of SNCA in our study. As far as we analyzed, SNCA gene dosage dose not seem to be altered in genomic DNA of MSA patients.

Mosaicism for supernumerary ring chromosome 17 resulting in partial trisomy of 17p11.1-17q23.3. *S.M. Purandare[1], J. Walman[1], N.J. Carpenter[2], J.J. Mulvihill[1], J.R. Seely[1]* 1) Department of Pediatrics, Division of Genetics, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma City, OK; 2) Center for Genetic Testing at Saint Francis, Tulsa, Oklahoma.

We present a case with mosaic partial trisomy 17 for the region 17p11.2-17q23.3, 47,XY+r[26]/46,XY[24].ish+r(17)(p11.1q23.3)(SMS- RARA+), due to a supernumerary ring chromosome (SRC) 17 in 52 % of cells analyzed. Clinical features included profound psychomotor delay, growth failure, feeding difficulties, microcephaly, atrial septal defect, ventricular septal defect, small hands and feet, bilateral single transverse palmar creases, partial syndactyly of 3rd and 4th toes, mild leg length discrepancy, scoliosis, bilateral inguinal hernias, hypospadias, cryptorchidism, hypotonia and other dysmorphic features including a large anterior fontanel, strabismus, malformed pinnae, broad nasal bridge and wide smooth philtrum. Other reported cases of supernumerary marker chromosome 17 involving 17q with partial trisomy of 17p11.2-17q23.3 have several clinical features similar to our patient, and those with the 17q duplication syndrome. Mosaicism and the size of the duplication likely account for the milder phenotype seen in our case. These observations further delineate the clinical features seen in partial trisomy 17q.

Insights from Three ChIP-on-chip Platforms. *D.O. Ricke[1], S. Wang[1,2], D. Cohen[2]* 1) Novartis Institutes for BioMedical Research, Inc. Genome and Proteome Sciences, Cambridge, MA; 2) Novartis Institutes for BioMedical Research, Inc. Strategic Alliances, Cambridge, MA.

Analysis of chromatin Immunoprecipation on microarrays (named chIP-on-chip) results allows new insights into regulatory networks and chromatin dynamics. These insights are affected by antibody used, experiment replicas, instrument settings (PMT), data normalization, and data analysis steps. Insights from the MIT Rick Youngs lab Hu19k platform, Affymetrix chromosome 21 and 22 platform, and Agilent 2-chip promoter platforms will be illustrated. Results can be influenced at multiple steps in the data processing. First, instrument PMT (photo-multiplier tube) settings non-linearly influence intensity measurements. Incorrect settings can lead to data saturation on one or both of the intensity channels. Second, another major source of variation can be introduced through data normalization. The number of antibody targets detected is dependent upon the biology of the antibody target. Third, for platforms that rely upon mismatch probes as controls, the PM-MM (perfect match minus mismatch) calculation introduces noise into the signal calculations. Also, the signal for half of the chips are arbitrarily discarded as PM-MM is negative for half of the probes. The introduction of this noise and loss of signal can be avoided by relying upon the PM signals in comparisons and calculations. Frequently, the experimental results from antibodies that detect large numbers of targets are often over-normalized because the normalization calculation includes the detected signal. Normalization based upon background or control signals enable improved comparisons across different antibodies. Fourth, after normalization, ratios between Cy5 and Cy3 dyes are frequently calculated. Skewed ratios often result from low Cy3 measurements. Eliminating these measurements reduces the number of false positives. Fifth, all of the platforms show good reproducibility between technical replicates. Correlation between biological replicates is influenced by the dynamics of the target protein biology. Insights from these three chIP-on-chip platforms will be illustrated and presented.

Annexin V interacts with Cystic fibrosis transmembrane conductance regulator. *P. Trouvé, M.A. Le Drévo, M. Kerbiriou, Y. Fichou, D. Gillet, C. Férec* INSERM, Brest, France.

The cystic fibrosis transmembrane conductance regulator (CFTR) which is involved in cystic fibrosis (CF) functions as a cAMP-activated chloride channel. The regulation of the CFTR function is involving protein-protein interactions. Nevertheless, the extent to which CFTR channels are regulated by interactions remains unknown. Based on the properties of Annexin V and CFTR and the overexpression of Annexin V in CF, we determine whether Annexin A5 was associated with CFTR and whether the association had implications upon the CFTR chloride channel function. Using co-immunoprecipitation and overlay experiments, we show that Annexin V is associated with the nucleotide binding domain 1 of the CFTR protein. Surface plasmon resonance was used to determine the KD values in the absence (4.3 nM) and in the presence of calcium and ATP (1.6 nM), indicating that the interaction is calcium and ATP dependant. Functional experiments were performed in A549 cells in which decreased expression of Annexin V was obtained using siRNA and was correlated to a decreased CFTR chloride channel function. The siRNA did not induce any modification in the mRNA accumulation and in the protein distribution of the CFTR protein, we concluded that Annexin V is necessary for the normal CFTR chloride channel activity via a direct mechanism. Furthermore, using confocal microscopy we show that CFTR and Annexin V are partially co-distributed in normal epithelial cells in human bronchias. In conclusion, we show for the first time that Annexin V is a CFTRs partner and is involved in the normal CFTR function.

Characterization of the *Mus musculus Tcof1* minimal promoter. *K. Shows, R. Shiang* Department of Human Genetics, Virginia Commonwealth University, Richmond, VA.

Treacher Collins syndrome (TCS) is an autosomal dominant mandibulofacial dysostosis caused by haploinsufficiency of the *TCOF1* gene product treacle. Mouse (*Mus musculus*) *Tcof1* is 74.3% identical to human *TCOF1*, and mouse Tcof1 is 61.5% identical to human treacle. Tissue-specific maximal expression of *Tcof1* mRNA is seen normally in the developing neural crest in mouse embryos; heterozygous knockout of *Tcof1* leads to severe craniofacial malformations. We have isolated the highly conserved core *Tcof1* promoter that is necessary and sufficient to direct maximal *in vitro* expression of a promoter/reporter construct in mouse P19 embryonic teratocarcinoma cells, which are of neural crest cell lineage. The putative transcription factor binding site CCAAT was shown to be essential to the maximal expression of the *Tcof1* promoter/reporter construct through transient transfection studies. In addition, the putative binding sites for transcription factors AP2-2, c-myb, and Sp1 have a modifying effect on regulation in combination with the CCAAT binding site.

High-resolution genomic profiling with Infinium Whole Genome Genotyping BeadChips. *D.A. Peiffer, T. Jenniges, F. Garcia, E. Chudin, K. Haden, E. Allen, F. Steemers, J. Le, D. Barker, R. Shen, K.L. Gunderson* Illumina, Inc., San Diego, CA.

High-density SNP genotyping technology (SNP-CGH) offers several advantages over traditional array-CGH using spotted BAC clones or oligonucleotides. Simultaneous measurement of signal intensity variations and changes in allelic ratios permits the detection of both copy number changes and copy-neutral loss of heterozygosity (LOH) events. Analysis of hundreds of thousands of SNP loci allows for chromosomal resolution of a few tens of kilobases. We demonstrate the utility of using multiple formats of high-density genotyping BeadChips (assaying 109K, 317K, and 550K SNPs) to detect chromosomal aberrations ranging in size from 90kb to several megabases in both constitutional and tumor samples. These include homozygous and heterozygous deletions, copy-neutral LOH, and amplifications. A subset of aberrations was verified using FISH, BAC array-CGH, and karyotype analysis. Genomic profiles were visualized with a genome browser displaying plots of log ratios of normalized intensities and allelic ratios along the chromosome. The browser allows expansion of the plots to any genomic region of interest, with links to genomic sequences, SNPs, and genes. The software also includes numerous other features including the ability to automatically detect and annotate aberrations. We developed two modes of quantitative analysis; one for single samples and the other for paired samples (i.e. tumor and matched normal). The single-sample mode utilizes a comparison to canonical genotype cluster data generated from ~120 reference samples. The benefits of paired-sample analysis and application of SNP-CGH arrays for profiling genomes from heterogeneous tumor samples will also be presented.

Peripheral Blood Gene Expression Signature Predicts Risk Profile of Thoracic Aortic Aneurysm. *R.R. Samaha[1], Y. Wang[1], C. Barbacioru[1], F. Chan[1], J. Blake[1], N.N. Mehmet[1], D. Shiffman[2], O. Iakoubova[2], S. Balasubramanian[3], D. Ngadimo[3], M. Tranquili[4], G. Albornoz[4], J.A. Elefteriades[4]* 1) Applied Biosystems, Foster City, CA 94404; 2) Celera Diagnostics, Alameda, CA 94502; 3) Celera Genomics, South San Francisco, CA 94080; 4) Department of Cardiothoracic Surgery, Yale University School of Medicine, New Haven, CT 06510.

Thoracic aortic aneurysm (TAA) is usually asymptomatic and associated with high mortality. Although adverse clinical outcome is preventable by surgical repair, identifying at-risk individuals is difficult. Our goal was to identify a potential gene expression signature in peripheral blood that may allow development of noninvasive screening tests to identify individuals at risk for TAA disease. Gene expression profiles of peripheral blood samples collected from 58 individuals diagnosed with TAA and 36 normal individuals were analyzed using the Applied Biosystems Expression Array Systems and Human Genome Survey Microarrays. Statistical analysis of gene expression profiles identified 1199 candidate signature genes (markedly up- or down-regulated) characterizing the TAA disease. Gene classification and biological pathway analyses of these signature genes revealed potential molecular mechanisms underlying this disease. Additionally, 30-gene prediction models for risk assessment of TAA were built for all-gender and male or female, respectively, using a training set containing 36 TAA patients and 25 controls. 10-fold cross-validation on these prediction models yield 82%, 90%, and 97% overall accuracy for each model, respectively. When these prediction models were applied on an independent testing sample set containing 22 TAA patients and 11 controls, the overall prediction accuracies for all-gender, male and female models were 79%, 70% and 77%, respectively. This study provides a comprehensive gene expression profile of peripheral blood cells from thoracic aortic aneurysm patients and normal individuals. In addition, our results also demonstrated a distinct RNA signature of aneurysm disease, setting the stage for a blood-based gene expression test that may facilitate risk assessment and early detection of thoracic aortic aneurysm disease.

Definition and Application of European Substructure Information. *M.F. Seldin[1], R. Shigeta[1], P. Villoslada[2], C. Selmi[1], L. Klareskog[3], P.K. Gregersen[4]* 1) Rowe Program Genetics, UC Davis, CA; 2) Cent Applied Med Res, Pamplone, Spain; 3) Karolinska Univ Hosp, Stockholm, Sweden; 4) North Shore-LIJ Res Inst, Manhasset, NY.

Our initial studies using over 5500 genomewide SNPs provided evidence that various cluster algorithms can distinguish northern vs. southern European(EUR)populations. To extend these studies additional samples (>1000 EUR-American subjects)were examined including most with 4 grandparent information. The samples were genotyped with 768 SNPs selected for EUR substructure information. Similar to our previous results, most individuals with ancestry from Western, Central and Northern EUR were distinguishable from those from Southern EUR. Clear membership in the corresponding north or south clusters was observed for 4 grandparent same country of origin: German, 22 subjects; mean 0.86 north; Irish 86 subjects, 0.97 north; Scandinavian, 6 subjects, 0.98 north; Italian 16 subjects mean 0.75 south; and Greek 7 subjects, mean 0.83 south. Although our initial results in this second panel of subjects showed more variability in the Eastern EUR group additional analyses suggested that most or all of the differences were explained when additional ethnic information was available. Individuals of Ashkenazi Jewish ancestry (38 individuals) clearly grouped with the southern European populations (mean =0.86 south) where as subjects with Eastern European ancestry including 16 subjects with 4 grandparent Ukrainian without Ashkenazi Jewish ancestry grouped with northern European populations (mean = 0.88 north). Finally, we examined the effect of controlling for population structure in analysis of putative RA susceptibility loci. These studies showed that significance of case control association tests were variably affected by structured association and subgroup analyses. Although most SNP candidates did not retain significance important exceptions included a confirmed disease susceptibility allele (for PTPN22) and one SNP with increased odds ratios and p values in a north only subset. These studies suggest that definition of population genetic substructure may potentially decrease both type 1 and type 2 errors.

Finemapping of a large multiplex neural tube defect (NTD) family at 2q and 7p. *D.S. Stamm[1, 2], L. Mehltretter[1], S. Slifer[1], B. Zhao[1], D. Siegel[1], T.M. George[1], J.R. Gilbert[1], M.C. Speer[1]* 1) Center for Human Genetics, Duke University, Durham, NC; 2) Curriculum in Genetics and Molecular Biology, UNC-CH, Chapel Hill, NC.

Background: Neural tube defects (NTDs) are a complex disorder with both genetic and environmental factors implicated. To date, no major causative genes have been defined in humans despite intensive investigations. NTD family 8776 is a large, multigenerational, Caucasian family that provides a unique resource for the genetic analysis of NTDs. We have performed a high density genomewide screen to establish regions of linkage in family 8776 (Stamm et. al. 2006, in press). The regions identified are proximal to the telomeres of chromosomes 2 and 7, mapping to 2q33.1-q35 and 7p21.1-pter respectively. Both regions had maximum lod scores of ~3.0 under multipoint nonparametric methods. Preliminary haplotyping performed in regions of interest confirmed the linkage regions. **Methods**: To further refine these regions of interest we genotyped nineteen microsatellite repeat markers for 2q and six microsatellite repeat markers for 7p. Microsatellite markers were prioritized based on high heterozygosity values and spacing (~ 2 cM) within the region. In parallel, we sequenced biologically plausible candidate genes mapping to 2q33.1- q35 and 7p21.1-pter. Primers were designed to flank the exons and include the splicing region to account for splice variants. **Results**: Further haplotype analyses narrowed the minimum candidate interval for both 2q and 7p. The haplotype for chromosome 2 extends from D2S425 to D2S433 (23.8 cM) mapping to 2q32.3-2q35; and for chromosome 7 the haplotype span D7S2508 to 7pter, a 31.4 cM interval mapping to 7p21.1-pter. Sequencing of exons in candidate gene CASP8 and of exons 1, 3-9 in the PAX3 gene showed no disease associated variants. **Conclusion**: Additional genes are being sequenced. Most importantly, this family has identified two specific regions of interest that may harbor NTD susceptibility genes. Identifying a gene in this family may aid in a directed search for a disease gene for other more typical NTD families.

Genome-wide association scan for type 2 diabetes in Finns. *L.J. Scott[1], W.L. Duren[1], L.L. Bonnycastle[2], H.M. Stringham[1], A.U. Jackson[1], M.L. Erdos[2], P. Chines[2], N. Narisu[2], C.J. Willer[1], K.F. Doheny[3], E.W. Pugh[3], N.L. Riebowl[2], T.T. Valle[4], J. Tuomilehto[4], R.N. Bergman[5], K.L. Mohlke[6], F.S. Collins[2], M. Boehnke[1]* 1) U Michigan, Ann Arbor, MI; 2) NHGRI, Bethesda, MD; 3) CIDR, J.H.U., Baltimore, MD; 4) National Public Health Institute, Helsinki, Finland; 5) U Southern California, Los Angeles, CA; 6) U North Carolina, Chapel Hill, NC.

Our goal is to identify genes that increase the risk of type 2 diabetes (T2D). The FUSION study group is carrying out a genome-wide T2D association scan in 2357 first-stage samples of a two-stage study, which will ultimately include genotyping a total of ~5400 geographically matched Finnish samples. The Center for Inherited Disease Research (CIDR) has performed the sample genotyping using the Illumina HumanHap300 BeadChip. We report here an intermediate analysis of the first stage, based on of 885 T2D cases and 885 normal glucose tolerant controls and 308,231 polymorphic autosomal SNPs. In this sample, using a conservative genome-wide significance level of .05/302K = 1.6 x 10^{-7}, we have 12% or 87% power to detect an additive OR of 1.3 or 1.5 with a risk allele frequency of 0.3. The average duplicate error rate per genotype was 0.002% and the average SNP completeness was 99.6%. We flagged 5357 SNPs for future consideration based on deviation from Hardy-Weinberg equilibrium, elevated error rates, low completion rates or low minor allele frequency. We analyzed 302,874 SNPs for T2D association using dominant, recessive and additive models and adjusted the results to account for the 3 tests. Our most significant SNP had a p-value of 5.5 x 10^{-6}. We examined results around SNPs with multiple reported associations with T2D. Near PPARG Pro12Ala, TCF7L2 rs12255372, and KCNJ11 Glu23Lys, the most significant SNPs had p-values of .005, .005, and .03, and were ranked 1648^{th}, 1831^{st}, and 10321^{st}, respectively. These results suggest that other T2D susceptibility SNPs may be detected after genotyping the top 1-3% of SNPs in the full two-stage sample. To increase the power of the analysis, we will incorporate statistical weighting based on annotation information, stratify the sample by age-of-onset, and combine results across multiple T2D studies.

Psychosocial Outcomes of Bone Marrow Transplant for MPS I Hurler Disease: Patient Self Report of Personality and Personal Adjustment. *C. Pitt*[1], *C. Lavery*[1], *N. Wager*[2] 1) Society for Mucopolysaccharide Diseases, Amersham, Buckinghamshire, United Kingdom; 2) Buckinghamshire Chilterns University College, High Wycombe, Buckinghamshire, United Kingdom.

Aims: To explore the composite scores, and clinical and adaptive scales of the Behaviour Assessment System for Children: Self Report of Personality (Reynolds & Kamphaus, 1998) in terms of norms for individuals affected by Mucopolysaccharidosis I Hurler Disease (MPS IH) post-bone marrow transplant (BMT). Particular attention was given to the Personal Adjustment composite and to its contributors. Method: Eighteen MPS IH patients post-BMT participated in this investigation, along with their mothers. Patients ages ranged from 8 to 25 years. Semi-structured interviews with patients mothers were utilised, and patients were administered tests of cognitive function and the BASC-SR. Results: Hierarchical multiple regression on the Personal Adjustment composite demonstrated that 95% of the variance could be explained ($F = 18.7413,2$, $p = .051$) by patient health and disability factors, and factors associated with the mother and the family environment. In terms of the clinical and adaptive scales, no overt behavioural difficulties were observed. However, possible trends emerged, which highlighted adjustment difficulties with school and feelings of inadequacy for the 8-11 year age group; and a tendency towards inhibition and withdrawal for the 12 years and over age group. Conclusion: The findings illustrate how aspects of parenting and the family, as well as aspects of the MPS disease, require attention when providing support to these patients. They also highlight the importance of appropriate and consistent classroom support, and question whether psychosocial support should be considered within the school environment.

Tetramelic monodactyly: rare autosomal dominant condition with high rate of gonadal mosaicism. *P. Wheeler*[1], *S. Rosenthal*[1], *M. Bamshad*[2] 1) Dept Genetics, Nemours Children's Clinic, Orlando, FL; 2) Dept Pediatrics, University of Washington, Seattle, WA.

The proband is a 34 y/o female who was born with absence of all digits except the 5th digits on both her hands and feet. Radii/ulnae and tibia/fibula are normal. Radiographs show a curved 5th digit with 3 phalanges for both hands and 3 metacarpals in the right hand and 4 in the left. She has 1 metatarsal bilaterally supporting the single 5th digit on each foot. She has normal intelligence and no other anomalies. Family history is pertinent for the proband having 4 full siblings including 1 sister with no limb anomalies. That woman has had 5 children all without birth defects. The other 3 siblings (a brother and 2 sisters) all have nearly identical limb anomalies to the proband. All the affected individuals have normal facial appearance and normal intelligence. None of the individuals with the limb anomalies has chosen to have biological children. The father of the proband has no limb anomalies. The mother was born with insertional polydactyly with an extra toe between the 1st and 2nd toes of the right foot. She also had preaxial polydactyly of the left hand described as a nubbins. Both limb anomalies were surgically corrected in infancy. Tetramelic monodactyly has been described previously in a few families. The physical features are consistent between families with all individuals having a single, in-curved 5th digit on each extremity. The majority of families reported have inheritance consistent with germline mosaicism of an autosomal dominant condition. There does not appear to be significant variable expressivity in this condition except for the current family, which is unique because of the limb abnormalities present in the mother of the affected siblings. It is possible that the mother has a separate condition from what her children have or the family could have variable expression of this disorder, although this has not been reported previously. Research testing including analysis of HOXD13 is planned due to the overlap of phenotypes reported with HOXD13 mutations (specifically insertional polydactyly) and the anomalies seen in this family.

A unique rearrangement involving the BCL6, MYC, IGH and BCL2 loci in diffuse large cell lymphoma. *L. Richmond, C. Aguilar, G.S. Bezzegh, J. Skierkowski, G. Hart, C. Berger, T.C. Brown* Genzyme Genetics, Phoenix, AZ.

Chromosomal abnormalities involving t(14;18)(q32.3;q21) and 3q27 (BCL6) are common in B-cell non-Hodgkin lymphoma of germinal center cell origin. B-cell lymphoma with concurrent t(14;18) and 8q24 (c-Myc) translocations fall within the morphologic spectrum of diffuse large B-cell (DLCL) and Burkitt lymphoma. These cases are extremely rare and are associated with an aggressive clinical course and poor prognosis. It has been suggested that t(14;18) and 3q27 rearrangements are mutually exclusive in lymphoma, however, our case not only has this combination but also involves c-MYC. We believe this to be the first study to report such a combination. Cytogenetic and fluorescence in situ hybridization (FISH) studies were performed on a bone marrow sample from a 90-year old female patient who was diagnosed as having diffuse large cell lymphoma. Chromosome analysis of metaphase cells revealed an abnormal karyotype: 47,XX,t(2;3)(q11.2;q27),add(14)(q32)x2,del(18)(q22q23),+mar[20]. Metaphase FISH analysis using whole chromosome paints for chromosomes 8, 14 and 18 showed chromosome 18 material on 3q, while 8q and 14q had additional unknown material. To further identify the unknown material, FISH was performed using the IGH/BCL2 (14;18), MYC/IGH (8;14), CCND1/IGH (11;14) and MALT1(18q) probes. The interphase FISH results were positive for both the IGH/BCL2 (76% of cells) and MYC/IGH probes (77% of cells). The CCND1/IGH probe revealed no evidence of a translocation, however 68% of the cells had an extra IGH signal. The MALT1 probe results were normal, allowing localization of the breakpoint on chromosome 18 at q21.32, between the MALT1 and BCL2 genes. The BCL6 break-apart probe at 3q27 confirmed this locus was disrupted by the 18q;2q material. The combination of both chromosomal analysis and FISH were essential in identifying the complex rearrangements in this lymphoma. FISH results enabled us to further clarify the cytogenetic findings and identify the following derivative chromosomes in this patient's bone marrow sample: der(3)t(2;18;3),der(8)t(18;14;8),der(14)t(8;14)x2 and der(18)t(14;18).

Clinical and molecular findings in Fraser syndrome; Report of an Iranian consanguineous family with two affected offsprings. *G. Vakili[1], Y. Shafeghati[1], M. Zenker[2]* 1) Genetics Research Center, University of Welfare Sciences and Rehabilitation, Tehran, Iran; 2) Institute of Human Genetics, Erlangen, Germany.

Background: Fraser syndrome is a very rare autosomal recessive disorder, characterized by major malformations such as cryptophthalmos or anophthalmia, acrofacial dysmorphism, laryngeal, genitourinary tract, musculoskeletal anomalies, and mental retardation. Materials & Methods: Here, we report the clinical and pathological findings and molecular analysis of two affected patients from an inbred Iranian family. We followed the second pregnancy by ultrasound, and on the weeks 25, multiple anomalies were detected in the fetus. Parents requested to terminate the pregnancy. Results: The clinical findings on this case were similar to the first sib. The fetus was sent for karyotyping and complete postmortem study. Extra findings in autopsy were: both lungs composed of two lobes (this is a new finding in this syndrome). Esophagus was narrow in its proximal part. Bilateral renal agenesis, small and hypo plastic bladder, and agenesis of internal genitalia were detected. Chromosome study was normal. Mutation analysis on FRAS1 gene was negative, but the patient turned out to be homozygous at FREM2 locus with a homozygous mutation. The mutation IVS1+1G>A destroys the splice donor of exon 14 and most likely leads to skipping of this exon, which would be predicted to result in frameshift and a premature stop codon. We are currently trying to figure out the consequences of the mutation on the transcript level. Conclusion: We should consider this rare syndrome in any fetus with IUFD and in stillborn babies with these complex anomalies. High resolution ultrasound is a very efficient tool for detection of the affected fetuses.

Chromosomal aberrations and its association with recurrent ART failure. *R. Prabu[1], R. Dada[1], R. Kumar[2], N. Gupta[2], K. Kucheria[1]* 1) Anatomy, AIIMS, N Delhi, India; 2) Urology Department, AIIMS, N Delhi, India.

Chromosomal abnormalities and aneuploidies are found to be associated with and have a higher prevalence in infertile males than in the general population. These aberrations not only result in partial or complete spermatogenic arrest but may also result in implantation failure and consequently failure of InVitro fertilization (IVF). Assisted Reproductive Technology (ART) has revolutionised the management of infertility and allows infertile couples to procreate. Cytogenetic and molecular analysis was done in 165 infertile males and 30 couples going in for IVF. Twenty five well spread G banded metaphases were karyotyped using image analyser (Cytovision, Applied Imaging). In mosaic cases 50 metaphases were analysed. Chromosomal abnormalities were found in 46 infertile males. We found 16 cases with Klinefelter Syndrome (KFS), 20 cases were KF mosaics and 6 were mosaic variants, three cases with 46,XY 1qh+ and one case with 46,XY16h+. In 5 of the 30 couples couples opting for assisted reproduction cytogenetic analysis in the female partner revealed 46,XXq- chromosomal complement in two cases and Yq microdeletion in the AZF region in 2 cases. The AZF loci deleted was AZFc. Deletion of long arm of X chromosome(Xq-) in the female partner in two cases might have resulted in repeated failure of blastocyt development. This couple had gone in for 4 IVF cycles which had failed due to failure of blastocyst development. The male partner was cytogenetically normal and also had no Yq microdeletion spanning the AZF loci. In cases with sex chromosomal and autosomal aberrations there is probability of poor embryo development and consequently poor implantation , which may be a result of high segregation abnormalities and may negatively affect the outcome of assisted reproductive techniques. Thus these infertile couples should be counseled prior to going in for ART about the importance of genetic analysis, and how the presence of genetic anomalies results in poor IVF outcome and vertical iatrogenic transmission of these anomalies to the offspring born through ART.

COMPARISON OF FLT-1 BINDING DOMIAN OF MODELLED VEGF-C WITH VEGF-A SHEDS LIGHT ON RECEPTOR SPECIFICITY. *M. Kasap, A. Sazci* Medical Biology and Genetics, University of Kocaeli, Faculty of Medicine, Kocaeli, Turkey.

Receptor specificity determines the role of vascular endothelial growth factors (VEGF) which either induce angiogenesis via VEGFR1 and VEGFR2 receptors or lymphangiogenesis via VEGFR3 receptor. Among the VEGF proteins, VEGF-A induces angiogenesis and VEGF-C induces both angiogenesis and lympangiogenesis. However, why VEGF-C is not able to bind VEGFR1 is not known. The tertiary structure of VEGF-C protein was modelled to answer this question and examined in great detail for validity and compared with the known VEGF-A tertiary structure. The overall topology of the modelled structure highly resembled to VEGF-A and consisted of a central four stranded -sheet, three loops and two helices. An additional -helix was detected within the VEGFR2 binding site in VEGF-C model suggesting that VEGFR2 binding efficiencies may be different between VEGF-A and VEGF-C. The key residues that involve in cysteine-knot motif formation located at the same position and in an identical orientation in both proteins indicating the presence of a VEGF-A-like VEGF-C homodimer. However, a VEGF-C homodimer created via monomer docking did not superimpose well with VEGF-A homodimer. The orientation of the VEGF-C monomers within the dimer with respect to each other differed from the orientation of VEGF-A monomers indicating that the residues that help interaction between monomers in both homodimers differ from each other in identity and orientation. In overall, VEGF-C occupied a wider space than VEGF-A. Rigid docking models of VEGF-C with VEGFR1 receptor revealed that in VEGF-C-VEGFR1 complex, the receptor-protein interacting residues were not correctly oriented to induce angiogenesis via VEGF-R1. Mapping the electrostatic surface potentials to the protein surfaces revealed noteworthy amount of dissimilarity between VEGF-A and VEGF-C indicating that in overall both proteins differ in their folding properties and stability.

Inference of polyphyletic SNP-ratio by zero-distance limit of fraction of SNP paris in complete linkage disequilibrium. *R. Yamada, K. Hirosawa, A. Yoshizumi, V. Renault, M. Yamaguchi, F. Matsuda* Hum Disease Gen,Ctr Gen Med, Kyoto Univ, Kyoto, Japan.

Linkage disequilibrium (LD) mapping with common SNPs has been widely applied to studies on various phenotypes with promising successes. The further achievements are to come along with development of public database and new technologies on SNP-based LD mapping. Although alleleic association in regular LD mapping is based on the assumption that the majority of genetic variations are monophyletic, fraction of monophyletic SNPs among common variations is unclear. Because D', one of LD indices, converges to 1 at the limit of inter-SNP distance being zero for pairs of monophyletic SNPs but because it does not for polyphyletic SNPs, we introduced an index, a fraction of SNP pairs with D' of 1, fr(D'=1), as a function of inter-SNP distance and characterized its limit at distance zero with Monte-Carlo simulation under a Wright-Fisher model modified with polyphyletic mutations. The limit of fr(D'=1) at distance zero increased grossly in proportion to effective population size and it gave estimates of floor of rate of polyphyletic SNPs in population. By applying the result to the HapMap project data, we estimated $\lim_{d0}$fr(D'=1|S(d)) in SNPs of HapMap project as 0.9426 in CEU (CEPH families), 0.9218 in CHB (Han Chinese), 0.9230 in JPT (Japanese) and 0.9216 in YRI (Yoruba, Africans), respectively. These result indicated some fraction of common SNPs seemed to be polyphyletic.

Additional Report of Late-Onset Friedreich Ataxia (LOFA). *H. Roberts[1], J. Bodurtha[2]* 1) Division of Medical Genetics, Children's Hospital of The King's Daughters, Norfolk, VA; 2) Department of Human Genetics, Medical College of Virginia, Richmond, VA.

Purpose: LOFA occurs in 25% of individuals with Friedreich ataxia (FRDA). Thirteen patients with LOFA were recently published in the literature. This report describes an additional patient with LOFA who is a compound heterozygote for a GAA triplet-repeat expansion and a point mutation in the *FRDA* gene.

Methods: The patient first noted at age 26 to have balance problems leading to ataxia. She subsequently developed dysarthria, diminished sensation in her feet, and decreased tone in her lower body. Her exam was significant for diminished sensation below the knee and complete loss of vibratory proprioception below the ankle. Strength in the upper extremities was 5/5 and 4/5 in the lower extremities. DTRs were 2+ in the upper extremities. Patellar reflexes were 3+, and no Achilles reflexes were elicited. Both toes were upgoing. Romberg sign was present. Her gait was widebased and ataxic with pes cavus. Brain MRI and ENT evaluation were normal. Nerve condution studies and EMG showed mild distal lower extremity axonal sensory neuropathy. Echocardiogram, EKG and glucose tolerance tests were normal. Results of molecular testing showed compound heterozygosity for GAA expansion and G130V point mutation in the *FRDA* gene.

Summary: Most compound heterozygotes are clinically indistinguishable from typical patients with FRDA who have homozygous GAA expansions. However, compound heterozygosity for G130V or D122Y missense mutations usually results in a mild phenotype. Despite these general genotype-phenotype correlations, it is not possible to predict the specific clinical outcome in individual cases. FRDA is an autosomal recessive disorder affecting the *FRDA* gene on chromosome 9q13 which encodes the protein frataxin. Deficient frataxin results in abnormal accumulation of intramitochondrial iron, defective mitochondrial respiration, and overproduction of free radicals causing intracellular damage. Antioxidant therapy by free radical scavengers is being considered for slowing the progression of FRDA.

The CHRNA5/A3/B4 gene cluster variability as an important determinant of alcohol and tobacco initiation in young adults. *I. Schlaepfer[1,2], A. Collins[1], R. Corley[1], T. Crowley[4], J. Hewitt[1,3], C. Hopfer[4], K. Krauter[5], J. Lessem[1], S. Rhee[1,3], M. Stallings[1,3], S. Young[1], J. Zeiger[1], M. Ehringer[1,2]* 1) Inst Behavioral Genetics, Univ Colorado, Boulder, CO; 2) Dept. of Integrative Physiology, Univ Colorado, Boulder; 3) Dept. of Psychology, Univ Colorado, Boulder,CO; 4) Division of Substance Dependence, Dept. Psychiatry, Univ Colorado, School of medicine, Denver, CO; 5) Dept. Molecular Cellular and Developmental Biology, Univ Colorado, Boulder, CO.

Understanding the genetic basis of the interaction between alcohol and nicotine holds great promise for elucidating strategies for treating the dual use of the drugs. One potential site of convergence of the nicotine and alcohol actions is the family of the neuronal nicotinic acetylcholine receptors. Our study examines the genetic association between variations in the genomic region containing the CHRNA5 A3 and B4 gene cluster (A5A3B4) and several phenotypes of alcohol and tobacco use in an ethnically diverse youth sample. We tested nine individual single nucleotide polymorphisms (SNPs) and haplotypes for association with various nicotine and alcohol phenotypes, including age of initiation and measures of subjective responses to the substances in the period shortly after initiation. SNP frequency calculations revealed ethnic-specific allele distributions in Caucasians, African-Americans and Hispanics. Therefore, analysis was conducted in the full sample, including ethnicities as covariates, and in each of the three ethnic sub-samples using the statistical genetics program WHAP. Our results suggest that genetic variation in the A5A3B4 gene cluster contributes to smoking initiation and use of alcohol and nicotine in young adults. In particular, three individual SNPs were significantly associated with age of initiation for both alcohol and tobacco variables. Haplotype analysis of the SNPs revealed that a fairly common (22%) haplotype may be protective ($p<0.01$) for both alcohol and tobacco initiation in the combined sample. This is the first report of an association between the locus containing the A5A3B4 gene cluster and tobacco and alcohol-related phenotypes.

Fetal Therapy : Issues of fetal selection ; when a prenatal ' isolated ' anomaly is really a postnatal ' sequence/syndrome '. *R..D. Wilson, M.P. Johnson, M.W. Bebbington, S. Kasperski, N.S. Adzick* Ctr Fetal Diag/Treatment/Wood, Children's Hosp Philadelphia, Philadelphia, PA.

Objective : Cases are presented which illustrate the difficulty of fetal selection for fetal surgical treatment as certain structural or functional anomalies cannot be easily identified prenatally by imaging , karyotype or other functional studies alone or in combination . Case reports : Fetal therapy by thoraco or vesico amniotic shunting may be successful in draining the fluid filled space but may not correct the underlying functional anomaly . Case 1: Normal fetal karyotype at CVS due to maternal age indication . Pleural effusions (PE) initially presented at 22 weeks and increased at 30 weeks with chylothorax . A thoracoamniotic shunt was placed at 30 weeks with right sided PE resolution but preterm delivery occurred at 33 weeks . A difficult neonatal course continued with respiratory issues , generalized oedema , lymphatic obstruction above the iliac level and death at 3 months of age . A diagnosis of congenital pulmonary lymphangiectasis (CPL) was made at autopsy with possible autosomal recessive inheritance . Case 2: Prenatal lower urinary tract obstruction (LUTO) at 20 weeks with serial vesicocentesis showing preserved renal function and normal karyotype . A vesicoamniotic shunt was placed at 22 weeks with continued hydrops (bilateral PE) but empty bladder. Polyhydramnios (44cm) developed at 28 weeks with subsequent moderate bilateral PE , mild ascites , empty bladder , and normal doppler waveforms . Induction of labour at 34 weeks due to non reassuring fetal assessment with early neonatal death . Autopsy showed a possible partial urorectal septum malformation sequence with sporadic or possible X linked inheritance . Conclusion: Prenatal identification of isolated anomalies only for fetal surgery is difficult . Fetal karyotype to rule out trisomy is not enough . Pattern recognition , other specific mutation analysis (if available) and prenatal search tools are required to assist in identifying appropriate fetuses for fetal therapy so that ' right diagnosis , right treatment ' can be accomplished. Multidisciplinary evaluation and consultation is required .

Complex chromosome rearrangement t(8;21)(p21;q22.1)inv(8)(p21q22.1), a novel variant of t(8;21) in acute myeloid leukemia. *J. Xu[1], L. Mak[1], C. Richmond[1], R.C. Lohmann[2]* 1) Cytogenetics; 2) Hematology, London Health Sciences Centre and University of Western Ontario, Canada.

We report a novel variant of 8;21 translocation in acute myeloid leukemia and propose cytogenetics mechanisms for this rearrangement. This is a case study by routine karyotyping and FISH using ETO (8q22)/AML1 (21q22) dual color, dual fusion translocation probe set (Vysis). A 72-year-old female patient was diagnosed with AML and 95% of her bone marrow cells were blast cells, confirmed myeloblasts on flow cytometry. Routine cytogenetics of the bone marrow cells showed a complex karyotype with 8;21 translocation and an inversion involving the translocated chromosome 8. The detailed karyotype is 46,XX,der(8)(21qter->21q22.1::8q22.1->8p21::8q22.1->8qter),der(21)(21pter->21q22.1::8p21->8pter[24]/46,XX[1]. FISH showed that the der(8p) had yellow ETO/AML1 fusion signal whereas the der(21q) had green AML1 signal but no ETO/AML1 fusion signal. We propose that this complex karyotype is possibly a result of 3 rearrangements: inversion, translocation and deletion. It might begin with a pericentric inversion of 8p21q22.1 with the breakpoint being distal to ETO at 8q22. This inversion would have relocated the entire ETO to the der(8p), as was evidenced by absence of orange ETO signal in the der(8q). This might be followed by 8p;21q translocation, as was evidenced by the presence of the ETO/AML1 fusion signal in the der(8p). There was deletion of ETO in der(21), as was indicated by no fusion/orange signals in the chromosome. This is a new variant to a list of reported cytogenetics variants of the classical t(8;21) involving various chromosome partners identified by routine karyotyping and submicroscopic variants of ETO/AML1 detected by FISH or RT-PCR. More case reports and further cytogenomics studies will help understand clinical and prognostic significance of such variants. Our finding emphasizes that caution be exercised in interpretation of variant ETO/AML1 signal patterns in interphase and metaphase FISH.

Mitochondrial DNA depletion syndrome. *S. Seneca[1], L. Van Haute[1], R. Van Coster[2], G. Van Goethem[3], A. Löfgren[3], J. Smet[2], J. Jaeken[4], M.C. Nassogne[5], B. François[6], P. Garcia[7], W. Lissens[1], A. Meulemans[1], I. Liebaers[1], L. De Meirleir[8]* 1) Center of Medical Genetics, AZ-Vrije Universiteit Brussel, Brussel, Belgium; 2) Pediatric Neurolgy & Metabolism, UGent, Belgium; 3) Department of Molecular Genetics, Neurogenetics Group, (VIB8), UIA, Belgium; 4) Department of Pediatric Neurology, KUL & University Hospital Gasthuisberg, Belgium; 5) Service de Neurologie Pédiatrique, UCL & Cliniques St-Luc, Belgium; 6) Pimocchio Centre, Cliniques Espérence, Montegnee, Belgium; 7) Unidade de Doenças Metabolicas, Hospital Pediatrico, Coimbra, Portugal; 8) Department of Pediatric Neurology, AZ-Vrije Universiteit Brussel, Brussel, Belgium.

The mitochondrial genome (mtDNA) is a 16.5 kb DNA molecule that is normally present in multiple copies in individual mitochondria. The mtDNA depletion syndrome (MDDS) is a disorder involving a quantitative defect of mtDNA, which is inherited in an autosomal-recessive mode. Patients are born after an uneventful pregnancy and are often normal at birth, but they deteriorate in the neonatal period or early childhood. There are two main clinical presentations : myopathic and hepatocerebral. In the first group children present with devasting myopathy and neurological abnormalities. In the second condition patients suffer from early progressive hepatic failure, hypotonia, hypoglycaemia, lactic acidosis and progressive neurodegeneration as described in Alpers syndrome. We used real-time PCR to quantify the level of mtDNA in fibroblast, blood, and muscle or liver tissue of patients suspected because of clinical and biochemical abnormalities. Of all patients with clinical suspicion of MDDS, mtDNA depletion was documented in ten. Molecular analysis of nuclear predisposing genes included sequencing exons of TK2, DGUOK and polG. Two patients had DGUOK mutations, while three other patients had recessive polG mutations. Mutations in these genes count only for a fraction of MDDS cases. Recently mutations in two other nuclear genes, SUCLA2 and MPV17, were identified in MDDS patients.

Mutalyzer - a tool to improve descriptions of DNA sequence changes in mutation databases and in literature.
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Unambiguous and correct sequence variation descriptions are of utmost importance, not in the least since mistakes and uncertainties may lead to undesired errors in clinical diagnosis. We have developed the Mutalyzer sequence variation nomenclature checker (http://www.humgen.nl/mutalyzer) as the first module of a package for sequence variation effect prediction. Mutalyzer handles all mutations following the recommendations of the Human Genome Variation Society (HGVS) for mutation nomenclature. Input for Mutalyzer is a GenBank accession number, a HGCN gene symbol and the mutation. Based on this input, Mutalyzer generates an output file containing a description of the mutation at DNA level, the effect on all annotated transcripts, its deduced outcome at protein level and gains or losses of restriction enzyme recognition sites. Mutalyzer is able to handle most mutation types, including splice site and frameshift mutations. Mutalyzer facilitates uploading of reference sequence files with user-modified annotation and a batch wise sequence variation checking. The latter tool can be used to quickly check all entries in existing locus-specific mutation databases (LSDBs). To investigate their current status regarding error-free description of sequence changes as well as how they adhere to the current mutation nomenclature rules we have used Mutalyzer to analyze the content of several LSDBs. The data show that Mutalyzer can facilitate curation of both existing and new data. The mutation checker module will be linked to the Leiden Open source Variation Database (LOVD) we have developed (see http://www.DMD.nl/LOVD/).

COMPLEX CHROMOSOMAL ABNORMALITIES IN A CHILD WITH ADVANCED NEUROBLASTOMA.

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A 6 year-old girl with negative family history was admitted with proptosis of both eyes, vomiting and abdominal pain. CT scan revealed a large right retroperitoneal mass and multiple metastatic lesions to spine, lung, brain and bone marrow. Fine needle aspirate of retroperitoneal mass, and bone marrow aspirate and biopsy showed small blue cells that were negative for CD45, CD99, cytokeratin, desmin, NSE, neurofilaments and positive for CD56, synaptophysin and chromogranin. FISH performed on interphase nuclei were negative for amplification of MYCN (chromosome 2p24.1) and negative for rearrangement or loss of MLL (chromosome 11q23). Cytogenetic analysis of metaphase tumor cells revealed multiple chromosomal abnormalities with many cell-to-cell variations. The composite karyotype of tumor cells disclosed, female, 46-47,XX,add(4)(q31.1),-6,del(6)(p21.3p25),+7,add(11)(p11.2),del(11)(p11.2p15),add(19)(p13.1),+1-7mar[cp9]/89,idemx2[1]. The primary adrenal tumor was resected after four cycles of chemotherapies and the immunostain of tumor cells are positive for NSE, chromogranin and synaptophysin. Neurofilament is randomly positive in stroma and S-100 is strongly positive in Schwannian stroma. Final diagnosis is Neuroblastoma, Stage 4. In this patient, there are no N-myc amplifications nor LOH of 11q (MLL) detected. However, there are notable structural anomalies on chromosome 4, 6, 11 and 19 in this patient. The genes involved in this patients genome regions of the chromosomal structural abnormalities involve numerous factors and oncogenes reported in other malignancies, including BAK, TNFSF2, WT2, ST5, CDKN1C, NUP98 and IGF2, which may trigger this patients tumorigenesis. Various molecular and cytogenetic factors have been implicated in the pathogenesis of neuroblastoma and useful in predicting clinical behavior and outcome. Chromosomal structural changes are found in more than 50% of neuroblastomas. Therefore molecular and cytogenetic characterizations of neuroblastoma become a routine part of the clinical evaluations that influence the clinical treatment and outcome.

Evidence of extra-telomeric effects of hTERT and its regulation involving a feedback loop. *T. Tollefsbol, S.R. Lai, A.P. Cunningham, V.Q. Huynh, L.G. Andrews* Biology, University of Alabama at Birmingham, Birmingham, AL.

The human telomerase reverse transcriptase (hTERT) is the catalytic subunit of the enzyme telomerase which is responsible for telomeric maintenance and extension. Using RNA interference to knock down hTERT mRNA expression, we provide evidence that hTERT exerts extra-telomeric effects on the cell cycle and on its own regulatory proteins, specifically: p53, p21, and c-Myc. We tested our hypothesis that hTERT regulates its own expression through effects on upstream regulatory genes using transformed human embryonic kidney HEK 293 cells, p53 and p16INK4a null human ovarian cancer SKOV-3 cells, and p53-null MDA-MB-157 human mammary cancer cells. In HEK 293 cells, hTERT knock down resulted in elevated p53 and p21 transcription and a decrease in cellular proliferation. Similar results were observed in the MDA-MB-157 cell line where p21 was up-regulated, correlating with cell growth inhibition. In contrast, we observed a decrease in expression of p21 and c-Myc in SKOV-3 cells with hTERT knock down and cell growth appeared to be unaffected. These findings suggest that hTERT may be involved in a feedback loop system, thereby playing a role in its own regulation.

Knock-down Analysis of 100 KAO-NASHI Genes. *A. Shimizu, S. Asakawa, T. Sasaki, N. Shimizu* Department of Molecular Biology, Keio University School of Medicine, Tokyo, Japan.

The human genome project has provided an computer-estimation of 23,000 protein-coding genes in the human genome. However, many of these protein-coding genes are not fully proven for their existence by experimental evidence. In general, proteins with known motifs are readily classified, but substantial numbers of protein have no obvious motifs in their sequences. We designated these genes/proteins without obvious motifs as KAO-NASHI (Face-less) and initiated a project to unveil their face (kao) by comparative genomics and knock-down analysis.

We extracted 1,000 KAO-NASHI genes from human genome sequence by step-wise filtration with InterPro motif analysis, BLAST homology search and PubMed document search. A small fish medaka (Oryzias latipes) was chosen as an experimental system to knock-down medaka orthologs of human KAO-NASHI genes with morpholino-antisense oligos. As an initial study, we designed antisense oligos to target translation initiation sites of 100 medaka kao-nashi genes. When these antisense oligos were micro-injected into medaka embryos, their morphogenesis at early developmental stages was disturbed and morphological changes were observed at certain rates. Thus, we obtained initial sketchy information how these medaka kao-nashi genes are involved in the embryonic process of patterning and organ formation. About 60 genes were found to cause embryonic defects and these were further classified in terms of developmental sub-stages and expression profiles. Thus, our approach using medaka seems effective and it will eventually provide functional information on the human KAO-NASHI genes/proteins.

Elevated level of common nonsynonymous variations in human EMR1 gene is consistent with balancing selection.

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The epidermal growth factor (EGF)-like module-containing mucin-like receptor, EMR1, is a member of the EGF-TM7 family of receptors that are predominantly expressed by cells of the immune system. EGF-TM7 receptors have a modular structure composed of an extracellular region containing EGF-like repeats that is joined to a seven-transmembrane membrane reminiscent of the G-protein coupled receptors. We resequenced all exons and flanking 5' and 3' regions of the EMR1 gene using DNA samples from 140 individuals of diverse geographical/ancestral origins (28 European-Americans, 28 Chinese, 28 Indians, 28 Malays and 28 Yorubans) as well as two chimpanzees. Population genetic analyses demonstrate an elevated level of nucleotide diversity in EMR1 that ranks among the most extremes of the empirical distributions, a skew in the allele frequency spectrum towards intermediate frequency alleles, positive Tajimas D values, an elevated nonsynonymous substitution rate within the human lineage, the presence of highly divergent intermediate haplotypes and a level of genetic differentiation that is lower than the global average. These data suggest that EMR1 gene does not evolve in a non-neutral fashion and is more likely to have experienced balancing selection. Because proteins containing EGF-like modules are typically involved in protein-protein interactions and the observation that 14 of the 20 nonsynonymous variations reside within the extracelullar portion of the receptor, the target of selection is probably directed against the EGF-like domain. Biological studies that seek to identify the interacting target(s) of the EMR1 receptor can shed further insight into the nature of the evolution of the gene.

Choreoathetosis gene mapping in a Native American family. *A.E. Shrimpton[1,2], J. Zeligman[2], C. Hubbell[1], J. Pellegrino[2]* 1) Clinical Pathology, SUNY Upstate Medical Univ, Syracuse, NY; 2) Pediatrics, SUNY Upstate Medical Univ, Syracuse, NY.

Blood has been collected from several members of a large four-generation pedigree of Native American extraction with non-progressive choreoathetosis (an involuntary writhing of the limbs). Onset is in infancy, is non-progressive and of variable severity with the more severely affected individuals being wheelchair-bound while less severely affected individuals remain fully ambulatory. Symptoms are not associated with any precipitating factors or improved with any specific medications or therapies. The affected individuals also have learning disabilities and are non-dysmorphic. The proband has a normal karyotype. Male to male transmission, vertical pattern of affected individuals and affected individuals of both sexes is consistent with an autosomal dominant mode of inheritance. Early onset non-progressive chorea with or without associated congenital hypothyroidism and neonatal respiratory distress has been reported in Benign Hereditary Chorea (BHC MIM 118700). A modest expansion in the CAG repeat in the Huntington's disease gene (IT15) has been reported in at least one BHC family and excluded in others. Investigation of the Huntington Disease gene (IT15) excluded CAG triplet repeat expansion as the cause of BHC in the present family. Several reports have described mutations in the TITF1 gene in several families with BHC. However a lack of linkage to 14q13 in other families indicates BHC locus heterogeneity. Missense and frameshift TITF1 mutations, as well as microdeletions, indicate TITF1 haploinsufficiency to be the cause of BHC in the 14q13-linked families. Sequencing of the TITF1 gene coding sequence, introns and promoter failed to detect an obvious pathogenic mutation, however TITF1 flanking and intragenic markers were consistent with linkage of choreoathetosis to TITF1 in this family (Maximum Lod of 1.5 at 0 cM). It is intended to collect additional family members so as to continue to investigate TITF1. Once sufficient family members have been collected and if TITF1 linkage is excluded, it is intended to use Affymetrix SNP microarrays to perform whole genome gene mapping.

Pharmacogenetic Analysis Reveals a New Role for Resistin in HIV Lipodystrophy. *K. Ranade, W. Geese, T. Delmonte, L. Hui, E. Emison, O. Flint, R. Parker, M. Noor* Pharmaceutical Research Institute, Bristol-Myers Squibb, Princeton, NJ.

Elevated lipid levels, insulin resistance and changes in body fat, collectively known as lipodystrophy, are common in HIV infected individuals on highly active anti-retroviral therapy (HAART). With the reduction in mortality resulting from HAART, the metabolic side-effects are of concern as they are well-established risk factors for cardiovascular disease. The mechanistic basis of HAART-associated lipodystrophy is poorly understood, however. For these reasons, we performed genetic analysis of 189 patients enrolled in a clinical trial of HAART. We clustered metabolic profiles of participants to identify a sub-group of patients (N = 47) who had normal mean triglyceride (176128 mg/dL), low density lipoprotein (LDL,120 30 mg/dL), total cholesterol (18233 mg/dL) and insulin resistance by homeostasis-model assessment (HOMA-IR, 21.5) at baseline but developed clinically significant elevations in mean triglyceride (+134 mg/dL, P<0.0001), LDL (+58 mg/dL, P<0.0001), total cholesterol (+81 mg/dL, P<0.0001) levels and HOMA-IR (+3, P=0.1) after 32 weeks of HAART. This high-risk cluster of patients also experienced significant body fat changes on HAART. In contrast, the normal cluster (N=142) had a similar metabolic profile at baseline and experienced clinically insignificant changes in these metabolic traits after HAART. Age, sex, race, baseline CD4 count and HIV copy number and drug treatment arm were not associated with cluster membership. Candidate gene analysis revealed that a common SNP in resistin, a gene previously implicated in obesity and insulin resistance, was highly significantly associated with clusters (P = 0.0003). The odds ratios for heterozygotes and homozygotes for high-risk cluster membership were 3 (95% C.I. 1.3-5.3) and 19 (95% C.I. 2-183) compared to wild-type, respectively. This SNP increased risk consistently in Caucasians, Hispanics and African-Americans. These results suggest a new role for resistin in HIV lipodystrophy, and demonstrate the importance of identifying less heterogeneous sub-groups prior to genetic analysis.

A Pharmacogenomic Study of Cholinergic Target Proteins in the Control of Cardiovascular Function. *A.M. Valle, K.Y. Ho, V. Mahboubi, M.T. Barragan, Z. Radic, B.K. Rana, D.T. O'Connor, P. Taylor* Depts of Pharmacology, Medicine and Psychiatry, Univ California, San Diego, La Jolla, CA.

The focus of this study is to identify polymorphisms in candidate cholinergic genes that may contribute to hypertensive disease states. Peripheral and central nervous system control of cardiovascular (CV) function mediated through the autonomic nervous system is critical in the homeostatic maintenance of blood pressure (BP) and responsiveness to exercise, postural alterations,and stress. Animal models have shown that cholinergic pathways in the spinal cord and higher brain centers modulate CV responses to influence basal BP and baroreflex pressor responses induced by external and internal stimuli. Currently we are investigating single nucleotide polymorphisms (SNPs) in acetylcholinesterase (AChE), the enzyme that regulates cholinergic neurotransmission by catalyzing the hydrolysis of acetylcholine. AChE sequence and structural makeup is relatively simple and encoded within 7.5kb. AChE can be found in blood enabling a biochemical phenotype in addition to correlating genotype with phenotypic physiologic responses.

Human whole blood samples were obtained from a twin registry of over 500 monozygotic and dizygotic twins and 80 unrelated individuals. Biochemical phenotype (AChE activity) was determined spectrophotometrically using the Ellman Assay. SNP discovery by the re-sequencing of *AChE* was performed on the unrelated panel and SNP genotyping was performed on the twin sample set. Site-directed mutagenesis was conducted to introduce non-synonymous coding SNPs that we found into a human *AChE* cDNA plasmid with subsequent transfection into HEK cells for protein expression. Protein purification was performed using affinity chromatography.

Biochemical analysis revealed over a 3-fold variance in AChE activity levels of sampled individuals. Correlation analysis showed a statistically significant relationship between enzyme activity and certain cardiovascular phenotypic endpoints. Characterization of the mutant enzymes revealed significant stability differences when compared with the wildtype AChE protein. (Supported by R37-GM18360 and U01-HL069758).

Usefulness of Buccal Smears in Rapid Diagnosis of Congenetial Myeloproliferative Disorders. *K. Patel, S. White, P. Hsu, J. Jacob, R. Kazi, AL. Zaslav* Department of Laboratory Medicine, Long Island Jewish Medical Center,the Long Island Campus of the Albert Einstein College of Medicine, New Hyde Park, NY 11040.

Neonatal myeloproliferative disorders (NMD) can represent a benign transient myeloproliferative disorder (TMD) associated with trisomy 21 Down syndrome (DS) or acute myeloid leukemia (AML) requiring aggressive management. It is important to identify true trisomy 21 so that these neonates can be treated conservatively. In NMD, the presence of trisomy 21 in peripheral blood may represent an acquired chromosome abnormality, which indicates secondary AML. Another tissue must be cytogenetically evaluated to determine this. We report two cases of NMD, in which trisomy 21 was first seen in peripheral blood. The clinical dilemma was resolved by rapidly identifying inherited trisomy 21 in epithelial nuclei from a buccal smear (BS) using FISH.

Two neonates without obvious DS phenotype or previously documented DS genotype presented with leukemic peripheral blood smears. Twenty trypsin-Giemsa banded metaphase spreads from peripheral blood revealed trisomy 21 in both cases. Fluorescent in situ hybridization (FISH) was performed on BS nuclei and peripheral blood cells using Aneu Vysion LSI 13/21 probe (Vysis, Downers Grove, ILL). In both the cases, the FISH analysis on interphase nuclei from blood and BS showed three signals for chromosome 21 in all cells indicating inherited trisomy 21. Based on the FISH results, the increased WBC count and blasts in the peripheral smear, the patients were diagnosed with DS-associated TMD and were managed conservatively. One of the two neonates showed resolution of myeloproliferative symptoms after four months of age and is disease-free one year after the initial diagnosis. The other expired at three months of age due to congenital heart defects.

This report demonstrates the use of BS in conjunction with FISH as an additional tissue to provide a quick method of determining acquired vs. inherited chromosome abnormalities. This technique was essential in assisting the clinicians with efficient, rapid, and accurate management of these cases.

Exploring gene joint effects and the clinical efficacy of morphine for cancer pain: OPRM1 and COMT gene. *C. Reyes-Gibby[1], S. Shete[1], T. Rakvåg[3], S. Bhat[1], F. Skorpen[3], E. Bruera[2], S. Kaasa[3], P. Klepstad[3]* 1) Epidemiology, U.T. M.D. Anderson Cancer Center, Houston, TX; 2) Department of Palliative Care and Rehabilitation, U.T. M.D. Anderson Cancer Center, Houston, TX; 3) Norwegian University of Science and Technology, Trondheim, Norway.

PURPOSE: The individual variation in opioid dose requirement for the treatment of cancer pain can be more than 1,000 fold, making it one of the largest observed variations in humans. Polymorphisms in genes coding for the mu-opioid receptor (A118G) and catechol-O-methyl transferase (Val158Met) may be important modulators of opioid efficacy. We assessed joint effects of OPRM1 and the COMT genes in predicting morphine dose for pain relief. PATIENTS AND METHODS: We used genotype and clinical data from a pharmacokinetic study of morphine in 207 inpatients. Patients were treated with stable morphine dose for at least 3 days by Palliative Medicine Specialists.RESULTS: Multivariate analyses showed Val158Met and A118G genotypes, months using morphine and time since cancer diagnosis as significant variables in predicting morphine dose. Significant joint effects were observed for OPRM1 and COMT genes. Adjusting for pain intensity, months using morphine and time since cancer diagnosis, carriers of the COMT Met/Met and OPRM1 AA genotype required 61% less morphine ($p<0.03$) relative to those with neither Met/Met nor AA genotypes (Met/Met and AA =92mg/24h, 95%CI=61,122; neither MetMet nor AA=150mg/24h;95%CI=109,192). CONCLUSION: Our preliminary findings suggest that genetic differences influence the clinical efficacy of morphine. Future studies with larger cohorts are needed to further characterize the role of genetic markers in predicting opioid dose. If our associations are confirmed, clinicians could potentially tailor morphine dose, based on the individuals genetic data, such that maximum effectiveness and fewer side effects could be achieved.

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Cleft Families Have a Higher Risk of Developing Several Types of Cancer. *R. Menezes, M.E. Cooper, K.M. Bardi, C.A. Brandon, A.R. Vieira, M.L. Marazita* Oral Biology and Center for Craniofacial and Dental Genetics, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA.

Individuals born with orofacial clefts have a shorter lifespan and a correlation between clefts and cancer has been proposed. The Pittsburgh Oral-Facial Cleft study begun in 1993 with the primary goal of identifying genes involved in nonsyndromic orofacial clefts and has recruited 185 families in the Pittsburgh area. Families with two or more individuals affected with orofacial clefts are preferentially recruited; therefore the study population is enriched by cleft multiplex families. Cancer history data were obtained from 168 families (75 cleft families and 93 control families) through a structured questionnaire. Out of the 75 cleft families, 70 families have two or more affected individuals. Chi-square and Fisher exact tests were used to determine statistically significant differences between cleft and control families. Overall, reported cancer history was higher in cleft families compared to control families ($p=0.0002$); further the occurrence of multiple types of cancers was also more common in the cleft families ($p=0.00001$). Among the 16 types of cancer reported in the study families, brain ($p=0.003$), breast ($p=0.009$), colon ($p=0.0009$), leukemia ($p=0.005$), liver ($p=0.02$), lung ($p=0.02$), prostate ($p=0.01$), skin ($p=0.01$), skin plus melanoma ($p=0.01$), and stomach ($p=0.04$) cancer types were found more commonly in cleft families than in control families. Our results suggest that individuals in multiplex cleft families have a higher susceptibility for specific types of cancer. Future studies will investigate the role of genes in which mutations have been associated with both cancer and clefts or other craniofacial anomalies (i.e. CDH1/E-cadherin, stomach cancer and clefts; AXIN2, colon cancer and tooth agenesis; CLPTM1, prostate and clefts). This research is supported by NIH Grants R01-DE016148, P50-DE016215, and M01-RR00084.

Dental Anomalies as an Extended Phenotype of Orofacial Clefts: Genome Wide Reanalysis of 12 Multiplex Filipino Families. *A.R. Vieira[1], G. Gamboa[2], M. Orbiso[2], T. Goldstein McHenry[1], M.E. Cooper[1], S. Daack-Hirsch[3], M.L. Marazita[1], J.C. Murray[3]* 1) Oral Biology and Center for Craniofacial and Dental Genetics, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA; 2) Phenomics Group of The Philippines, Consolacion, Cebu, The Philippines; 3) Pediatrics, University of Iowa, Iowa City, IA.

Cleft individuals have up to 6 times more dental anomalies than individuals from the general population. We are revisiting 154 Filipino families with 2 or more cleft affected siblings that participated in previous genome wide studies and collecting complete dental information. To date, we have re-visited 12 families. One of the families was found to have an undiagnosed syndrome and was not included in this analysis. Genotypes from 390 microsatellite markers at 10cM intervals were reanalyzed in the remaining 11 families to incorporate the dental anomaly data. There were 32 affected individuals with clefts and an additional 9 people that were added as affected with the addition of the dental phenotype. In the first pass of two- and multipoint parametric linkage analysis (FASTLINK, SIMWALK2) and nonparametric two- and multipoint analysis (MERLIN), only individuals with clefts were assigned as affected. In a second pass, non-cleft-affected relatives with a dental anomaly were also assigned as affected. In the first pass, 9 chromosomes (2,3,4,7,8,13,14,16,17) presented regions with LOD>1.0. In the second pass, 9 chromosomes (1,4,7,8,9,10,14,16,18) presented regions with LOD>1.0. The most significant results in the cleft affected only scan were a LOD of 2.98 at D4S2394 (4q28.2) under a recessive model and a LOD of 2.05 at D8S592 (8q24.11) under a dominant model. When dental anomalies were added as affection status in the analysis, the most significant results were also at 4q (4q31.21, D4S1625, LOD=2.36, and 4q28.2, D4S2394, LOD=2.18). The LOD score for D8S592 dropped to 1.51. Our preliminary results confirm a candidate region for clefts at 4q and support the hypothesis that some loci may contribute to both clefts and congenital dental anomalies. Supported by NIH Grants R21-DE016718, R37-DE08559, P50-DE016215, R01-DE016148 and CIDR N01-HG-65403.

Therapy of PKU by AAV-1 based transfer of PAH and BH4-cofactor genes into skeletal muscle. *B. Thony[1], C.O. Harding[2], A. Rebuffat[1], L. Elzazouk[1], J.A. Wolff[3], Z. Ding[1]* 1) Dept Ped, Div Clin Chem/Biochem, Univ Zurich, Zurich, Switzerland; 2) Dept Ped, Dept Molec/Med Genet, Oregon Health/Science Univ, Portland, OR, USA; 3) Dept Ped, Dept Med Genet, Univ Wisconsin, Madison, WI, USA.

Phenylketonuria (PKU) is caused by an autosomal recessive deficiency of the hepatic phenylalanine hydroxylase (PAH) leading high serum Phe and irreversible damage of the brain. PAH catalyzes the hydroxylation of Phe to tyrosine with tetrahydrobiopterin (BH4) as cofactor. BH4 biosynthesis requires the consecutive action of the enzymes GTPCH and PTPS, with dihydroneopterin triphosphate as the first intermediate. Here we aimed at expressing the PAH system in skeletal muscle to degrade serum Phe, as this tissue is abundant, easy accessible, and persistent due to its post-mitotic nuclei. However, BH4 is scarce in skeletal muscle as the cofactor-synthesizing enzyme GTPCH is absent in muscle tissue, and PTPS is expressed at low levels. We first demonstrated that transgenic PKU mice that had no liver PAH and expressed coordinately PAH along with GTPCH in skeletal muscle tissue accumulated dihydroneopterin triphosphate and remained hyperphenylalaninemic unless synthetic BH4-cofactor was supplied by intraperitoneal injections. Thus, PTPS activity is definitely limiting in skeletal muscle to synthesize sufficient BH4 and to support Phe hydroxylation. A recombinant triple-cistronic AAV2-based pseudotype 1 vector expressing PAH along with the two cDNA-genes for BH4 biosynthesis, GTPCH and PTPS, was then generated. Upon single injections of 3.5x10e12 recombinant triple-cistronic AAV2/1 vector particles into each of the gastrocnemius muscles of the hind legs of the PKU mouse model Pah-enu2 resulted in long-term clearance of blood Phe, including complete phenotypic reversion. A similar therapeutic effect was achieved when a combination of two AAV2/1 vectors expressing individually PAH and GTPCH-PTPS were co-injected into the same hind leg muscles. As a control, an AAV2/1-vector expressing only PAH with GTPCH was not therapeutic. This non-invasive application is the basis to develop an efficient therapy for PKU using a triple-cistronic gene transfer into skeletal muscle.

Provisional QTL mapping of prepulse inhibition (PPI) of tactile startle response in recombinant congenic strains of mice and comparison with acoustic PPI. *A. Torkamanzehi[1], P. Boksa[2,3], R. Joober[2,3,4]* 1) Department of Cellular and Molecular Biology, University of Sistan and Baluchistan, Zahedan, Iran; 2) Douglas Hospital Research Centre, Montreal, Canada; 3) Department of Psychiatry, McGill University, Montreal, Canada; 4) Department of Human Genetics, McGill University, Montreal, Canada.

Introduction: Prepulse inhibition (PPI) of the startle response is a psychophysiological measure of sensorimotor gating believed to be cross-modal between different sensory systems. PPI has been extensively used as an endophenotype measuring sensorimotor gating which is known to be abnormal in a number of psychiatric disorders including schizophrenia. Methods: Using light as a prepulse stimulus, we analyzed the tactile startle response (TSR) and PPI of TSR (tPPI), in the mouse strains A/J and C57BL/6J and 36 recombinant congenic strains (RCSs) derived from them. We then performed a provisional QTL mapping for loci modulating TSR and tPPI using 620 SSLP markers informative for A/J and C57BL/6J. Results: Parental strains were significantly different for TSR, but were comparable for tPPI. Among the congenic strains, variation for TSR was significant in both genetic backgrounds, but that of tPPI was significant only for the C57BL/6J background. SSLP markers associated with tPPI appeared on chromosomes 2, 4, 6, 10, 11,18 and 1, 8, 11, 17, 19, 20 in the A and B backgrounds, respectively. Conclusion: Comparing mapping data from a previous study, on acoustic startle responses (ASR) and PPI of ASR (aPPI), no common markers for aPPI and tPPI were identified. However, some markers were significantly associated with both ASR and TSR, at least in one genetic background. These results indicate cross-modal genetic regulation for the startle response but not for PPI, in these mouse strains.

Impact of Cytogenetic Evaluation on Prognosis of Patients with AML. *S. Viditio[1,3], K. Zamkoff[1,3], T. Mercado[2,3], D. Gladstone[1,3], AL. Zaslav[2,3]* 1) Department of Blood And Marrow Stem Cell Transplatation Program; 2) Department of Clinical Pathology, Cytogenetics Laboratory; 3) SUNY at Stony Brook University Medical Center, Stony Brook NY 11794.

Non-random chromosome abnormalities are important, independent prognostic factors in AML. We report a study of 27 adults with AML. Cytogenetic aberrations were correlated with the median overall survival (MOS) of all patients (pts). Clinical features and outcome were examined. Cytogenetic analyses on 24, 48 and 72-hr cultures of bone marrow or unstimulated blood were performed using G-banding and/or FISH on 20 metaphase cells or 200 nuclei.

Among 27 pts, five (19%) had a normal karyotype and 22 (81%) had 1 clonal abnormalities. The pts were grouped according to the presence of a recurrent abnormality and were categorized as F, I or A risk groups.

F had six pts median age (MA) of 43 yr. Four had a t(8;21)(q22;q22) and two had a t(15;17)(q22;q22). The MOS in this group was 10.6 mo.

I had 16 pts (59%), MA of 60 yr. Five had normal karyotypes (18.5%), nine had +8 (33%). MOS was 7.8 mo. Nine of 27 had +8; and MOS of 8 mo. Five had +8 and additional abnormalities; MA of 65 yr and OS 4.9 mo. Trisomy 8 was also analyzed as a separate risk group, (nine pts). Four pts, MA of 49 yr, had only +8(15%) with MOS of 8.3 mo and five pts, MA of 65 yr, had +8, and other aberrations. MOS was only 4.9 mo. The difference in the outcomes of the two subgroups was significant. Pts with +8 and additional abnormalities had a worse MOS than patients with only +8.

A had 19 pts(70%). Ten pts had complex abnormalities. A had a MA of 63 yr and MOS 6.2 mo.

In summary, chromosome analysis is a critical independent determinant of the clinical outcome of AML. This study though limited, yielded significant data and was consistent with results from prior studies. We plan to incorporate a more aggressive approach to the use of cytogenetic data for diagnosis, treatment and prognosis in our practice.

Desmin splice variants causing cardiac and skeletal myopathy. *A. Shatunov[1], M.C. Dalakas[1], K-Y. Park[1], A. Dagvadorj[1], F. Muntoni[2], H.H. Goebel[3], L.G. Goldfarb[1]* 1) National Institute of Neurological Disorders and Stroke and National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892, USA; 2) Dubowitz Neuromuscular Centre, Imperial College, Hammersmith Hospital, London, UK; 3) Mainz University Medical Center, D-55131 Mainz, Germany.

Desminopathy is a hereditary or sporadic cardiac and skeletal myopathy characterized by intracytoplasmic accumulation of desmin-reactive deposits in muscle cells. We characterized splice site mutations in desmin gene resulting in deletion of the entire exon 3 during pre-mRNA splicing. Sequencing of mRNA and genomic DNA from patients diagnosed with desminopathy identified 1) a heterozygous A-to-G change at the +3 position in the splice donor site of intron 3 (IVS3+3A->G) in two families and a single patient with the same (IVS3+3A->G) mutation generated de novo; 2) a G-to-A transition at the highly conserved -1 nucleotide position of intron 2 affecting the splice acceptor site (IVS2-1G->A) in two brothers affected with cardiomyopathy or a combination of cardiac and skeletal myopathy; 3) an A-to-T change at -2 nucleotide of intron 2 (IVS2-2A->T) in a patient with cardioskeletal myopathy; and 4) a G-to-A transition at the highly conserved +1 nucleotide (IVS3+1G->A) in a mother and son pair with highly aggressive illness. Aberrant splicing leads in each of the patients to an in-frame deletion of 32 codons, from 214 through 245, and is predicted to result in mutant desmin lacking 32 amino acids in the 1B segment of the alpha-helical rod. None of the 150 tested control individuals carried a splice variant distinct from the wild type desmin gene sequence. Expression of genomic DNA fragments carrying the IVS3+3A->G and IVS2-1G->A mutations in SW13 (vim-) cells confirmed that these mutations cause exon 3 deletion. Functional analysis of the mutant desmin lacking the 32 amino acids in SW13 (vim-) cells demonstrated aggregation of abnormal coarse clumps of desmin-positive material dispersed throughout the cytoplasm.

Ryanodine Type 1 Receptor mutations in Neuromuscular Disorders. *N. Sambuughin[1, 2], S. Muldoon[2], B. Brandom[3], T. Nelson[4], L. Goldfarb[1]* 1) NINDS/NIH, Bethesda, MD; 2) USUHS, Bethesda, MD; 3) Children's Hospital, Pittsburgh, PA; 4) Wake Forest University of Medicine, Winston Salem, NC.

The Ryanodine Receptor 1 (RYR1) is skeletal muscle specific intracellular calcium channel which plays a central role in muscle contraction. The RYR1 receptor is localized at the terminal cisternae of the sarcoplasmic reticulum and regulates calcium release into myoplasm. Mutations in the RYR1 gene have been identified in several neuromuscular disorders with underlying pathophysiology involving calcium regulation in skeletal muscle. To date, more than 120 mutations in the 15,117bp coding region of the RYR1 gene have been found to segregate with Malignant Hyperthermia (MH) and Central Core Disease (CCD), while a few of these mutations are also associated with Multiminicore Disease (MmD). MH is a life-threatening hypermetabolic crisis occurring in susceptible individuals as a result of exposure to inhalational anesthetics or depolarizing muscle relaxants. CCD and MmD are congenital myopathies characterized by the presence of cores in muscle. Clinical presentations of CCD and MmD may overlap and differential diagnosis is based on the nature of the cores and the type of affected muscle fibers. The majority of RYR1 mutations appear to be clustered into three regions or mutational hot spots: N-terminal, Central and C-terminal. The functional role of the first two regions is unclear, while the C-terminal region of RYR1 forms the transmembrane channel. We have screened the entire coding region of the RYR1 gene in 30 MH individuals from North America. Nine previously reported and nine novel RYR1 mutations have been identified accounting altogether for 70% of screened patients. Some of the new mutations were located outside of the known mutational hot spots, suggesting that RYR1 contains previously unknown mutation-prone areas requiring further analysis. The North American MH population exhibits notably different RYR1 mutational pool compared to the European MH populations and is characterized by a higher RYR1 allelic heterogeneity.

Haplotype and copy number variation in the low affinity Fc receptor 3B in the Pima Indians of Arizona. *K.M. Schroeder, V.M. Ossowski, L. Baier, C. Bogardus, M. Prochazka* PECRB, NIDDK, Genomics Unit, Phoenix, AZ.

The Pima Indians of Arizona have the worlds highest reported prevalence rate of type 2 diabetes (T2D), and our prior genetic linkage study indicated a susceptibility locus for this disease on Chr1q21-q25. As part of ongoing positional candidate gene analysis within this region of linkage, we analyzed the low affinity Fc receptor 3B (FCR3B), located within 2 Mb centromeric to the peak of linkage. FCR3B contains a well-validated haplotype system whose predominant alleles, FCR3B*1 and FCR3B*2 (formerly referred to as NA1 and NA2, respectively), are associated with a number of immune-related diseases. FCR3B can also vary in copy number, and a lower number was recently shown to be associated with predisposition to lupus nephritis. To genotype the FCR3B*1/*2 system in Pima Indians, genomic DNA from 208 subjects who contributed to the linkage was directly sequenced using allele-specific primers. Genotype frequencies for the 186 subjects for whom product was obtained indicated an excess of heterozygosity (0.80) and deviation form Hardy-Weinberg equilibrium (HWE; $p < 0.001$), suggesting higher copy numbers. Consistent with this, sequencing revealed an intronic single nucleotide polymorphism using either of the allele-specific primer sets. Based on heterozygosity at this site, 39 % of the Pima subjects had at least three alleles, in contrast to a Caucasian sample ($n = 49$) where only 4 % of subjects had more than two alleles, and where genotype frequencies did not deviate from HWE ($p = 0.85$). Our results show that Pima Indians, unlike most other populations studied to date, show excess FCR3B*1/*2 heterozygosity that can be attributed to higher gene copy numbers. We are currently attempting to measure copy number of each of the two haplotypes using quantitative real-time PCR to determine whether haplotype copy number contributes to susceptibility to T2D in Pima Indians.

Recessive alleles and Longevity among Kurichians: a tribal population of Kerala, India. *P.K.R. Thavanati[1], K.R.R. Kanala[2], A. Escoto de Dios[3], P.R. Alaharai[2], M.G. Lopez Cardona[1], N.O. Davalos Rodriguez[1], J.M. Cantu Garza[1]* 1) Instituto de Genetica, Departamento de Biologia Molecular y Genomica, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, 950 Sierra Mojada, Col. Independencia, Guadalajara. Jal. 44340,Mexico; 2) Department of Anthropology, School of Biological & Earth Sciences, Sri Venakateswara University, Tirupati - 517 502. Andhra Pradesh. India; 3) Genetica, Centro de Investigacion Biomedica de Occidente, IMSS, 900 Sierra Mojada, Col. Independencia, Guadalajara. Jal. 44340,Mexico.

Recessive lethal/semi-lethal genes will be eliminated at a faster rate from the population that maintains higher rate and prolonged practice of inbreeding wherein, the deleterious genes are effective in post-natal stages of life. The present study is aimed to evaluate the levels of inbreeding and its effects including the ancestral consanguinity effect, along with the levels of lipid peroxidation and DNA damage among Kurichias, a tribal population of Kerala, India to understand their structure who are leading a healthy long life. Personal interview method has been done on 300 families of Kurichian tribe of Kerala, using a questionnaire alongwith 10 ml intravenous blood sample (healthy 600: 404 males and 196 females) for the analysis of lipid peroxidation and DNA damage. The high levels of inbreeding among Kurichians would have been eliminating the deleterious genes, and under interaction with living conditions, such high inbreeding leading to increase in homozygosity of many of the genes that might have adapted to those conditions would explain the increased survivial of the individuals under inbreeding. Further, the effects of parental consanguinity of both the parents of the couples suggest the practice of inbreeding had already reduced the burden of deleterious genes. The values of lipid peroxidation and the DNA damage explains less susceptibility to oxidative stress leading to many degenerative diseases resulting to mortality. Therefore, inbreeding under favourable heredity does not reduced the level of longevity, where healthy aged and centenarians are found in the study population.

Large-scale blinded comparison of a commercially available array CGH platform to FISH for the analysis of subtelomeric abnormalities. *E. Thorland, T. Gliem, P. Gonzales, A. Wiktor, R. Ketterling* Cytogenetics, Dept of Lab Medicine & Pathology, Mayo Clinic, Rochester, MN.

Array comparative genomic hybridization (aCGH) allows the detection of copy number differences at varying sensitivity across the genome, depending on the number and density of probes utilized. aCGH chips targeting clinically defined loci that are recurrently deleted or duplicated are quickly becoming a clinical tool used for testing patients with unexplained mental retardation, developmental delay, or congenital anomalies. While this technology is not likely to replace standard chromosome analysis in this patient group, it may substitute for the numerous FISH tests that are often performed in an attempt to make a diagnosis, including FISH for the 41 clinically relevant subtelomeric sites which is very labor intensive and expensive. We have utilized our extensive clinical database of patients with previously defined subtelomere abnormalities, representing deletions and duplications of the majority of the 41 clinically relevant sites, to initiate a blinded study using the Spectral Genomics Constitutional Chip 3.0. This will allow a determination of the sensitivity and specificity of this aCGH platform compared to standard subtelomere analysis by FISH. The Spectral chip contains multiple BAC clones at all of the subtelomeric regions, in addition to clone coverage at numerous other clinically relevant loci. Patient samples that were normal by chromosome analysis or had subtle chromosomal rearrangements that necessitated further confirmation by subtelomere analysis were preferentially chosen for the blinded study. This strategy minimizes detection bias by limiting the number of abnormal proximal, non-subtelomere clones that could artificially enhance the ability of the aCGH chip to detect dosage diffences in this patient subgroup. To our knowledge, this is the most comprehensive comparison of subtelomere FISH to any aCGH platform that has been reported. This study will provide data as to the efficacy of aCGH as a replacement for subtelomeric FISH studies in a clinical setting.

Associated malformations in cases with neural tube defects. *m-p. roth, y. alembik, b. dott, c. stoll* genetique medicale, faculte de medecine, strasbourg, France.

Objective: Infants with neural tube defects(NTD)may have other associated con¬genital defects. The reported incidence and the types of associated malfor¬mations vary between different studies. The purpose of this investigation was to assess the prevalence of associated malformations in a geographically de¬fined population. Method: The prevalences at birth of associated malformations in infants with NTD were collected between 1979 and 2003 on all infantss born in the area covered by the registry of congenital anomalies of Northeastern France in 334,262 consecutive births. Results: Of the 295 infants with NTD born during this period, 18,6% had associ¬ated malformations. Associated malformations were more frequent in infants who had encephalocele(24,2%) than in infants with anencephaly(18,8%) or infants with spina bifida(12,5%). Malformations in the cardiovascular system and in the skeletal system were the most common other anomalies, fol¬lowed by oral clefts and malformations in the digestif system. Weight, length, and head circumference of children with NTD and multiple associated malformations were lower than in controls, as was the weight of the placenta. Conclusion: The overall prevalence of malformations, which was one in more than six infants, emphasizes the need for a thorough investigation of infants with NTD. A routine screening for other malformations especially skeletal, and cardiac defects may need to be considered in infants with NTD,and genetic counseling seems warranted in most of these complicated cases.

Empirical evaluation in 8 diverse populations reveals that common variation is well-captured by transferred tags, but tag combinations are highly divergent from specified tests. *E. Zeggini, N.W. Rayner, L.R. Cardon, M.I. McCarthy for the International Type 2 Diabetes 1q Consortium* WTCHG, University of Oxford, UK.

Universal applicability of the HapMap relies on tag SNP portability across populations. We addressed the issue of tag transferability by studying 3304 SNPs across 21 Mb of chromosome 1q, a replicated region of linkage to type 2 diabetes (T2D), in 8 case-control sets from diverse populations (4 European-descent, 2 East Asian, 1 African-American and 1 Native-American). Sample sizes were standardized to 64 (controls for training and cases for test sets). Tags were selected from each population and capacity to capture variation was tested in all 8. Tag method (pairwise or aggressive) was consistent between selection and evaluation steps. Importantly, neither pairwise nor multimarker tests were specified for the evaluation stage, thus permitting full use of the underlying LD structure. To reflect realistic genotype success rates, random 10% tag subsets were dropped from each set and capture was re-evaluated. Irrespective of tagging method, tags were portable across datasets (63%-97% common SNPs captured), but with a marked decrease in capture when evaluated in African-Americans (45%-69%). The average drop in observed common SNP capture with 90% of tags at $r^2>0.8$ was substantial (>7%) and overall comparable between pairwise and multimarker-defined tags. Only 20.6% of aggressively-captured variation was tagged by the SNP combination specified at the selection stage ($r^2>0.8$, with similar results for $r^2>0.5$). Interestingly, up to 12% (16.9% for 90% tag subset) of tests do not match the specified tests for pairwise-defined tags. We demonstrate that common SNP tags are transferable across most of the diverse populations studied, but that variation capture can decline substantially when even a small proportion of tags is not successfully genotyped, or when downstream analyses are restricted to specified tests.

A Genome-Wide High Ancestry Informative SNP panel for African American Admixture Mapping. *C. Tian*[1], *D.A. Hinds*[2], *R. Shigeta*[1], *R. Kittles*[3], *D.G. Ballinger*[2], *M.F. Seldin*[1] 1) Rowe Program in Human Genetics, Univ California Sch Medicine, Davis, CA; 2) Perlegen Sciences, Mountain View, CA; 3) Comprehensive Cancer Center, Ohio State Univ, Colombus, OH.

Admixture mapping requires a genome-wide panel of relatively evenly spaced markers that can in admixed individuals distinguish the ancestral origins of chromosomal segments. Using the results of the HapMap study (including over 3.5 million SNPs) and specific selection criteria the current study has examined the ability of selected SNPs to extract continental ancestry information in African American subjects and to explore parameters for admixture mapping. Genotyping of two disparate African populations (Bantu speaking and Nilo-Saharan ethnic groups), European Americans and African Americans with 5300 SNPs validated a genome-wide set of 4222 SNP ancestry informative markers (AIMs) with mean and median Fst values > 0.59 and mean and median Fishers information content >2.5. In contrast, the intra-African and intra-European mean Fst values were < 0.02. This set of AIM SNPs extracted a larger amount of ancestry information in the African American than previous reported SNP panels and provides more uniform coverage of the genome. ADMIXMAP was used to examine the admixture information in the genotyped African American samples (96 samples from Coriell set). When considering an interpolated genome-wide Decode map, the 4222 SNP AIMs extract >60% of the admixture information for over 98% of the genome, >70% for over 90% of the genome, and >80% for >66% of the genome. Moreover, simulations using different marker sets show that this more informative AIMs panel can improve power for admixture mapping in African Americans compared with smaller AIMs panels. This is particularly evident when the ethnicity risk ratio was below 1.75. This may have practical importance in the application of admixture mapping in complex genetic diseases in which the individual ethnicity risk ratios of many relevant susceptibility genes are unlikely to be very great.

The study of DNA hypervariability detected by DNA of phage M13 with the purposes of ecological monitoring.

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Ecological intensity in industrial regions conducts to contact of the population with potentially harmful polluting factors of environment, which can enhance the mutation influence, can damage a genome in gametes and somatic cells of the man. Sterlitamak is large industrial city of the Bashkir Republic, where are submitted the potent chemical industry and oil processing. The bank DNA of the townspeople is being created, which now contains 397 samples. The experimental group include of the members 53 families (both of parents and them children), selected with allowing for professional contact with toxic materials even of one of the parents during not less half-year up to a conception of the child. The control group - 30 families, where the parents by virtue of the professional work had no similar contact. The fingerprints with DNA of phage M13 of the members 14 experimental and 6 control families is carried out. The fields of 11 radioautographs with lengthes from 2027 up to 21226 p.n. were used for visual comparison. We believed that the bands is identical, if in an electrophoretic profile the bands differed less, than on 1 mm. We were considering as mutation events the absence of fragment in a profile of child in comparison with the strip at the level of parents strips and presence at him of the restriction fragment which is not conterminous on molecular weight with the strips of the parents. Amount of bands in a profile of children, which were not referred to parents, was unexpectedly above the concerning similar researches: 1,55 new bands for the child in experimental group and 3,10 - in monitoring. This fact can be conditioned as by methodological features of the given research, so by actual effect on gametes of high concentrations of mutagenes of environment.

Short isoform of Myoclonin1/EFHC1 in human and monkey but not in mouse. *T. Suzuki[1, 2], A. Delgado-Escueta[3], K. Yamakawa[1]* 1) Lab. for Neurogenetics, RIKEN Brain Science Institute, Saitama, Japan; 2) Special Postdoctoral Researchers Program; 3) Epilepsy Genetics/Genomics Laboratories, Comprehensive Epilepsy Program, David Geffen School of Medicine, UCLA, and VA GLAHS-West Los Angeles Epilepsy Center of Excellence, Los Angeles, California, USA.

Juvenile myoclonic epilepsy (JME) is a life-long disorder accounting for 10-20% of all epilepsies. In 2002, we mapped a JME gene on chromosome 6p12-p11 (EJM1) and in 2004 we identified JME causing mutations in a novel gene myoclonin1/EFHC1. Myoclonin1/EFHC1 encodes 640 amino acids protein (isoform A) and 278 amino acids protein (isoform B). EFHC1 revealed five missense mutations in isoform A co-segregating with 21 affected members of six JME families that did not appear in 382 healthy control individuals. Recently, we screened 111 new JME families from Mexico, Honduras and Japan for mutations. We identified additional novel heterozygous missense mutations in isoform A, and frameshift and nonsense mutations in specific regions of isoform B. Because mutations of isoform B appear in three JME families, we further characterized Myoclonin1/EFHC1 isoform B in mice and monkeys. Northern blot analysis of mouse multiple tissues including brain at various developmental stages was performed with 3' end (nt 1670-1990) of Efhc1 as a probe. A ~2.3 kb transcript appeared in multiple tissues including brain but most strongly in lungs. Hence, the mouse either does not have isoform B or it is expressed at levels below that detectable by Northern blots. Northern blots of monkey Efhc1 showed multiple size transcripts (~2, 3, 4.4 and 6 kb) in cerebellum. The 3 and 6 kb transcripts were detected by human isoform B specific probe. We also isolated monkey Efhc1 cDNA clones by RT-PCR. These cDNA clones contained several forms of Efhc1 including isoform B. Further studies on monkey isoform B may advance understanding of the pathophysiology of seizures in human JME.

A Japanese patient with a mild variant of Lenz-Majewski syndrome. *D. Sumito[1], T. Kondoh[1], G. Nishimura[2], K. Motomura1[1], K. Yoshiura[3], A. Kinoshita[3], H. Kuniba[1], Y. Koga[4], H. Moriuchi[1]* 1) Dept Pediatr, Nagasaki University, Nagasaki, Japan; 2) Dept Radiol, Tokyo Metropolitan Kiyose Childrens Hospital, Tokyo, Japan; 3) Dept. Hum Genet, Nagasaki University, Nagasaki, Japan; 4) Dept. Dev and Reconstr Med, Nagasaki University, Nagasaki, Japan.

Lenz-Majewski syndrome (LMS) is a rare skeletal dysplasia comprising progressive skeletal sclerosis, mental retardation, failure to thrive, enamel hypoplasia and loose atrophic skin with prominent cutaneous veins, and its prognosis is considered to be poor. Here we report a 17-year-old Japanese boy with a mild variant of LMS. He showed cutis laxa and prominent scalp veins in infancy. Dentition started at age 2 years; however, permanent teeth of the upper jaw had not been erupted. At the age of 13 years, he was admitted to our hospital for purpose of jaw reconstruction. Facial appearance was peculiar with progeroid-looking, sparse hair, large auticles, maxillar hypoplasia, high-arched palate and severe enamel dysplasia. He had mild mental retardation of 60 intelligence quotients. His skin was loose with prominent veins in abdominal wall. His extremities lacked major anomaly except for interdigital webbing and mild cubitus valgus, and his motor function was entirely normal. He had neither hearing impairment nor impingement on other cranial nerves. Skeletal roentgenograms demonstrated symmetric hyperostosis of calvaria, skull base, vertebrae, pelvis, carapals, and long bones, which were pronounced in epiphysis. At the age of 17 years, clinical findings appeared stable, but radiological abnormality was slowly progressive. No abnormality was detected in his TGFB-1 and LRP5 genes, which are candidate genes of two similar disorders, Camurati-Engelmann disease and endosteal hyperostosis, respectively. Clinical features and radiological findings of our patient were overlapped with those of LMS, but milder and less progressive than those of the previously reported LMS patients. It is suggested from our experience that LMS may be phenotypically variable. Further investigation is required to better understand etiology and pathogenesis of LMS and related disorders.

Voiding dysfunction - a previously unrecognized but surprisingly frequent complication of Down syndrome. *A. Tanaka[1], T. Kondoh[1], M. Noguchi[2], T. Hatada[2], S. Tohbu[2], T. Matsuo[2], M. Matsuo[2], H. Kanetake[2], H. Moriuchi[1]* 1) Dept Pediatrics, Nagasaki Univ, Nagasaki, Japan; 2) Dept. Urology, Nagasaki Univ, Nagasaki, Japan.

Genitourinary anomalies such as small penis and small scrotum have been associated with Down syndrome (DS) patients; however, voiding function has not been precisely evaluated in DS patients. We therefore investigated voiding function in 74 DS patients (36 women and 38 men), aged 2-39 years. Voiding function was evaluated by urinary frequency/volume chart for 3 consecutive days, uroflowmetrical examination by the uroflowmeter, residual urinary volume after micturition by ultrasonography, urinalysis and checking their history of voiding status. We divided the subjects into three groups: group A (23 patients younger than 10 years old), group B (14 patients aged 10-15 years), and group C (37 patients older than 15 years old). According to their voiding history, 52 (70%) and 10 (14%) patients reported normal and reduced desire to urinate, respectively. Seventeen patients (23%) presented with urinary incontinence. Urinary incontinence was infrequent in group C patients, as compared to other groups. On the other hand, 16% of group C patients had significant amount of residual urine, while group A patients had no residual urine. The urine frequency in 24 hours was lower and mean voided volume per one time was larger in group C patients than in group A or B patients. Uroflowmetrical analyses were informative in 40 of 74 patients. Maximum and average flow rates were highest in group B and lowest group C patients. Twenty nine (73%) DS patients presented with abnormal flow pattern such as straining to void. Thus, voiding function of DS patients is characterized by decrease in urinary frequency and flow rate as well as increase in residual urinary volume with aging. In other words, the bladder of DS patients tends to retain urine with aging, ultimately resulting in detrusor dysfunction. In conclusion, surprisingly many DS patients are affected by voiding dysfunction, which should decrease quality of their lives. We should recognize such important complication and monitor their voiding function regularly.

Multi Information for Testing Association of Gene-Gene Interaction Networks with Disease. *M. Xiong, L. Luo*
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Complex diseases are caused by multiple genes, primarily through nonlinear gene interactions and gene-environment interactions. Complex gene interactions are organized into networks. It has been found that the number of interactions per gene varied from 1 to hundreds, with average of 34 interactions in some model organisms. Genetic interaction networks must be ubiquitous in common diseases. A major challenge is to develop quantity for characterization of the genetic interaction networks which take high order gene-interaction into account and statistics for testing association of the genetic interaction networks with diseases. In this report, we present novel concept of multi information that is the extension of the mutual information or correlation between two random variables in formation theory to the multiple variables and the network and reveal the relationship between multi information and linkage disequilibrium at multiple loci. We study the distribution of multi information and develop a novel statistic based on multi information to test association of the genetic interaction networks or biochemical pathways with the diseases. The null distribution and power of the statistic are evaluated. The proposed statistic is applied to the genetic studies of atherosclerosis where overall 1518 SNPs from 123 candidate genes in inflammatory pathway, antioxidant pathway, coagulation pathway and lipoprotein metabolic pathway in 916 samples (492 atherosclerosis patients and 424 controls) were typed and identify a genetic interaction network with total 84 interactions within the pathway and 36 interactions between the pathways associated with atherosclerosis. The development of the multi information theory will provide a general framework for study of gene-gene and gene-environment interaction.

Genome-wide association mapping under the Malecot model and composite likelihood. *W. Zhang[1], A. Collins[1], W. Jia[2], C. Hu[2], N.E. Morton[1]* 1) Human Genetics Div, Univ Southampton, Southampton, United Kingdom; 2) Shanghai Diabetes Institute, Shanghai Jiaotong University Affiliated Sixth Peoples Hospital, Shanghai, 200233, China.

The combination of the evolutionary model of the Malecot and composite likelihood has been successfully applied to candidate gene association mapping. Here, we extended our method to genome-wide scans. The underlying logic is that a chromosome (a unit of the genome) is divided into segments according to the LD map expressed in linkage disequilibrium (LD) unit (LDU), a metric of genetic distance between markers monotonic to cM in a linkage map. Tentatively we defined 10 LDUs as the length of a segment assumed to have extensive LD that is useful for association mapping and minimum of 30 SNPs in a segment. The observed association between the phenotype and a marker SNP was calculated from the data. The expected association was predicted by the Malecot model, which is a function of the distance in LDU or kb between the hypothesized disease variant and the marker and a few other parameters. Composite likelihood was applied to combine association at multiple loci, substantially reducing the burden of multiple testing encountered in single SNP tests. By fitting the Malecot model and maximizing the composite likelihood, we can detect association and if there is an association, to estimate the location of the disease variant and its confidence interval. Starting with a case-control sample with thousands of SNPs for a large region, we simulated the case/control status based on genotypes of a randomly chosen causal SNP in a segment with some constrains. We estimated the significance levels empirically through simulation under the null hypothesis of no association by randomly shuffling the case/control status, and assigned a P value for each segment in a replicate according to the rank order of the statistic to ensure uniform distribution. We explored three association statistics by contrasting different hierarchical models. We demonstrate that our method is both practical and efficient for whole genome association scans and we also discuss other issues, such as the false discovery rate and meta-analysis.

Expression profiling supports peroxisome proliferator-activated receptor alpha (PPAR) activation in livers of 70kDa Peroxisomal Membrane Protein (PMP70) deficient (*Abcd3*-/-) mice. *I. Silva-Zolezzi*[2,3], *S. Bradley*[1], *D. Valle*[1], *G. Jimenez-Sanchez*[2] 1) McKusick-Nathans Institute of Genetic Medicine and Howard Hughes Medical Institute, Johns Hopkins University, School of Medicine, Baltimore, MD; 2) National Institute of Genomic Medicine, Mexico DF, Mexico; 3) Ph.D. Program in Molecular Biomedicine, CINVESTAV-IPN, Mexico.

There are 4 known human half ABC transporters in the peroxisomal membrane: ALD, ALDR, PMP70 and P70R, encoded by *ABCD1, 2, 3*, and *4*. Mutations in *ABCD1* cause X-linked adrenoleukodystrophy while the function and disease associations of the other three are unknown. PMP70 is thought to be a transporter for very long chain branched chain fatty acids into peroxisomes. *Abcd3*-/- mice have reduced hepatic glycogen, dicarboxylic aciduria, secondary carnitine deficiency and cold intolerance. Despite these metabolic alterations, mice are healthy and have a normal lifespan. In order to understand the molecular mechanisms underlying this metabolic phenotype, we evaluated the role of (PPAR) activation by surveying hepatic gene expression using murine U74Av2 Affymetrix arrays. In pooled samples of liver total RNA of *Abcd3*-/- and *Abcd3*+/+ mice, we identified 221 transcripts, with an AFC in mRNA level of at least 1.7. Of these, 97 were increased and 124 were decreased. Using designations of Gene Ontology and information from public resources, we organized genes into 13 categories. Our results show expression changes in genes of lipid metabolism, inflammation and cell growth similar to those observed when PPAR is activated. In addition, 65% of the altered genes have not been reported to be affected by PPAR activation, some of these may be novel PPAR targets, while others may represent downstream effects. Interestingly, we found reduced expression of two genes lipocalin 2 (*Lcn2*) and serum amyloid P-component (*Apcs*), both reported to be increased in hepatocellular carcinomas (HCCs) from *Acox1*-/- mice, a peroxisomal -oxidation enzyme, and in mice subjected to long term exposure to PPAR synthetic agonists. Our results are consistent with our hypothesis that *Abcd3*-/- metabolic phenotype is a consequence of inappropriate and chronic PPAR activation.

Expression of cAMP-responsive element modulator (CREM) in Rat Testes Following the Root of *Panax ginseng* Treatment. *W.M. Yang, D.Y. Shin, B.H. Lee, W-N. Kim, D.R. Kim, W. Park, S.K. Park* Prescriptionology, Kyung Hee Univ. College of Oriental Medicine, Seoul, Korea.

Ginseng Radix (GR), the root of *Panax ginseng* has been used as a tonic in Asia for more than 2,000 years, and is now widely used around the world. Previous reports have shown GR to improve survival rate and sperm quality; inhibit lipid peroxidation; protect against testicular toxicity. However, few studies have used rat testes to examine the effects of GR on male reproductive functions. After Rats were treated GR for 56 days consecutively. Total RNA was extracted from rat testes using Trizol method. Then we carried out Western blotting assay using anti-CREM antibody. CREM mRNA levels were significantly increased in testes from the rats treated with GR (p 0.05). The relative expression of CREM mRNA in the GR treated group was 174% than that in the normal group. There was significantly enhanced expression of CREM-immunoreactive bands in the GR treated group compared to the vehicle treated group (p 0.05). The relative expression of CREM in the GR treated group was 108% than that in the normal group. In this study, GR has the enhancing effect of CREM expression in rat testes especially at mRNA level. These findings suggest that GR may have a role of improving male infertility related with the sperm alterations and proliferating or differentiating of germ cells closely related with CREM gene expression.

Leptin and adiponectin in Gaucher disease. *A. Rosenberg[1], G. Litvin[1], G. Altarescu[2], A. Zimran[1], D. Elstein[1]* 1) Gaucher Clinic, Shaare Zedek Medical Center, Jerusalem, Israel; 2) Genetic Unit, Shaare Zedek Medical Center, Jerusalem, Israel.

Although genotype may be predictive of phenotype in a broad sense in type I (non-neuronopathic) Gaucher disease, there is considerable phenotypic variability. Research suggests that adipose tissue may secrete hormones that take part in energy metabolism and have anti-atherogenic and anti-inflammatory properties since adipocyte products such as leptin and adiponectin have been implicated in pathological processes in liver, spleen, bone, lung, and brain. The purpose of this study was to ascertain whether either or both of these hormones are predictive of various disease specific parameters, including hematological and bone markers, body mass index, inflammatory indices, lipid profiles, and glucose levels. All adult patients arriving at the Gaucher Clinic for routine follow-up during January-June 2005 and for whom laboratory tests were available, were included. There were 99 patients (45 men, 54 women). Mean values for leptin in male patients only showed borderline higher values than controls (p=0.082). There were no other statistically significant correlations between leptin and any of the other parameters, nor with adiponectin. Patients with Gaucher disease had statistically significant lower values of adiponectin (p=.0007 and p=.0001 for men and women, respectively) with a trend to a statistically significant difference between men and women (p=.06). Adiponectin was not correlated with any of the other disease parameters. There was no significant difference in leptin levels or in adiponectin levels between patients with the N370S/N370S genotype versus other genotypes or between patients treated with enzyme replacement therapy versus untreated patients. We speculate that Gaucher-induced liver pathology even if not clinically overt may be related to adiponectin levels as in other liver diseases. However, we cannot explain why lipid accumulation in Gaucher disease is correlated with abnormally low adiponectin values, albeit without clinical evidence of insulin resistance.

Correlation between inflammatory cytokines genes polymorphisms and Mainz Severity Score Index (MSSI) in patients with Fabry disease. *R. Safyan[1], C. Whybra[2], D. Elstein[3], G. Chicco[3], M. Beck[2], G. Altarescu[1]* 1) Genetic Unit, Shaare Zedek Medical Center, Jerusalem, Israel; 2) Universitäts- Kinderklinik2, Mainz, Germany; 3) Gaucher Clinic,Shaare Zedek Medical Center, Jerusalem, Israel.

Fabry disease is an X-linked disorder associated with early-onset stroke, cardiomiopathy, and progression to end-stage renal failure. Correlations between IL-6 inflammatory cytokine gene polymorphisms and MSSI scores have been shown. Therefore, polymorphisms of other key pro- and anti-inflammatory cytokines and correlation to clinical manifestations (MSSI scores) were attempted in order to build haplotype descriptions for Fabry disease. Genotyping for IL-10[819C/T; -592C/A]; IL-1beta[+3954 C/T; -511C/T]; IL-1alpha[-889C/T]; and TNF-alpha[-308G/A] was performed in 76 patients and correlated with MSSI sub-scores and with enzyme (alpha-galactosidase A) levels. Fifty normal volunteers, age- and sex-matched, were also genotyped. Of 76 patients, 31(41%) were males and 45(59%) were females. There was no correlation between enzyme levels and any cytokine polymorphisms. Statistically significant differences were found in prevalence of TNF-alpha[-308G/A] genotypes: 84%GG in patients versus 63%GG in controls ($p=0.038$) and for IL-1alpha[-889C/T] genotypes: 94%CC in patients versus 21%CC in controls ($p<0.001$). Statistically significant differences were found in ratio between the two polymorphisms of IL-10 ($p<0.0001$), between the two polymorphisms of IL-1beta ($p=0.001$); between IL-1alpha[-889C/T] and IL-1beta [3954C/T] ($p=0.002$); and between IL-10[-592C/T] and IL-1beta [3954C/T] ($p=0.041$). Correlations between TNF-alpha[-308G/A] and both kidney and neurological MSSI sub-score (both: $p=0.06$) and between IL-10[-819C/T] and the MSSI neurological score ($p=0.03$) were noted. We speculate that sequence variations in regulatory DNA of genes coding for important members of the interleukin inflammatory family are associated with differential effects in Fabry disease and with increased sample size, haplotype blocks might be constructed.

Nosology and Classification of Genetic Skeletal Disorders. *A. Superti-Furga[1,3], S. Unger[2,3], and the ISDS Nosology Group* 1) Dept of Pediatrics, Univ of Freiburg, Germany; 2) Inst of Human Genet, Univ of Freiburg, Germany; 3) ISDS International Skeletal Dysplasia Society, www.isds.ch.

Objectives - Revision of the Nomenclature of Genetic Disorders of Bone to incorporate newly recognized disorders and to reflect new molecular and pathogenetic concepts.

Methods -Criteria for inclusion of disorders were (1) significant skeletal involvement corresponding to the definition of skeletal dysplasias, metabolic bone disorders, dysostoses, and skeletal malformation and/or reduction syndromes, (2) publication and/or MIM listing, (3) genetic basis proven or very likely, and (4) nosologic autonomy confirmed by molecular or linkage analysis and/or distinctive diagnostic features and observation in multiple individuals or families.

Results - 370 different conditions were included and placed in 37 groups defined by molecular, biochemical and/or radiographic criteria. Of these conditions, 213 were associated with one or more of 138 different genes. Nosologic status ranged from final (mutations or locus identified) to probable (pedigree evidence) to bona fide (multiple observations but no pedigree or locus evidence yet).

Discussion - The number of recognized genetic disorders with a significant skeletal component is growing and the distinction between dysplasias, metabolic bone disorders, dysostoses, and malformation syndromes is blurring. Clinical and radiographic evaluation remains essential. Molecular evidence leads to confirmation of individual entities and to constitution of new groups, but also allows for delineation of new entities and indicates an unsuspected heterogeneity of molecular mechanisms; thus, molecular evidence does not necessarily simplify the Nosology, and a further increase in the number of entities and growing complexity is expected.

Conclusions - By providing an updated overview of recognized entities with skeletal involvement, the new Nosology can provide practical diagnostic help, facilitate the recognition of new entities, and foster research in skeletal biology and genetic disorders.

Identification of a novel role of excess copper in enhancing apoptotic potential of Arg72 variant of p53: New insights into molecular pathophysiology of Indian Childhood Cirrhosis. *R. Prasad[1], N. Sharma[1], B.R. Thapa[2], G. Kaur[3]* 1) Biochemistry, PGIMER, Chandigarh, India; 2) Pediatric Gastroenterology, PGIMER, Chandigarh; 3) Physiology, Government Medical College, Chandigarh.

We purified a major copper binding protein (MCuBP ~50 kDa) from ICC patients. N-terminal sequencing of this protein matched to p53. Cloning of MCuBP gene was accomplished by polysome isolation, immubolization of polysome-antibody (pantropic p53) complex, elution of specific MCuBP mRNA, dscDNA synthesis, annealing of dC tailed cDNA with dG tailed pBR322 and transformation of competent E.coli dH5 with recombinant plasmid. Sequence of plasmid DNA bearing MCuBP gene matched to p53 cDNA. C residue at nucleotide position 350 was substituted by G, resulting in Argnine instead of Proline at position 72. Using gene specific primers, approximately 1.2 kb p53 cDNA was synthesized by RT-PCR. Sequence analysis confirmed Arg72 polymorphism in p53 gene of ICC patients. The p53 Arg72 was cloned into pcDNA 3.1 vector for expression studies which showed increased expression of p53 mRNA by RT-PCR analysis and increased p53 staining intensity on immunocytochemical analysis with increasing concentrations of copper. Western blot analysis showed p53 protein in liver and p53 antibodies in plasma of ICC patients. Exon 4 was amplified from the genomic DNA of 11 ICC patients, 8 were homozygous and 3 were heterozygous for Arg at position 72. We demonstrated an increased expression of CD95 in ICC liver by immunoblot and RT-PCR. We conclude that in ICC, consumption of milk in brass/copper utensils results in hepatocytes death by increased tumor suppressor activity of Arg72 form of p53 through activation of CD95. Role of copper in transcriptional or translational regulation of Arg72 p53 deserves further investigations.

Two novel bi-allelic MMR gene mutations: further evidence for a distinct syndrome with constitutional inactivation of DNA mismatch repair system. *Q. Wang[1,4], J. Auclair[1], D. Leroux[5], F. Desseigne[2], J-C. Saurin[6], C. Lasset[3], V. Bonadona[3], S. Pinson[4], M-O. Joly[7], G. Montmain[1,4], A. Puisieux[1]* 1) Dept Oncologie Moleculaire,Centre Leon Berard,; 2) Dept de médecine, Centre Leon Berard,; 3) Dept de Santé Public, Centre Leon Berard, Lyon, France; 4) Plateforme Mixte de Génétique Constitutionnelle HCL-CLB, Lyon, France; 5) Service de génétique, CHU Grenoble, Grenoble, France; 6) Service d'Hépato-Gastroentérologie, Centre Hospitalier Lyon-Sud, France; 7) Laboratoire Central d'Anatomie et de Cytologie Pathologiques, Hôpital Edouard Herriot, Lyon, France.

Hereditary nonpolyposis colon cancer (HNPCC) is characterized by early onset of colorectal cancer and certain extracolonic cancers, caused by a heterozygous germline mutation of one of the DNA mismatch repair genes (MMR). Such mutations are also implicated in HNPCC-related syndromes, i.e. Muir-Torre syndrome associated with skin malignancies and Turcot syndrome associated with tumors of central nervous system. Recent studies suggested the existence of a distinct HNPCC-related syndrome caused by a bi-allelic germline mutation of MMR genes. A total of 18 cases have been reported so far, involving either MLH1, MSH2, MSH6 or PMS2. Mutation carriers developed frequently, in their early childhood, brain tumors and haematological malignancies, with the presence of café-au-lait spots (CALS). Microsatellite instability was often observed indicating a MMR deficiency. Herein we reported two novel compound heterozygous mutations in respectively MSH6 and PMS2 gene from families not suggestive for HNPCC. In addition to CALS, mutation carriers displyed early onset of brain tumor, colorectal neoplasia, and endometrial carcer. However, no deleterious mutation was detected in NF1 gene. Our observation further evidence the existence of this distinct, although rare, HNPCC syndrome variant with recessive inheritance. The phenotype and genotype of all similar cases are compared, in an attempt to draw a profile of this syndrome which will be of great help for genetic counselling for those families.

A novel HEXB gene mutation producing a dual pathogenic mechanism results in the Sandhoff motor neuron phenotype. *M. Santoro[1], G. Silvestri[1], A. Modoni[1], M. Sabatelli[1], F. Piemonte[2], E. Ricci[1], P. Tonali[1]* 1) Neuroscience, Catholic University, Rome, Italy; 2) Laboratory of Molecular Medicine Bambino Gesù Hospital, Rome, Italy.

GM2 gangliosidosis type Sandhoff(MIM 268800) is an inherited lysosomal storage disorder caused by mutations in the HEXB gene, encoding the beta subunit of Hexosaminidase (Hex). We report a novel HEXB gene mutation associated with a familial, chronic Sandhoff lower motor neuron (LMN) phenotype. HEX activity in leukocytes was done by spectrophotometric assays. HEXB gene sequencing and PCR-RFLP analysis for the c.1556A>G transition were performed on DNA, and RT-PCR studies on RNA extracted from leukocytes. Data were analysed by ESE-finder, RESCUE-ESE, BLASTP and Cn3D 4.1 softwares. The biochemical diagnosis of Sandhoff disease was made in two affected family members showing a reduction of total HEX,lack of Hex-B and near normal Hex-A activity; an unaffected member showed total HEX and Hex-B enzyme activities compatible with heterozigosity.HEXB gene sequencing documented a novel c.1556 A>G transition in exon 12, homozygous in the two affected individuals and heterozygous in the unaffected one. The mutation, not detected in 100 Italian controls, changes a highly conserved aspartic acid to glycine at position 494 (D494G). The mutation does not affects Hex cathalitic domain, but it is part of the interface I,which regulates Hex dimers assembly and their affinity for the natural substrate,GM2-activator complex. Moreover, amplification of a cDNA fragment including exon 12 from the RNA extracted from leukocytes of the two affected family members showed a normal 538 bp and a shorter 447 bp fragment, corresponding to an aberrantly spliced RNA in which skipping of exon 12 had occurred, producing a frame-shift with stop codon 78 nucleotides downstream from exon 11. ESE-finder and RESCUE-ESE analyses revealed that the mutation disrupts an ESE motif in exon 12 for human SR proteins SC35 and SRp55. Conclusions: Analysis of clinical, biochemical and molecular data in this family suggest that the D494G mutation would affect HEXB gene expression both at the transcriptional and the post-traductional level.

Cryptic ins(4;11)(q21;q23q23): a variant of t(4;11)(q21;q23) in an infant with an immature B-ALL. *C.A. Tirado[1], A.M. Meloni-Ehrig[1], T. Edwards[1], J. Scheerle[1], K. Burks[1], C. Repetti[2], J.C. Kelly[1], N.C. Christacos[1], J. Grenberg[3], C.D. Croft[1], D. Heritage[1], P.N. Mowrey[1]* 1) Cytogenetics, Quest Diagnostics Nichols Institute, Chantilly, VA 20151; 2) Flow Cytometry, Quest Diagnostics Nichols Institute, Chantilly, VA 20151; 3) Arlington-Fairfax Hematology-Oncology, Arlington, VA.

We report on a 4-year-old female with a WBC of 120,000 and thrombocytopenia. The blood findings revealed a Hb of 10 g/dL, a hematocrit of 29%, and a platelet count of 47 K/L. The flow cytometry report identified abnormal blasts which were CD10-, CD19+, partial dim TdT, HLA-DR +, and CD15+ consistent with an immature B-cell lineage acute lymphoblastic leukemia. Routine chromosome analysis detected an isochromosome 7q resulting in loss of the short arm and gain of the long arm of chromosome 7, as well as a possible loss of material from 11q23. FISH studies on both interphase nuclei and metaphase cells using the LSI MLL Dual Color, Break Apart Rearrangement probe (Abbott Molecular, Inc.) were instrumental in the characterization of an *MLL* gene rearrangement, which was cryptic by conventional cytogenetic analysis. Specifically, the FISH pattern was consistent with an insertion of the 5' region of the *MLL* gene into one chromosome 4 at band q21. Insertions of the *MLL* gene into various partner chromosomes have been reported previously. To our knowledge, this particular insertion of the MLL gene into chromosome 4 has only been reported once. We assume that this cryptic insertion results in the fusion of the 5' portion of the *MLL* gene with the *AF4* gene at 4q21, as is seen in the typical t(4;11)(q21;q23). This translocation has been reported mostly in very young children (1-2 years of age, 50% are under 4 years), as well as adults and it is usually associated with an unfavorable prognosis. The presence of the i(7q), which is an additional abnormality seen in approximately 10% of ALL cases with t(4;11)(q21;q23), strengthens the possibility that this insertion is a variant of the typical t(4;11)(q21;q23). This case exemplifies the importance of FISH in the further characterization of cytogenetic abnormalities in hematologic oncology cases.

Genetic and reproductive knowledge among adolescents and adults with cystic fibrosis. *N.H. Robin[1,2], G.H. Houser[1], H. Gutierez[2], J.P. Clancy[2], K.R. Young[3], C. Holt[3]* 1) Dept Genetics; 2) Pediatrics; 3) and Medicine, Univ Alabama at Birmingham, Birmingham, AL.

Cystic fibrosis (CF) is now becoming a disease of adulthood. In 2002, 40% of the 30,000 CF patients in the United States were over 18, and it is expected that in the next 10 years over 50% of CF patients will be adults. Therefore, issues of transition care are becoming more important. One such issue is reproduction. Many studies have shown that CF patients have a good understanding of the medical issues of CF, but a poor understanding of the genetics. No study has looked at their knowledge of reproductive options. This is because, until recently, issues of genetics and inheritance were not relevant. Most CF patients died very young, older male patients were almost all sterile, and women had also had diminished fertility. However, these issues are now very important, as improving health and advances in assisted reproductive technology have made reproduction a viable option for many CF patients. We conducted structured interviews with 18 CF patients age 16-25 yrs., 7 male and 11 female. All had a good understanding of the medical issues of CF, such as common symptoms, organs affected, and complications, and all knew genes were somehow involved in causing CF. However, there was poor understanding of inheritance. Only 4/18 could explain that their parents each carried a CF allele, and that that they had a 25% chance of having a CF child; 6/18 did not know they inherited a CF mutation from each parent; 15/18 did not know their risk for having a child with CF; and 15/18 responded incorrectly to basic questions about the genetics of CF. Only 12 (5 males, 7 females) knew that males are sterile, and 9/12 (3 males, 6 females) did not know why. There was also confusion concerning female reproductive capabilities. Finally, 9/18 (2 males, 7 females) did not know that there are reproductive options available if they do want to have children in the future. Based on these results, genetic and reproductive education should be incorporated into the transitional care of adolescents and young adults with CF.

A-Richness and the Repression of L1 Protein Expression. *N. Wallace, P. Deininger* Epidemology, Tulane University, New Orleans, LA.

Long interspersed element-1 or L1 composes approximately one-fifth of the average mammalian genome. It is necessary to constrain the activity of this retroelement in order to minimize genomic damage. The repression of L1 expression and subsequent retrotransposition has been shown to occur through multiple cellular mechanisms. It has been demonstrated that premature polyadenylation, and improper splicing function to suppress the expression of human L1 proteins. In addition to these ways, the use of very A-rich open reading frames (ORFs) may play a suppressive role, as well. This excessive A-richness results in extremely suboptimal codon use in each of the two L1 proteins, ORF1p and ORF2p. In addition to poor translation efficency, the A-richness may also result in altered RNA production, transport or stability. We have demonstrated that removing the A-richness found in the ORF1 region results in an increase in RNA production, and a dramatic increase in protein production. Similarly, the removal of the ORF2 A-richness results in increased RNA levels. Orf2p levels, as measured via a functional assay, also increase. These findings are consistent with our hypothesis that the A-richness of the L1 ORFs contributes negatively to the translation as well as transcription of L1.

Mortality in Achondroplasia study: A 40 year follow-up. *J. Wynn[1], M.J. Gambello[1], T.M. King[1], A. Scott[3], K. Waller[2], J.T. Hecht[1]* 1) Department of Pediatrics, The University of Texas Health Science Center at Houston, Houston, TX; 2) The University of Texas School of Public Health, Houston, TX; 3) Houston Shriners Hospital, Houston, TX.

Achondroplasia (ACH) is a well-studied, common dwarfing condition that affects approximately 12,000 persons in the US. In studies of the natural history, an increase in overall mortality, age specific mortality up to age 34 years and cardiovascular disease related mortality was identified in a large cohort of ACH individuals. Concern about premature death, particularly in young adults, has continued to be a worry in the ACH population. This study was undertaken to follow up the original ACH mortality study to assess the patterns of mortality in a more contemporaneous population. Seven hundred eighteen ACH individuals from the original study and 75 new ACH individuals ascertained through the University of Texas Medical School at Houston were assessed for vital status using the National Death Index, Social Security Administration Death Master File and the Little People of America list of deceased members. The results showed that the overall mortality and age specific mortality at all ages remains significantly increased. Rates of death were similar in the first and second 20 years of follow-up suggesting that higher death rates were still occurring in this ACH population. Accidental, neurological, and cardiac deaths were all significantly increased in adults. Cardiovascular disease related mortality, in ACH individuals between ages 25 to 35, was more than 10 times higher than the general population. Overall survival and the average life expectancy were decreased by fifteen years for this ACH population. This study demonstrates that despite advances in the knowledge of the natural history of ACH and health care of this population, mortality remains significantly increased. The high rate of cardiovascular related deaths illustrates the need for risk factor assessment in the ACH population to identify specific risk factors and to develop treatment interventions.

A Novel Approach To Detect Parent-Of-Origin Effects From Pedigree Data. *S. Shete[1], R. Elston[2], Y. Lu[1]* 1) Dept Epidemiology, Univ Texas MD Anderson CA Ctr, Houston, TX; 2) Department of Epidemiology and Biostatistics, Case Western Reserve University School of Medicine, Cleveland, OH.

The parent-of-origin phenomenon in humans is now well recognized, and the deregulation of imprinted genes has been implicated in a number of human diseases. Recently, several linkage analysis methods have been developed to allow for parent-of-origin effects in the analysis of pedigree data. However, in general, one does not know a priori if disease-causing loci are imprinted or not. Linkage methods that allow for imprinting can lose power if there is no imprinting. Conversely, linkage methods that do not allow for imprinting will lose power if there is imprinting because of penetrance values not being correctly specified. Therefore, it is important to know whether imprinting is a possible mode of disease inheritance before performing linkage analyses. In this paper, we describe a simple covariate-coding scheme to test for the presence of parent-of-origin effects and provide a formula for calculating parent-specific penetrance values prior to any linkage analysis. Our coding scheme was successful in detecting parent-of-origin effects and in leading to more accurately estimated penetrance values. The use of accurate penetrance values in a linkage analysis that allows for imprinting can provide higher power in the case of a disease locus that is imprinted.

Analysis of the dopamine hypothesis in schizophrenia reveals significant main effects and interactions between the dopamine transporter and dopamine D3 receptor genes. *M.E. Talkowski, K. Chowdari, H. Mansour, J. Wood, B. Shirts, B. Devlin, V.L. Nimgaonkar* Departments of Human Genetics and Psychiatry, University of Pittsburgh Graduate School of Public Health and School of Medicine.

We investigated the genetic basis for the dopamine (DA) hypothesis of schizophrenia (SZ) in a two-stage design using the intersection of functional biology and SZ linkage to prioritize our analyses. We first analyzed functional DA genes using database SNPs. We then conducted comprehensive tag SNP analyses of associated genes and genes localized to linked regions based on a published meta-analysis of SZ linkage.

In phase I, 18 DA genes were evaluated among 150 SZ trios and 280 controls. Two genes met suggestive criteria for associations after corrections for multiple testing ($p<0.0028$; *SLC6A3* (DAT) and *DRD3*). Phase II involved follow-up analyses in an enlarged case sample (n=478) and new control sample (n=500). We included the four DA genes localized to linked regions (*COMT, DRD2, VMAT1*, *NR4A2*), as well as *SLC6A3* and *DRD3*. Tag SNPs ($r^2>0.8$ between loci) were derived using HapMap data alone (*DRD2, NR4A2, VMAT1, SLC6A3*; 148 total SNPs/58 tags) or in combination with novel SNPs detected by sequencing known functional regions (*COMT*; 58 SNPs/20 tags). At *DRD3*, we sequenced the entire gene to identify all common variation and determine LD patterns (69 total SNPs detected, 15 novel SNPs, 20 tags). A genomic control panel was also evaluated.

Phase II results continued to implicate *SLC6A3* and *DRD3* ($p<0.05$: 6 SNPs and 5 SNPs associated, respectively). The associations could not be attributed to population substructure and were detectable using different analytic designs. Post-hoc logistic regression with 6 SNPs at these two genes detected significant interactions between two locus pairs ($p<0.05$). A previously reported interaction between COMT polymorphisms and gender in both SZ and Alzheimer's disease was significantly replicated in this cohort (rs737865; $p<0.01$ in females). These analyses represent a novel evaluation of dopaminergic genes in SZ genesis. .

Simultaneous Preimplantation Genetic Diagnosis (PGD)for Tay Sachs and Gaucher Disease. *P. Renbaum[1], B. Brooks[2], E.J. Margalioth[2], T. Eldar-Geva[2], M. Patt[1], Y. Kaplan[1], E. Levy-Lahad[1], G. Altarescu[1]* 1) Zohar PGD Lab, Genetic Unit, Shaare Zedek Medical Center, Jerusalem, Israel; 2) IVF Unit, Shaare Zedek Medical Center.

PGD for single gene defects is presented for a family in which each parent is a carrier of both Tay Sachs (TS) and Gaucher disease (GD). This childless couple were both compound heterozygotes for the GD-GBA and TS-HEXA genes (N370S/ IVS2+1G>A and IVS12+1G>C/ Gly269Ser respectively). We developed a multiplex fluorescent PCR protocol simultaneously amplifying all four familial mutations and 10 (2 sets of 5) closely spaced, highly polymorphic informative microsatellite markers surrounding these genes, to be used in polar body or blastomere based PGD. In one cycle of PGD, blastomeres from 7 embryos were analyzed for both diseases using the 10 informative markers (while mutation analysis was informative for polar body analysis, due to possible allele drop out none of the mutations were fully informative in blastomere analysis). Amplification was observed in all 7 blastomeres, and a conclusive diagnosis for both TS and GD was reached in 6/7 embryos, based on a minimum of 4 markers encompassing each gene. We observed ADO in 3 of 34 reactions (8% ADO rate). Of the 6 diagnosed embryos, one was wild type for both TS and GD, while another three were wild type for GD and carriers of TS. The two remaining embryos were compound heterozygotes for TS. While both of these embryos were wild type for GD, one showed recombination in the GD-linked markers. Only 2 of the 4 transferable embryos developed into blastocysts (wt/wt and wt/TS car) and both were transferred on day 5. This single cycle of PGD resulted in an ongoing singleton pregnancy. The occurrence of allele drop-out in single cell analysis and undetected recombination events (when mutation testing is either unavailable or non-informative) are primary causes of misdiagnosis in PGD. These obstacles emphasize the necessity of using multiple polymorphic markers flanking both sides of the gene/mutation in order to prevent misdiagnosis. To our knowledge this is the first report of concomitant PGD for two frequent Ashkenazi Jewish recessive disorders.

LOC387715 is associated with increased risk of neovascular age-related macular degeneration in the Utah population. *Z. Yang[1, 2], Z. Tong[1], Y. Zhao[1], A. Praggastis[1], E. Brinton[1], J. Baird[1], Y. Chen[1], D.J. Cameron[1], E. Pearson[1], P.S. Bernstein[1], G. Brinton[3], C. Wang[1], K. Howes[1], N.J. Camp[4], K. Zhang[1]* 1) Ophthalmology Research, EIHG, Salt Lake City, UT; 2) Sichuan Medical Science Academy & Sichuan Provincial Peoples Hospital, Sichuan 610071, Peoples Republic of China; 3) Retina Associates of Utah, Salt Lake City, Utah 84103; 4) Division of Genetic Epidemiology, Department of Biomedical Informatics, University of Utah School of Medicine, Salt Lake City, UT 84108.

Purpose Age-related macular degeneration (AMD) is the most common cause of irreversible vision loss in the developed world. Recently, it has been proposed that a second locus at 10q26.13 increases the risk for AMD. The precise gene involved at this locus has not been determined. The purpose of this study is to confirm the association at 10q26, determine the principal variant which explains this association, and to determine its contribution to wet AMD risk. Additional we examined the risk at 10q26 in the context of risk attributed by CFH at 1q31.3. Methods We genotyped 260 Utah patients with wet AMD, and 297 age and ethenity matched control subjects. Three single nucleotide polymorphism (SNPs) at 10q26.13, LOC387715 rs10490924, PLEKHA1 rs2421016, and PLEKHA1 rs4146894, in addition to CFH Y402H were genotyped and analyzed. Results We showed that the A69S (rs 10490924) in LOC387715 was significantly associated with wet AMD. In addition we showed that the association for LOC388715 rs10490924 was the principal variant for 10q26.13, and that it drives the secondary associations found for PLEKHA1 rs2421016 and PLEKHA1 rs4146894. Association analyses for genotype at LOC388715 rs10490924 conditional on genotype at CFH Y402H also demonstrated significant association indicating its independence effect from that of CFH. We showed that the interaction term was non-significant and that the risk of wet AMD at LOC387715 was independent and additive with CFHY402H. Conclusions Our results confirm the findings of Rivera et al (2005) suggesting that LOC387715 as the second major susceptibility locus for wet AMD. Together with CFH, they represent a major risk for AMD.

Beneficial effects of oral miglustat on skeletal symptoms in Type 1 Gaucher disease: meta-analysis of a 2-year clinical trial follow-up. *G.M. Pastores[1], D. Elstein[2], M. Hrebicek[3], A. Zimran[2]* 1) Department of Neurology and Paediatrics, New York University School of Medicine, New York, USA; 2) Gaucher Clinic, Department of Medicine, Medical Centre, Jerusalem, Israel; 3) Institute of IMD, Prague, Czech Republic.

Bone disease has a substantial impact in patients with Type I Gaucher disease (GD1). The aim of this study was to assess the effectiveness of oral miglustat (Zavesca) on skeletal complications in GD1 over two years. Prevalence and incidence of bone pain (BP), bone crises (BC), pathologic fractures, and avascular necroses (AVN) were assessed. Bone mineral density (BMD) measurements were performed at the lumbar spine and/or hip at 6 months, then annually. 72 patients (49% females) were included in the study. Mean age SD was 41.213.2 years. Mean miglustat exposure SD was 20.53.6 months. 57% of patients had been previously treated with enzyme replacement therapy (ERT) for a mean duration SD of 6424 months. At baseline 63% of patients had BP and 15% had a recent history of BC. AVN had occurred in 12.5% of patients. 68% of patients were classified as osteoporotic and 7% were receiving bisphosphonates. Among those who had BP at baseline, only 14.6% and 7.3% had new BP in the first and second years after treatment with miglustat, respectively. There were no differences in the incidence of new BP between treatment-naive and former ERT-treated patients. Amongst patients without BP at baseline, 8.3% developed new BP in the first year and none in the second year. No cases of BC, AVN and pathologic fractures occurred over the 2-year follow-up period. Spine and hip BMD measurements in patients (excluding those taking bisphosphonates) revealed increased BMD. Amongst naive patients, spine and hip BMD increased in 29% and 43% of patients at 6 months, and in 77% and 73% at 24 months. In former ERT users, spine and hip BMD increased in 48% and 83% of patients at 6 months and in all patients at 24 months. This study demonstrates that miglustat treatment is associated with early improvement in bone manifestations and increased bone density in GD1 patients.

***CYP2D6* worldwide genetic variation shows high frequency of altered activity variants and no continental structure.** *J. Sistonen[1], A. Sajantila[1], O. Lao[2], J. Corander[3], G. Barbujani[4], S. Fuselli[1,4]* 1) Department of Forensic Medicine, University of Helsinki, Helsinki, Finland; 2) Department of Forensic Molecular Biology, Erasmus University Medical Center, Rotterdam, The Netherlands; 3) Department of Mathematics and Statistics, University of Helsinki, Helsinki, Finland; 4) Department of Biology, University of Ferrara, Ferrara, Italy.

CYP2D6, a member of the cytochrome P450 superfamily, is responsible for the metabolism of about 25% of the commonly prescribed drugs. Its activity ranges from complete deficiency to excessive activity, potentially causing toxicity of medication or therapeutic failure with recommended drug dosages. Here we report on global variation at the *CYP2D6* locus, on the basis of 52 worldwide-distributed populations, typed at 12 highly informative variable sites, as well as for gene deletion and duplications. Phenotypes were predicted on the basis of haplotype combinations. Our study shows that (1) *CYP2D6* diversity is far greater within than between populations and groups thereof, (2) null or low-activity variants occur at high frequencies in various areas of the world, (3) linkage disequilibrium is lowest in Africa and highest in the Americas. Patterns of variation, within and among populations, are similar to those observed for other autosomal markers (e.g. microsatellites and protein polymorphisms), suggesting that the diversity observed at the *CYP2D6* locus reflects the same factors affecting variation at random genome markers.

Oral miglustat in Niemann-Pick type C (NPC) disease: a 1-year interim analysis. *M.C. Patterson[1], D. Vecchio[1], H. Prady[2], L. Abel[3], N. Ait Aissa[4], J.E. Wraith[2]* 1) Departments of Neurology and Paediatrics, Columbia University, New York, USA; 2) Royal Manchester Children's Hospital, Biochemical Genetic Unit, Manchester, UK; 3) Department of Optometry and Vision Sciences, University of Melbourne, Melbourne, Australia; 4) Actelion Pharmaceuticals Ltd., Allschwil, Switzerland.

NPC is an inherited neurodegenerative disorder characterised by an intracellular lipid-trafficking defect with secondary pathological storage of glycosphingolipids (GSLs). Oral miglustat (Zavesca) reversibly inhibits glucosylceramide synthase, which catalyses the first committed step of GSL synthesis. Due to its physicochemical properties, miglustat is able to cross the blood-brain barrier. Adult and juvenile NPC patients (n=29, age12 yrs) were randomised to receive miglustat, 200 mg t.i.d. (n=20) or standard care (n=9). Patients were then treated with miglustat for an additional year. Twelve paediatric patients (age<12 years) were included in a separate substudy: all received miglustat at a dosage adjusted for body surface area. Changes in horizontal saccadic eye movement was the primary study endpoint, based on the correlation between the severity of supranuclear gaze palsy and NPC disease progression. At baseline, nearly all individuals suffered from cognitive impairment, as shown through extensive cognitive measurements including mini-mental state examination (MMSE) scores. At 1 year, saccadic velocity improved in treated adult-juvenile patients vs. controls; results were statistically significant when patients taking benzodiazepines (a known confounder) were excluded (p=0.03). Improvements in swallowing capacity, auditory acuity and MMSE scores were also observed. A beneficial effect on horizontal saccades was also shown at 1 year in the paediatric cohort. The safety profile of miglustat was consistent with previous trials in Type I Gaucher disease, where half the dose was used. In conclusion, these findings suggest that miglustat may induce some degree of restoration of neuronal function after 1 year of treatment. These preliminary data suggest that miglustat may represent a major advance in the clinical management of NPC disease.

The first association study between G72/G30 and unipolar depression in a large sample of patients and controls of German descent. *T.G. Schulze[1], A. Georgi[1], F. Schirmbeck[1], A. Karpushova[2], S. Hoefels[3], R. Abou Jamra[4], J. Schumacher[4], W. Maier[3], L. Beckmann[5], P. Propping[4], M.M. Nöthen[2], S. Cichon[2], F. Henn[6], M. Rietschel[1]* 1) Division of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Mannheim, Germany; 2) Dpt. of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany; 3) Dept. of Psychiatry, University of Bonn, Bonn, Germany; 4) Institute of Human Genetics, University of Bonn, Bonn, Germany; 5) Division of Genetic Epidemiology, German Cancer Research Center (DKFZ), Heidelberg; 6) Dpt. of Psychiatry and Psychotherapy, Central Institute of Mental Health, Mannheim, Germany.

G72/G30 is considered a strong susceptibility gene for both schizophrenia (SZ) and bipolar disorder (BD). We recently reported association between identical G72/G30-haplotypes and SZ, BD and panic disorder (PD). An association study on major depression has not yet been conducted. We studied a German sample of 500 MD patients and 1030 population-based controls. We were interested whether our previously identified risk haplotype of markers M22, M23, and M24 was also associated with MD. To further explore any relationship between G72/G30 and MD, we genotyped 9 additional SNPs highlighted in other studies. The haplotype C-C-T was significantly more frequent in MD patients than in controls (40.5 % vs. 36.1 %; p=0.027; OR=1.2). The exploratory analysis on 9 further G72/G30 SNPs of interests yielded significant associations for M12 (p=0.038), M14 (p=0.046), while M15 (p=0.090) and rs1935062 (p=0.055) did not reach significance. Permutation analysis adjusting for the 9 SNPs yielded a global p=0.176 This is the largest case-control study on G72/G30 and MD to date. We found an association between MD and the same risk haplotype that we previously found associated with SZ, BD, or PD. We also found suggestive associations with other variants. Given that depressive symptoms are present across these diagnostic groups, G72/G30 might predispose to core symptoms prevalent in all four disorders. Further studies will be needed to elucidate these core symptoms.

Heritability of carotid intima-media thickness: A twin study. *J. Zhao[1], F. Cheea[1], J. Bremner[1], J. Goldberg[2], S. Su[1], H. Snieder[3], C. Maisano[1], L. Jones[1], N. Murrah[1], V. Vaccarino[1]* 1) Dept. of Medicine,Emory University, Atlanta, GA; 2) VETR, Seattle, WA; 3) Georgia Prevention Institute, August, GA.

Carotid intima-media thickness (IMT) is widely used as a surrogate marker for atherosclerosis. Several studies reported the heritability estimates of carotid IMT using family studies, however, these estimates may be biased due to confounding factors in unmatched samples. The twin study design totally eliminates these confounding effects due to the exact match for demographic and early environmental influences (if the twins are raised together), thus is ideal for heritability estimation. In this study, we report the results of heritability estimation of carotid IMT using 98 middle-aged male twin pairs (55 monozygotic [MZ] and 43 dizygotic [DZ]) from the Vietnam Era Twin Registry. All twins were free of overt cardiovascular disease. Carotid IMT was measured by ultrasound. Bivariate and multivariate analyses were used to determine the association of traditional cardiovascular risk factors with carotid IMT. Intraclass correlation coefficients and structural equation modeling were used to determine the relative contributions of genes and environment to the variation in carotid IMT. We found that age, systolic blood pressure (SBP), diastolic blood pressure (DBP) and high-density lipoprotein (HDL) were strongly associated with carotid IMT in bivariate analysis. In multivariable analysis, age, SBP and HDL were significantly associated with carotid IMT. The intraclass correlation coefficients for carotid IMT were 0.66 (95% CI, 0.62-0.69) in MZ and 0.37 (95% CI, 0.29-0.44) in DZ twins, suggesting a genetic influence. After adjusting for traditional cardiovascular risk factors, the heritability of carotid IMT was 0.66 (95% CI, 0.48-0.78). This classic twin study demonstrates that genetic factors account for a significant proportion of variation in carotid IMT.

Alcohol dependence is associated with the *ZNF699* gene, a human locus related to *Drosophila hangover*, in the Irish Affected Sib Pair Study of Alcohol Dependence (IASPSAD) sample. *B. Riley[1,2], G. Kalsi[1], P.-H. Kuo[1], V. Vladimirov[1], D.L. Thiselton[1], J. Vittum[1], B. Wormley[1], M.S. Grotewiel[2], D.G. Patterson[3], P.F. Sullivan[4], E.J. van den Oord[1], D. Walsh[5], K.S. Kendler[1,2], C.A. Prescott[6]* 1) Dept. of Psychiatry, Virginia Commonwealth University, Richmond, VA; 2) Dept. of Human Genetics, Virginia Commonwealth University, Richmond, VA; 3) Shaftesbury Square Hospital, Belfast, Northern Ireland; 4) Dept. of Genetics, University of North Carolina, Chapel Hill, NC; 5) Health Research Board, Dublin, Ireland; 6) Dept. of Psychology, University of Southern California, Los Angeles, CA.

Because tolerance is an important aspect of alcohol dependence (AD) in humans, recent evidence showing that the *Drosophila* gene *hangover* is critically involved in the development of alcohol tolerance in the fly suggests that variation in related human loci might be important in the etiology of alcohol-related disorders. The orthology of hangover in mammals is complex, but a number of human gene products (including ZNF699) with similar levels of amino acid identity (18-26%) and similarity (30-37%), are consistently identified as the best matches with the translated hangover sequence. We tested for association between the dichotomous clinical phenotype of alcohol dependence and seven single nucleotide polymorphisms (SNPs) in *ZNF699* in our sample of 565 genetically independent cases and 496 siblings diagnosed with AD, and 609 controls. In analyses of genetically independent cases and controls, four of the seven single markers show strong evidence for association with AD (Fishers exact p=0.00003-0.001), and the most significant single marker, rs7254880, tags an associated haplotype with frequency 0.071 in cases compared to 0.034 in controls (chi-square 15.563, p<0.00008, 5000 permutation p<0.001, OR 2.17); inclusion of affected siblings gives similar results. Expression analyses conducted in independent postmortem brain samples show that expression of *ZNF699* mRNA is significantly reduced in the dorsolateral prefrontal cortex of individuals carrying this haplotype compared with other observed haplotype combinations.

Genotype-phenotype correlation for age of onset in patients with nephrotic syndrome and *NPHS2* mutations.

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Nephrotic syndrome (NS) is defined by proteinuria, edema, hypoalbuminemia, and hyperlipidemia which can progress to end-stage renal failure. Genetic causes for pediatric cases of NS have been elucidated and include autosomal recessive mutations in *NPHS1* (nephrin), *NPHS2* (podocin), and *LAMB2*; as well as autosomal dominant mutations in the *WT1* gene. Approximately 28% of NS cases are caused by mutations in *NPHS2*. Standard treatment for NS includes administration of steroids. We have previously described that patients with mutations in *NPHS2* exhibit steroid resistant nephrotic syndrome (SRNS)(1). In order to study a possible genotype-phenotype correlation for age of onset in SRNS we examined a worldwide cohort of 433 children with SRNS for *NPHS2* mutations. The *NPHS2* mutations were grouped into 4 classes: A) 2 mutations, at least one of which is truncating (stop codon, frame shift, or obligatory splice site); B) homozygous R138Q mutation (founder mutation); C) 2 missense mutations, and D) single heterozygous mutations only. Within this group we confirmed a total of 112 out of 433 (26%) individuals had mutations in *NPHS2*. We found that the 4 groups ranked as follows in increasing age of onset: A, 1.0 yr; B, 1.7 yrs; C, 4.2 yrs; D, 7.2 yrs. Age of onset (overt nephrosis or significant proteinuria) was significantly different between all groups of mutations, with the exception of groups A) vs. B). These data confirm the previously described strong pathogenic role of R138Q mutations. Additionally, truncating mutations of *NPHS2* also lead to early onset of SRNS. We demonstrate a statistically significant genotype-phenotype correlation regarding age of onset in children with SRNS due to different *NPHS2* mutations.

(1) Ruf *et. al.* (2004) *J Am Soc Nephrol.* 15(3):722-32.

Increased nuchal translucency necessitates full chromosome analysis. *M.H. Quigg, A. Decker, A. Adeyinka, J. Roberson, B. Wolf* Dept Medical Genetics, Henry Ford Health System, Detroit, MI.

Increased nuchal translucency (NT) in the first trimester and increased fetal nuchal fold measurement in the second trimester of pregnancy are associated with fetal chromosome abnormalities. Measurement of these parameters has been incorporated into screening for Down syndrome, trisomy 13 and 18, and sex chromosome abnormalities. There is growing sentiment that it is cost-effective to assess these pregnancies by performing only aneuploidy FISH analysis We report a fetus with increased NT that was found to have an unbalanced translocation that would not have been ascertained if only aneuploid FISH assessment was performed.

The mother is a 25-year-old female who had a fetus in a previous pregnancy with multiple fetal anomalies and IUGR.This fetus was found to have an unbalanced translocation, 46,XY,der(7)t(4;7)(q33;q34)pat producing a functional partial monosomy 7 and a partial trisomy 4. The father was a carrier of a balanced translocation, 46,XY,t(4;7)(q33;q34). This fetus expired shortly after birth.

In the current pregnancy, the mother had a CVS at 11 weeks gestation. NT was abnormal at 4.7 mm. Aneuploidy FISH analysis was normal. Chromosomal analysis found that the fetus had an unbalanced translocation, 46,XX,der(4)t(4;7)(q33;q34).ish der(4)t(4;7)(qter-,qter+)pat producing a partial monosomy 4 with partial trisomy 7. Fetal demise occurred two weeks after the CVS.

Unbalanced chromosomal translocations may be associated with increased NT. Although the risk for an unbalanced translocation was known in advance in this case, it is frequently not known until after prenatal diagnosis. Aneuploidy FISH analysis as the sole method of assessing pregnancies with increased NT would not have detected this abnormality. Caution should be given when informing a family about normal aneuploid FISH results in a pregnancy with increased NT.

SOD2 association with Alzheimers disease: False positive, or the result of non-monotonic genotypic risk? *H.W. Wiener[1], R.T. Perry[1], G. McGwin[1], L..E. Harrell[2], R.C.P. Go[1]* 1) Epidemiology, University of Alabama at Birmingham, Birmingham, AL; 2) Neurology, University of Alabama at Birmingham, Birmingham Alabama.

Lack of replication of association results in genetic studies is a widely recognized phenomenon. We propose a non-monotonic genetic model as one possible explanation for such results. When the genetic model has this form, we show that the results of association studies can vary with allele frequency at the affective locus, and even show diametrically opposed results for different allele frequencies. As a possible example of this type of genetic model, we present results from an association study of superoxide dismutase 2 (SOD2) with Alzheimers Disease (AD). The gene for this enzyme is located within a region on 6q where evidence of linkage to AD has been reported. We genotyped four SNPs in SOD2 in three different populations: probands and families of the NIMH AD Genetics Initiative, a sample of African American AD cases and age-matched controls, and a set of Caucasian controls from a study of age related macular degeneration. One SNP, which lies in intron 3 of SOD2, was significantly associated with onset of AD in all three samples. A conditional logistic regression analysis for development of AD versus genotypes at this locus showed significant excess risk for the heterozygotes. The allele that was transmitted in excess to individuals with AD in the NIMH dataset was protective against AD in both the African American case/control dataset and in the comparison of NIMH cases versus Caucasian controls Similar results were noted at the other genotyped loci. While the association results seen here could be explained by chance, the data is consistent with a non-monotonic disease model.

Improving Estimates of Linkage Maps by Combining Information over Studies. *W.C.L. Stewart* Department of Biostatistics, Center for Statistical Genetics, School of Public Health, University of Michigan, Ann Arbor, MI.

In parametric multipoint linkage analysis, map misspecification leads to a loss in power when linkage is present and can lead to an increase in type 1 error when linkage is not present (Daw et al. 2000 Genet Epidemiol **19**, 366-380). To improve the accuracy of existing maps, a meta-analysis of map estimates is an attractive approach. It has the potential to reduce sampling error by incorporating information from multiple studies, and since the sampled genotypes are not needed, it avoids the difficulties associated with the sharing of human-subjects data. Also, when data sets have study specific parameters and missing data, it can yield more accurate estimates than existing methods which analyze the pooled data.

I present an efficient estimator of the linkage map that combines map estimates from different studies using weights that vary inversely in proportion to the variance of each map estimate. I apply the proposed estimator to simulated marker data on nuclear families, three-generation families, and 239 families modelled after the large linkage study of Abkevich et al. (2003, Am J Hum Genet **73**, 1271-1281). I compare it to two estimators that analyze the pooled genotypic data directly: the maps estimated by the programs LM_MAP and CRIMAP.

The results indicate that when the constituent map estimates share a a common panel of markers, the proposed estimator and the maximum likelihood estimator have similar performance in terms of absolute bias and variance. Furthermore, in the presence of missing data, both estimators outperform CRIMAP. Also, the results demonstrate that the weights can be used to estimate accurately the variance of the proposed estimator. Remarkably, when the constituent map estimates do not share a common marker panel, the proposed estimator performs at least as well as CRIMAP.

Addiction molecular genetics: 639,401 SNP whole genome association reveals many "cell adhesion" gene variants. *G.R. Uhl, Q.R. Liu, T. Drgon, D. Walther, C. Johnson* Molecular Neurobiology, NIH IRP, NIDA, Baltimore, MD.

Addictions are substantially-heritable complex disorders. We now report whole genome association studies that identify 89 genes that are likely to contain variants that contribute to vulnerability to addictions. These studies follow validation of pooling methods for early acvess versions of affymetrix 100 and 500k arrays. These genes are identified since each contains clustered single nucleotide polymorphisms (SNPs) that display significant allele frequency differences between abusers and controls in each of two samples (n = 1460) studied with 639,401 autosomal SNP Affymetrix array sets and confirmatory SNPs from each of two other abuser/control samples (n = 450) studied using 100,000 SNP Afymetrix arrays. Primary data analyses use Monte Carlo simulations and secondary analyses use permuation and false discovery rate approaches. Each indicates that these results are very unlikely to be due to chance. The genes identified here are implicated in interesting functions. We focus on genes that contribute to "cell adhesion" processes that help to establish and maintain neuronal connections of special relevance to addiction's memory-like features. These genes are all expressed in the brain, with most displaying striking expression in hipposampus, cerebral cortex, and other memory-associated areas. Most of these genes display rich patterns of alternative splicing and alternative transcriptional start site variations and individual differences in these expression patterns in studies of cDNA prepared from postmortem brain samples. These observations support polygenic contributions of evolutionarily old allelic variants to human addictions to substances in several different chemical classes. They support the idea that the brains of indiduals at most risk for addiction are likely to differ from those at less risk.

Distant Relative Identical-By-Descent Mapping in Autism. *S. Strom* Dept Human Genetics, Univ California, Los Angeles, Los Angeles, CA.

Autism Spectrum Disorder (ASD) is a set of complex neurological disorders presenting with a range of deficits in social and language development and repetitive behaviors. ASD exhibits a complex inheritance pattern, and there are likely many genetic and environmental risk factors. While little is known of the molecular or genetic mechanisms underlying this disorder, twin concordance studies have strongly implicated heredity as a major risk factor.

Using an effected cousin pair strategy, analyzing high-density single nucleotide polymorphisms (SNPs) across the genome in related probands allows the determination of shared genomic regions. Relatives share large blocks of regions identical-by-descent (IBD), which can be readily identified with a dense marker set. Regions shared at a significantly increased rate in probands may be linked to the disease. This method potentially allows for the mapping of disease genes or risk alleles without the need for phase information. While observation of IBD between unrelated individuals is rare, observing an elevated rate of IBD sharing in these comparisons may identify regions of ancestral IBD.

Many groups are utilizing high-density marker sets to perform whole-genome association, which rely on trios. Distant relative IBD analysis provides a convenient and cost-effective alternative that produce similar results to whole-genome association while testing fewer individuals. Additionally, there may be specific genetic risk factors enriched in such families. Such factors would likely be either strong or common due to the influx of new genetic material via married-in parents. Importantly, this technique may demonstrate the potential for a novel usage of high-density SNP genotyping in identifying genomic regions of interest for complex traits and diseases.

The Genetic Basis of Lactase Persistence in Africa; Evidence for Convergent Adaptation Due to a Shared Cultural Trait in Europeans and Africans. *S.A. Tishkoff[1], F.A. Reed[1], A. Ranciaro[1,2], B.F. Voight[3], C.C. Babbitt[4], J.S. Silverman[4], K. Powell[1], J.B. Hirbo[1], H. Mortensen[1], M. Osman[5], M. Ibrahim[5], S.A. Omar[6], S. Bumpstead[7], J.K. Pritchard[3], G.A. Wray[4], P. Delouckas[7]* 1) Dept. of Biology, Univ of Maryland, College Park, MD; 2) Dept. of Biology, Univ of Ferrara, Ferrara, Italy; 3) Dept. of Human Genetics, Univ of Chicago, Chicago, Illinois, USA; 4) Dept. of Biology and Inst. for Genome Sciences & Policy, Duke Univ, Durham, NC 27708, USA; 5) Dept. of Mol. Biol., Inst. of Endemic Diseases, Univ of Khartoum, Khartoum, The Sudan; 6) KMRI,(CBRD) Nairobi, Kenya; 7) Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, CB10 1SA, UK.

The ability to digest milk as adults (lactase persistence, LP) is a cis-regulated genetic trait. Although a mutation associated with LP was previously identified in Europeans, the genetic basis of LP in African populations remained unknown. We collected phenotype data from 470 individuals originating from 43 populations in Tanzania, Kenya, and the Sudan using a Lactose Tolerance Test (LTT). We have identified two novel SNPs located ~14 kbp upstream of the lactase gene (*LCT*) that are significantly associated with the LP trait; one common in Tanzanian and Kenyan pastoralist populations and the other common in the Beja from northern Sudan. Expression assays indicate that the Kenyan/Tanzanian LP-associated SNP enhances transcription from the *LCT* promoter *in vitro*. Genotyping of 122 SNPs across a 3 Mbp region in these populations demonstrated that these African LP-associated mutations exist on haplotype backgrounds that are distinct from the European LP-associated mutation and from each other. In addition, we observe haplotype homozyogisty extending > 1.8 Mbp on chromosomes with the LP-associated mutation most common in Kenya/Tanzania, consistent with an ongoing selective sweep over the past 12,000 years (time of origin of this mutation ranges from ~2000 - 12,000 ybp). These data indicate a striking example of convergent evolution due to strong selective pressure (selection coefficients range from .05 - .13) resulting from shared cultural traits (*e.g.* cattle domestication and adult milk consumption) in Europeans and Africans.

Changing neurocognitive outcomes in Hurler syndrome with advancements in therapy. *E. Shapiro, K. Bjoraker, P. Orchard, L. Charnas, C. Doyen, C. Whitley* Dept Pediatrics, Univ Minnesota, Minneapolis, MN.

Hematopoietic stem cell transplant (HSCT) halts decline in rate of cognitive growth in Hurler syndrome. Without treatment, cognitive development slows by age 2 with decline in developmental quotient (DQ), eventual loss of skills after age 3, and the mean age of death is 5 years. At the Univ. of Minnesota, the first bone marrow transplant (BMT) for Hurler syndrome was performed in 1983. Initially only HLA matched siblings were donors for BMT, then unrelated donors. New protocols decreased risk and improved mortality. In 1998, umbilical cord (UCB) started to be used with routine use in 2001. In 2004 combination of ERT and HSCT began. While mortality has improved, we explore the change in morbidity associated with advancement in therapies. Data for 49 children who are alive, engrafted and have 1 or 3 years of follow up data after HSCT are presented; 24 prior to 1997, 16 from 1995-2002, and 9 after 2002. Within this data set, 10 who had UCB were matched with 10 with BMT. Of children seen before 1997 median DQ was 85, receptive language (RL) was 73 and expressive language (EL) was 82 (normal 8515). Of the 18 children seen between 1997 and 2002, median DQ was 90 and RL and EL were both 85 with a correlation of 0.52 between DQ and HSCT date. One year post-HSCT, the median loss of DQ points was one standard deviation (15 points) for all HSCT types. Between one and three years after HSCT no change in developmental quotient and RL was seen, but improved EL was found in those transplanted more recently. No difference in outcome was found between UCB and BMT. We conclude that all children show a decline of one standard deviation over the 1st year post transplant and stability thereafter. DQs and language quotients of children seen more recently are more intact due to a variety of factors 1) children are being diagnosed slightly earlier and 2) they are physically healthier at baseline with better DQs and language (partly because of increased use of early intervention and therapies). No difference was found in outcome by source of cells. The challenge is to find methods to prevent the cognitive decline during the first year post transplant.

Age-at-Onset Anticipation in Intracranial Aneurysms: An Analysis of 103 Finnish Families. *S. Wills[1], H. Kuivaniemi[1,2], A. Ronkainen[3], K. Helin[3], M. Niemelä[4], J.E. Jääskeläinen[3], J. Hernesniemi[4], G. Tromp[1]* 1) Center for Molecular Medicine and Genetics, Wayne State University,School of Medicine, Detroit, MI; 2) Department of Surgery, Wayne State University,School of Medicine, Detroit, MI; 3) Department of Neurosurgery, University of Kuopio, Kuopio, Finland; 4) Department of Neurosurgery, University of Helsinki, Helsinki, Finland.

Age-at-onset (AO) anticipation (AOA) occurs when the AO of a disease decreases or disease severity increases with successive generations or both. The goal of the study was to determine if AOA exists for familial intracranial aneurysms (IA) in the Finnish families. Families with two or more members having verified diagnoses of spontaneous rupture of IA and vertical transmission were recruited from the Kuopio and Helsinki IA registries in Finland. Families having heritable disorders associated with IA were excluded. Birth year, AO of spontaneous rupture, and generation data were collected. Statistical analysis for AOA was performed using the method reported by Vieland and Huang (Am J Hum Genet. 1998; 62:1212-1227) with an implementation of their algorithm by Dr. Huang and R, an open-source statistical computing environment. The algorithm was run for 1000 iterations with change criterion of 0.000001. We identified 103 (24.4%), IA families with vertical transmission in this collection of 422 Finnish families, in which AO information was available. There were 224 individuals with spontaneous rupture of IA. The mean AO in the 224 individuals was 49.3 13.9 years which is the same as that of the overall Finnish estimates for AO in the population. We found strong evidence for AOA ($P < 4.2 \times 10\text{-}6$). Truncation has a profound effect on AO of disease between parent-child pairs; however, with an appropriate statistical test correcting for truncation, our results show the existence of anticipation in Finnish IA families. This is the largest collection to date reporting on AOA in familial IAs. Anticipation suggests trinucleotide repeats may be involved in the disease. Our findings also suggest that screening at a younger age may be necessary in cases with a family history.

In preeclampsia invasive cytotrophoblasts have a lower rate of hyperdiploidy. *J. Weier[1, 2], C. Ferlatte[1], C. Jung[3], Y. Zhou[3], V. Winn[3], H. Nguyen[1], H. Weier[2], R. Romero[4], K. Bianco[4], S. Fisher[1,3,5]* 1) Dept OB/GYN & Repro Sci, University of California, San Francisco (UCSF), CA; 2) Life Sciences Division, University of California, E.O. Lawrence Berkeley National Laboratory, Berkeley, CA; 3) Dept Cell and Tissue Biology, UCSF, CA; 4) Perinatology Research Branch, NICHD, Wayne State University/ Hutzel Hospital, Department of Obstetrics and Gynecology, Detroit, MI; 5) Dept Anatomy & Pharm Chem, UCSF, CA.

Preeclampsia is associated with defects in the (placental) cytotrophoblast (CTB) differentiation pathway that leads to uterine invasion. Anchoring villi and invasive CTBs from patients with preeclampsia show specific morphological alterations: endovascular invasion is consistently shallow, and fewer spiral arteries are modified. Using a molecular cytogenetic method [i.e., fluorescence in situ hybridization (FISH)], we found that in normal pregnancy more than 50% of invasive CTBs were hyperdiploid without evidence of endoreduplication (Weier et al., Dev. Biol. 279:420-432, 2005). These data suggest that hyperdiploidy is an important component of normal placentation, perhaps limiting the proliferative and invasive potential of CTBs. Here we tested the hypothesis that preeclampsia is associated with a shift in the normal hyperdiploid state of these cells. Accordingly, we used FISH and immuno¬staining to study the aneuploidy rates of CTBs in placental tissues obtained from patients with preeclampsia. The results showed lower overall rates of hyperdiploid CTBs as compared to normal control samples. Specifically, the average rate of hyperdiploid CTBs involving chromosomes X, Y and 16 in preeclampsia samples was not significantly different from that of normal controls [36.5% (PE) vs. 40.2% (control)]. However, scoring of chromosomes 13, 18 and 21 showed significantly fewer hyperdiploid cells [11.7% (PE) vs. 44.7% (control), $p < 0.01$]. In conclusion, the results of this study offer genetic evidence that corroborates an association between preeclampsia and incomplete CTB differentiation along the pathway that leads to uterine invasion. Furthermore, these data support the concept that abnormal CTB differentiation proceeds the onset of the clinical syndrome.

Sequence divergence between *Mus Spretus* and *Mus Musculus* across a 13Mb region on chromosome 12. *A.E. Toland[1,2,5], K.L. Cooper[1,5], A. Dworkin[1,3,5], N.P. Gladman[6], H.-Y. Cho[4,5], J.-H. Mao[7], A. Balmain[7]* 1) Molecular Virology, Immunology & Medical Genetics, Human Cancer Genetics; 2) OSU Comprehensive Cancer Center, Department of Internal Medicine; 3) Integrated Biological Science Graduate Program; 4) Molecular, Cellular, & Developmental Biology Program; 5) The Ohio State University, Columbus, OH; 6) Wittenberg University, Springfield, OH; 7) UCSF Comprehensive Cancer Center,University of California, San Francisco, CA.

Mus. spretus and *Mus. musculus* diverged from each other approximately 1-3 million years ago and are estimated to have an average of one single nucleotide polymorphism (SNP) every 50bp. In addition to a high degree of sequence diversity, *spretus* and *musculus* show a high degree of phenotypic variability and have been used for QTL mapping for cancer susceptibility and inflammation. Using 3 different *spretus* x *musculus* F1 backcrosses, we mapped 13 loci for skin cancer susceptibility. One locus on chromosome 12, *Skts5*, shows linkage for skin cancer susceptibility in all three crosses. To determine the degree of genetic diversity between *musculus* and *spretus* derived strains at *Skts5* and to identify potential candidate genes for cancer susceptibility, we sequenced all 51 named and unnamed genes mapping to the peak area of linkage in *Spret/EiJ* and *FVB/NJ*. Regions housing polymorphisms between the strains were sequenced in the other strains used for linkage analysis: *outbred spretus*, *SEG/PAS* and *NIH/Ola*. All 51 genes contained at least one polymorphism between *spretus* and *musculus*. The majority of changes were synonymous or located in 5 or 3UTRs. Of interest, we identified 69 amino acid substitutions in 25 genes. To determine the potential significance of the amino acid substitutions identified, protein alignments between *Mus. musculus* (*FVB/NJ*, *NIH/Ola*), *Mus. spretus* (*Spret/EiJ*, outbred *spretus*, and *SEG/PAS*), other mouse strains for which sequence was available, and a minimum of 8 additional species were completed. A few amino acids that differ between musculus and spretus at *Skts5* are highly conserved across taxa; these will be considered as candidate polymorphisms for cancer susceptibility.

Haplotype inference for absent-present genotype data for clustered genes using identified haplotypes and/or sub-haplotypes. *Y. Yoo[1], J. Tang[2], R.A. Kaslow[2,3], K. Zhang[1]* 1) Departments of Biostatistics; 2) Medicine; 3) Epidemiology, UAB, Birmingham, AL, 35294.

The majority of killer cell immunoglobin-like receptor (KIR) genes are detected as either present or absent using locus-specific genotyping technology. This detection scheme leaves missing data: whether a detected gene is present on one or both chromosomes remains unknown. Thus, the performance of methods for haplotype inference (e.g., PHASE) for KIR genes may be compromised. To accommodate this, we developed an EM-based method for haplotype inference by incorporating previously identified haplotypes and/or sub-haplotypes. Specifically, a set of reported haplotypes is used and then extended to resolve as many as possible samples using a greedy algorithm. Haplotype is then inferred on he basis of this extended set of haplotypes via the EM algorithm. We compared our method with HAPLORE and PHASE based on three measures: 1) SAD, the sum of absolute difference between the true and estimated haplotype frequencies; 2) IE, the portion of individuals whose estimated haplotypes are not correct, and 3) SE, the Hamming distance between the true and estimated haplotypes in an individual. Our method outperformed the two other techniques by all three measures when at least 60% of known haplotypes were incorporated and by SAD even when only 25% were known (Table). From these results, our method appears more useful than existing techniques in assigning haplotypes for genes detected only as present or absent.

Table - Comparison of three methods for haplotype inference for KIR genes designated present or absent

	EM Using 100% known haplotypes	EM Using 60% known haplotypes	EM Using 25% known haplotypes	HAPLORE	PHASE
SAD	0.11	0.13	0.18	0.31	0.86
IE	0.17	0.20	0.34	0.29	0.64
SE	0.02	0.03	0.13	0.03	0.09

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Quantitative analysis using decreasing amounts of genomic DNA to assess the performance of the Agilent oligo comparative genome hybridization microarray system. *S. SONG, D. Ilsley* Genomics Division, Agilent Technologies Inc., Santa Clara, CA.

Comparative genomic hybridization (CGH) is a technique for studying chromosomal changes in cancer. As cancerous cells multiply, they can undergo dramatic chromosomal changes, including chromosome loss, duplication, and the translocation of DNA from one chromosome to another. Chromosome aberrations have previously been detected using optical imaging of whole chromosomes, a technique with limited sensitivity, resolution, quantification, and throughput. Efforts in recent years to use microarrays to overcome these limitations have been hampered by inadequate sensitivity, specificity and flexibility of the microarray systems. The Agilent oligonucleotide CGH microarray system overcomes several scientific hurdles that have impeded comparative genomic studies of cancer. This new system can reliably detect single copy deletions in chromosomes. The system includes a whole human genome microarray, genomic DNA Labeling kit for sample preparation, an optimized microarray processing protocol, and software for data analysis and visualization. In this study, we determined the sensitivity, accuracy and reproducibility of the new system. Using this assay, we find that the performance of the complete system was maintained over a range of input genomic DNA (non-amplified) from 5 ug down to 0.2 ug with the array signal to noise ratio higher than 70:1.

Mutation screening and phenotype analysis is 48 patients with a clinical diagnosis of CFC or Costello syndromes and no mutation in PTPN11 and HRAS. *A. Verloes[1], C. Nava[1], M. Gérard-Blanluet[1], C. Baumann[1], T. Niihori[2], Y. Narumi[2], Y. Aoki[2], Y. Matsubara[2], B. Arveiler[3], D. Lacombe[3], H. Cavé[1]* 1) Department of Genetics, AP-HP Robert Debre University Hospital, Paris, France; 2) Department of Medical Genetics, Tohoku University School of Medicine, Sendai, Japan; 3) Department of Genetics, CHU Pellegrin, Bordeaux, France.

CFC syndrome is a serious clinical condition clinically related with Noonan syndrome, which has been very recently linked to mutations in 4 genes from the RAS signalling pathway. We performed mutation analysis for KRAS, BRAF, MEK1, MEK2 in 19 patients with CFC syndrome referred to our lab for molecular screening of PTPN11, and a clinical diagnosis of CFC, and 16 patients from the french Costello family support group without HRAS mutations. We further incorporated in this presentation the 13 CFC patients initially analysed by Niihori & al, and already published in Nature Genetics, as their were a part of our French/Belgian Noonan-CFC pool. Direct bidirectional sequencing of KRAS (exons 1, 2, 3, 4a, 4b), BRAF (exons 6, 11, 12, 14, 15), MEK1 and MEK2 (exons 2, 3). We found a) 20 mutations in BRAF: 13/32 CFC patients (40,6%) and 7/16 Costello HRAS-neg patients (44%) - b) 2 mutations in KRAS 1/32 CFC (3%) and 1/16 (6%) Costello HRAS-neg. c) 6 MEK1 mutations : 2/32 CFC (6%) and 4 / 16 (25%) Costello HRAS; d) at least 2 MEK2 mutations : CFC : 2/13 (analysis still ongoing.) We will present the molecular and clinical data related to this series, and possible genotype/phenotype correlations. Although most patients have been sent by trained clinical geneticists, it clearly appears that distinction between CFC and Costello, and to a lesser extend between CFC and Noonan remains difficult, at least in younger children.

Physician Survey on Asthma Genotyping. *I.W. Yu[1], T. Stith[1], A. Ferreira-Gonzalez[2], L.B. Schwartz[3], A-M. Irani[4], B.L. Bukaveckas[1,2]* 1) School of Pharmacy; 2) Department of Pathology; 3) Division of Rheumatology, Allergy and Immunology, Department of Internal Medicine; 4) Division of Rheumatology, Allergy and Immunology, Department of Pediatrics at Virginia Commonwealth University, Richmond, VA.

Our aim was to test physician understanding of and perceived utility for a new lab assay described on a reference card. The test is for position 16 ArgGly in the 2 adrenoceptor gene (ADRB2). Asthmatics with the Arg/Arg genotype (20-25% of the population) have decreased lung function from long-term, regular use of 2 agonist bronchodilators. To aid clinicians we developed an ADBR2 test information card with steps to avoid future decreased lung function in asthmatics based on test results. **Methods**: We distributed a reference card and questionnaire to 81 physicians in the VCU Health System, Richmond, VA. There were five multiple choice comprehension questions as well as seven visual analog scale response questions. Scalar responses were converted to continuous variables prior to analysis, and are reported here as (mean standard deviation %). **Results**: 17 physicians returned surveys (21% response rate). More than 80% had correct answers for all comprehension questions. The physicians agreed (76, 18.9%) that the goal of the test was significant to asthmatics, and did not think the test without utility (77.6, 23.4%). Physicians were also in agreement with the benefit of the test for physicians and patients, (72.2, 22.2% and 78, 15.7% respectively). There was strong agreement that the test would result in better treatment for asthma patients (78.4, 16.4%), and that using the test was a straightforward process (81.6, 13.2%). Perhaps most telling is that almost 2/3 of the physicians plan to use the test (62.9, 26.7%). The results of the survey are limited and potentially biased by respondent self-selection. **Conclusion**: Our survey demonstrated that physicians understand and concur with the content and intent of the ADBR2 assay pocket reference card. The card was an aid to comprehension and acceptance of the ADBR2 test. Ms. Stith was supported by the VA-Nebraska Alliance during this project.

Universal detector assay for measuring DNA copy number changes. *A.R. Tobler, A.J. Broomer, R.T. Koehler, D.C. Merrill, K.J. Guegler, C. Chen* Molecular and Cell Biology R&D, Applied Biosystems, Foster City, CA.

Understanding the genetic basis of human phenotypic differences requires the study of an increasing variety of human genetic variations. Detection of single nucleotide polymorphisms (SNPs) has been at the center of efforts to characterize the genetic components of diseases and traits. However, emerging evidence suggests that intermediate and large-scale DNA copy number changes in a genome are prevalent and account for an important source of genetic variation between individuals. Several methods have been developed to measure DNA copy number changes. Most methods require the enzymatic manipulation of genomic DNA and the analysis of fluorescently labeled DNA fragments by either array hybridization or capillary electrophoresis. For the analysis of a small number of loci, conventional TaqManÒ assays can be designed as well. We report here a new assay called Universal Detector (UD) for accurately measuring copy number changes. The UD assay utilizes a 96-plex oligonucleotide ligation assay (OLA) followed by 48 duplex TaqManÒ assays for analyzing up to 96 genetic loci. Our results show accurate identification of duplicated sequences in samples with known chromosome duplications. We further tested DNA samples for copy number changes in drug metabolizing enzyme genes, and for a 1.5 MB duplication responsible for a neurodegenerative disease. Samples with gene duplications and deletions were identified. The UD assay can be completed in less than a day and is suitable for genetic studies requiring a low to medium sample throughput. UD is a powerful, new tool for accurately measuring DNA copy number changes.

Prevalence of 47,XXX, 47,XXY and 47,XYY in prenatal diagnosis. *M. Thangavelu, B. Huang* Genzyme Genetics, Orange, CA.

Trisomy for chromosomes 13, 18 and 21 and monosomy X are the chromosome abnormalities most frequently observed during prenatal diagnosis and are associated with an "adverse" phenotype in the fetus. Unlike these abnormalities, 47,XXX, 47,XXY and 47,XYY are not associated with advanced maternal age, abnormal maternal serum screen or ultrasound abnormalities. Because of the mild and variable phenotypes, they come with their own challenges of counseling for the clinical team and decision making for the patients. We attempted to estimate the prevalence of these sex chromosome anueploidies for groups of patients with different indications during prenatal diagnosis. A data base of 306,487 prenatal specimens was analyzed for the incidence of sex chromosome polysomies compared to trisomy for chromosomes 13, 18 and 21 and monosomy X for various clinical indications was estimated. The combined incidence of +13, +18, +21 and XO was 2% for advanced maternal age, 3% for abnormal maternal serum screen and 20.6% for abnormal ultrasound. The combined incidence for XXX, XXY and XYY was 0.1% for each of the groups, irrespective of clinical indication. Of the three aneuploidies, 47,XXY, a karyotype associated with infertility was the most frequent (46.7%), followed by 47,XXX (35.5%) and XYY (17.8%). Uniform prevalence of these sex chromosome aneuploidies in the different groups is not surprising, as these abnormalities are not associated with either maternal age (as a factor in their etiology) or anatomical abnormalities detectable on prenatal ultrasound evaluation.

Validation of a molecular technique (MLPA) for prenatal screening in amniotic fluid. *M.M. Weiss, L. Vijfhuizen, M.J.V. Hoffer, C. Ruivenkamp, E.M.J. Boon, N. denHollander, E. Bijlsma, E. Bakker, M.H. Breuning* Clinical Genetics, Leiden University Medical Center, Leiden, Zuid-Holland, Netherlands.

Diagnosis of chromosomal abnormalities on amniotic fluid samples by karyotyping or FISH is time consuming and labor intensive. It can be expected that a DNA-based technique will replace the FISH test and karyotyping in those prenatal cases where there strictly is no indication for karyotyping, for example, advanced maternal age with no structural abnormalities of the fetus. We are currently validating Multiplex Ligation dependent Probe Amplification (MLPA) for advanced maternal age in a national collaboration with the other cytogenetic laboratories in The Netherlands. Therefore, we performed a prospective study on 1296 amniotic fluid samples by analysing these both with MLPA and karyotyping. The commercially available kit (P095, MRC Holland, The Netherlands) contains eight probes for each of the chromosomes to be tested (13, 18, 21, X), as well as four probes on chromosome Y. There were 1133 conclusive MLPA tests. In 1113 test, results were concordant with those of karyotyping. The twenty disconcordant cases were all cases that could theoretically not be detected with this MLPA kit. Of these twenty cases only five were referred for advanced maternal age, the fifteen others were screened because of a high-risk based on ultrasound abnormalities, or serum screening and/or nuchal translucency measurement or because of a familial chromosomal translocation. After collecting follow-up data (from four cases), two healthy children were born, one pregnancy resulted in IUD, and one was considered a culture artefact after re-analysing a 2nd amniotic fluid by karyotyping. We correctly identified all 33 autosomal trisomy cases, and 11 sex chromosomal copy number aberrations (45,X; 47,XXX; 47,XXY; 47,XYY). In conclusion, in this study, for advanced maternal age, no chromosomal aberrations would have been missed by MLPA that would have consequence for the fetus. In a clinical setting, MLPA can be used for a rapid and reliable determination of common chromosomal aneuploidies in uncultured amniocytes for advanced maternal age.

Mutations of PRSS1 and SPINK1 Genes in Korean Patients with Idiopathic Recurrent or Familial Pancreatitis.

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BACKGROUND: Hereditary pancreatitis (HP) is a rare inherited disorder, characterized by recurrent episodes of pancreatitis often beginning in early childhood. It has been found that mutations of cationic trypsinogen gene (PRSS1) and serine protease inhibitor, Kazal type 1 gene (SPINK1) increase the susceptibility of chronic pancreatitis (CP) and pancreatic cancer. We investigated the genotype of PRSS1 and SPINK1 genes and clinical characteristics in idiopathic recurrent or familial pancreatitis with PRSS1 or SPINK1 mutations in Korea. METHODS: Clinical data was obtained from 19 patients with a family history (2 or more family members) of pancreatitis or with early-onset (40 years of age or younger) idiopathic recurrent acute pancreatitis (AP) or CP. Entire coding regions with intron-exon boundaries of the PRSS1 gene and SPINK1 gene were sequenced by PCR-direct DNA sequencing RESUTLS: Six patients were heterozygote for PRSS1 (p.R122H: 5, p.D20G: 1, p.G208A: 1) mutations and 4 of them had a family history of early-onset pancreatitis. Mutations in SPINK1 (c.194+2T>C: 5, p.N34S: 1) were identified in 6 patients, and 2 of them had a family history of early-onset pancreatitis. The median age at diagnosis in patients with PRSS1 mutation was 10 years of age, 26 years with SPINK1 mutation, and 29 years in mutation negative patients. Mean peak serum amylase and lipase level, the male to female ratios and the frequency of complications of the patients with mutations were not significantly different compared with the patients without mutations. Median age at diagnosis of pancreatitis is younger (P=0.04) and pancreatic duct stones are more frequently observed in mutation positive patients. CONCLUSIONS: PRSS1 and SPINK1 mutations are related to the development of idiopathic recurrent or familial pancreatitis in Korea. HP caused by PRSS1 or SPINK1 mutations is considered as an etiology of early-onset idiopathic recurrent AP or CP, especially when a family history is present.

Evolution of the Y chromosome *AZFc* region in the great ape lineage. *P. Yen[1], Y.-H. Yu[1], Y.-W. Lin[1], W. Schempp[2]*
1) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) Institute of Human Genetics and Anthropology, University of Freiburg, Germany.

The *AZFc* region on the long arm of the human Y chromosome is one of the least stable regions in the human genome. It consists of several very long repeats (amplicons) and is prone to rearrangement. Numerous *AZFc* architectures have been identified, and deletion of the entire *AZFc* region is present in about 10% of azoospermic men and is a major genetic cause of male infertility. Within the *AZFc* region are four *DAZ* genes that encode RNA-binding proteins with one, two, or three RNA-binding motifs (RBMs) and 8-24 copies of the DAZ repeat. *DAZ* orthologs are present only on the Y chromosomes of Old-World monkeys, great apes and humans, whereas its two autosomal paralogues, *BOULE* and *DAZL*, are found in all vertebrates. It has been proposed that the Y-linked *DAZ* genes arose from the autosomal *DAZL* gene by duplication and translocation. We used Southern analyses to study the evolution of the *AZFc* region in the great ape lineage. We found that Bonobos (*Pan paniscus*), chimpanzees (*Pan troglodytes*) and gorillas (*Gorilla gorilla*) have two *DAZ* genes encoding various copies of RBMs, whereas orangutans (*Pongo pygmaeus*) have at least 6 *DAZ* genes encoding a single RBM. The results suggest that the ancient Y chromosome of the great ape lineage had only two *DAZ* genes, and subsequent duplication of the *DAZ* gene pair occurred independently in the human and the orangutan branches to give rise to the present day structural spectrum of the *DAZ* genes. We also found that all great apes have the "red" and the "green" amplicons, but not the "gray" amplicon within their *AZFc* regions, indicating that the "gray" amplicon was translocated from an autosome to the human Y chromosome after the separation of the human and the chimpanzee lineages.

Acquired microcephaly: patterns, causes & effects. *A.S. Rigby[1], P. Baxter[2], M. Rotsaert[2], B. Steele[2], I. Wright[3]* 1) Academic Cardiology, University of Hull, Hull, UK; 2) Sheffeld Children's Hospital, Sheffield, UK; 3) Department of Psychology, University of Sheffield, UK.

Acquired microcephaly is a condition where a child's head circumference is within the normal range at birth but then does not increase as fast as normal and falls below the 2nd centile. There is a lack of literature on this acquired form of microcephaly. Retrospective study of 58 children (29 M) aged 0.7-11.3 years (median 4.5 years) referred over a 10 year-period. Measurements included neonatal discharge examinations and community measurements. Centile charts/growth data were based on a UK reference sample. Cause was judged from standard assessments including head size in the immediate family. Growth pattern was made by inspection of centile charts by 2 independent observers. 34 families agreed to further assessments including: IQ, BSID-II, WPPSI-R, WISC-III, literacy, numeracy, specific abilities and behaviour (WORD, WOND, NEPSY,CBCL). Causes were divided into following: idiopathic (n=8) - no specific cause; familial (n=15) - parent/sib had a head circumference below 2nd centile; syndromic (n=15) - with associated other anomalies; symptomatic (n=16)- following a pathologic event; other (n=4). In the familial group affected relatives were mainly mothers (n=12) and brothers (n=8). In the syndromic group 5 had recognised symptoms including Rett (n=2) and Angelman (n=1). In the symptomatic group causes included EBV infection, trauma and failure to thrive. Growth patterns could be classified into 4 types: type A (n=36) - initial fall below 2nd centile then parallel to the centiles; type B (n=12) - continued fall across all centiles; type C (n=5) - later recovery after falling below normal range; type D (n=10) - insufficient data to classify. More symptomatic cases had type B (6/12) than type A (9/26) but type B could occur in all causal groups. Idiopathic had mainly type A (6/8). Final Z-scores were strongly related to initial Z-scores ($p<0.001$). There was no correlation between IQ and final Z-scores. Parents can be advised that there are 4 main causal groups. Some of these may have genetic implications. There was poor correlation between causes and patterns.

5-aza Cytidine mediated CAG expansion in vitro. *Ravindra. Varma[1,2], K. Sreelatha[1], V. Sharmila[2], Sudharthi[2], Gita Sharma[2], Zacharia Mathew[2], Qurratulain Hasan[1]* 1) Department of Genetics, Kamineni Hospitals, Hyderabad, India; 2) Assay Development Unit, MAGENE Lifesciences, Hyderabad, India.

Spinocerebellar ataxias (SCAs) are a heterogeneous group of neurodegenerative disorders, inherited in an autosomal dominant manner. CAG repeat expansion in the causative genes has been identified as the basic cause of most types of SCAs. Studies from different groups have identified cis-elements, such as tract length, sequence purity and orientation relative to replication origins and trans-acting factors, such as DNA repair and replication proteins as agents that can critically affect the stability of repeats. Nevertheless, the mechanisms by which such agents collaborate to bring about intergenerational repeat instability remain unclear. AIM: The main aim of these experiments was to determine if exogenous agents play a role in modifying the somatic mutational dynamics of expanded CAG repeat sequences. We tested a genotoxic agent, 5-aza Cytidine that is known to have an effect on DNA. METHODS: Two stable cell lines with 17 CAG repeats (Q17) and 65 CAG repeats (Q65) were developed using HEK293 cell lines. Both the cell lines were grown in triplicates for 90 days in presence of 10uM 5-aza Cytidine. Control cultures were also maintained through out the experiments with no chemicals added. RESULTS: We identified that 5-aza Cytidine significantly enhanced the CAG repeat number in case of Q65 cells where as no change in repeat number was observed in Q17 cells treated with the same chemical. CONCLUSION: Although much progress had been made in understanding the molecular and cellular mechanisms that underlie diseases with CAG repeats, cause for their instability is unknown. Our results indicate that 5-aza Cytidine plays a role in triggering expansion at the CAG repeat size beyond a certain length. Methylation may be the critical mechanism in triggering expansion. The system we have developed offers the potential to investigate the role of other exogenous agents that mediates somatic expansions by interfering with specific aspects of DNA metabolism.

Mitotic recombination as evidence of alternative pathogenesis of gastrointestinal stromal tumors (GISTs) in neurofibromatosis type 1. *D.R. Stewart[1], C.L. Corless[2], B.P. Rubin[3], M.C. Heinrich[2], L.M. Messiaen[4], L.J. Kessler[5], P.J. Zhang[6], D.G. Brooks[7]* 1) NHGRI/NIH, Bethesda, MD; 2) OHSU, Portland, OR; 3) U. Washington Medical Center, Seattle, WA; 4) UAB, Birmingham, AL; 5) U. Pennsylvania, Dept. Medical Genetics, Phila, PA; 6) U. Pennsylvania, Dept of Pathology, Phila, PA; 7) Merck and Co, Whitehouse Station, PA.

Background: Neurofibromatosis type 1 (NF1) is a neurocutaneous disorder resulting in the growth of a variety of tumors and is inherited in an autosomal dominant pattern. Gastrointestinal stromal tumors (GISTs) are mesenchymal tumors that commonly harbor oncogenic mutations in KIT or PDGFRA and are thought to arise from the interstitial cells of Cajal (ICC; the pacemaker cells of the gut). We characterize two patients with NF1 and GISTs. Methods: Both patients were genotyped for germline mutations in NF1. GIST tumors from both patients were genotyped for somatic mutations in KIT and PDGFRA. Loss of heterozygosity (LOH) of NF1 in one GIST tumor was assessed by genotyping seven microsatellite markers spanning 2.39 Mb of the NF1 locus in the tumor and in genomic DNA. The known germline mutation in NF1 was confirmed in GIST tumor DNA by sequencing. The copy number of the mutated NF1 allele was determined by MLPA. Results: GIST tumors from both patients were wild-type for mutations in KIT and PDGFRA. In the one GIST tumor with adequate DNA, all seven markers were informative and showed a loss of heterozygosity (LOH) at the NF1 locus; sequencing of NF1 from that GIST showed no wild-type sequence, suggesting it was lost in the tumor. The MLPA analysis showed that two copies of all NF1 exons were present. Conclusions: This is the first evidence of mitotic recombination resulting in a reduction to homozygosity of a germline NF1 mutation in an NF1-associated GIST. The LOH of NF1 and lack of KIT and PDGFRA mutations are evidence of an alternative pathogenesis in NF1-associated GISTs. The significant clinical differences between sporadic and NF1-associated GISTs are compatible with the proposed alternative pathogenesis.

An Age-Related Macular Degeneration Susceptibility Locus on Chromosome 16p12. *K. Spencer[1], S. Schmidt[2], M.A. Hauser[2], W.K. Scott[2], L.M. Olson[1], P. Gallins[2], N. Schnetz-Boutaud[1], A. Agarwal[3], E.A. Postel[4], M.A. Pericak-Vance[2], J.L. Haines[1]* 1) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN; 2) Center for Human Genetics and Department of Medicine, Duke University Medical Center, Durham, NC; 3) Vanderbilt Eye Institute, Vanderbilt University Medical Center, Nashville, TN; 4) Duke University Eye Center and Department of Ophthalmology, Duke University Medical Center, Durham, NC.

Age-related macular degeneration(AMD) is a complex disease caused by both genetic and environmental risk factors. As the disease progresses from the presence of large, soft drusen to geographic atrophy and/or choroidal neovascularization, legal blindness may result. The risk associated with the Y402H variant in the complement factor H gene on chromosome (chr) 1 and a variant in the LOC387715 gene on chr.10 have been well-documented. Consistent with the results of several genomic screens, we also have evidence of another AMD susceptibility locus that resides on chr.16p12. We genotyped 312 SNPs across chr. 16 in a dataset of 127 multiplex families as part of a genome-wide screen. In an independent dataset of 584 cases and 248 unrelated controls we genotyped a subset of 137 SNPs that are concentrated between 10-31 Mb to follow up on linkage results in this region. Our peak multipoint nonparametric LOD* score of 1.33 occurred at 22.8 Mb. Ordered Subset Analysis (OSA) considering smoking, the proportion of risk alleles in affecteds for Y402H or the rs10490924 variant in LOC387715 did not significantly change the LOD score. Using the association in the presence of linkage APL method in the family-based dataset and tests of allelic and genotypic association in the case-control dataset, a cluster of SNPs between 28 and 30 Mb were significant at <=0.01 level. Stratifying by Y402H or rs10490924 risk alleles did not have a large impact on the association results in the case-control dataset, consistent with the family-based OSA analysis. The association results in the independent case-control dataset confirm the linkage data and further localize the risk gene to a 2 Mb region.

Human genetic association study of 232 osteoarthritis candidate genes in patients with radiographically confirmed knee OA. *J. Richmond[1], L. Wood[1], K. Durham[2], S. Yocum[3], D. Aguiar[4], A. Seymour[1]* 1) Pharmacogenomics, Pfizer, Groton, CT; 2) Statistics, Pfizer, New London, CT; 3) Inflammation, Pfizer, Ann Arbor, MI; 4) Molecular Pharmacology, Pfizer, St Louis, MO.

Osteoarthritis (OA) is the most common form of arthritis with 1 in 3 people expected to have radiographic evidence of joint destruction by age 75. While the multifactorial nature of OA is well recognized, genetic factors comprise an essential component of the etiology of the disease. The genetic component of OA has been estimated via familial aggregation and twin studies with heritability ranging from 20 to 65%. A human genetic approach to identify associations between biologically relevant candidate genes and OA can provide early confidence in these genes as drug targets. Multiple genome wide linkage and candidate gene association studies have identified several loci contributing to the disease, confirming the complex heterogeneity of this disease.

We have extended genetic studies to also include RNA work to focus on particular candidate genes. 232 candidate genes, selected on the basis of differential RNA expression in cartilage tissue, were tested for genetic association in a large OA cohort. 422 subjects with clinical and radiographic knee OA and 437 age and gender matched controls were screened for 636 SNPs using a pooled approach on the Sequenom Mass Spectrometer. 104 SNPs representing 68 genes were selected from the pool based on a first pass allelic association of p0.05. These 104 SNPs were then genotyped across each subject individually to test for allelic and genotypic associations. The results of this study identified twelve putative candidate genes (NR1D1, SPARCL, LMOD1, PPAP2B, CXCR4, CLIC5, BMP6, PECAM1, RS6K2, SERPINE1, STK39, and HIST1H2A1) for follow-up evaluation based on a combination of gene expression and genetic association data. These results provide preliminary evidence of genetic association in a human knee OA population. However, as with any genetic association study, further validation in an independent population is warranted to better assess the role these genes play in disease risk.

Chediak Higashi syndrome: a genotype-phenotype correlation. *W. Westbroek[1], D. Adams[1], A. Koshoffer[2], H. Dorward[1], A. Helip-Wooley[1], M. Huizing[1], J. Parkes[1], R. Kleta[1], R. Boissy[2], W.A. Gahl[1]* 1) Medical Genetics Branch, NHGRI/NIH, Bethesda, MD; 2) Univeristy of Cincinatti College of Medicine, Cincinnati, OH.

Chediak Higashi syndrome (CHS) is a rare autosomal recessive disorder caused by mutations in the CHS1 gene. Clinical characteristics include skin, hair and eye hypomelanosis, recurrent infections, a mild bleeding diathesis and late-onset progressive neurological impairment. We report two unrelated patients, CHD4 and CHD6, with vastly different clinical presentations. CHD4 had severe symptoms in early childhood and carried two truncating CHS1 mutations; a nonsense (p.R514X)) and a frameshift mutation (p.F3298fsX3304). CHD6 had mild adult-onset neurological symptoms and carried a truncating frameshift (p.E805fsX) and a relatively milder missense mutation (p.N3376S) located in the BEACH domain of CHS1. Whole mount electron microscopy of normal and patients platelets revealed absence of dense bodies in most platelets of CHD4 and few dense bodies for CHD6. Laser scanning confocal microscopy and electron microscopy on cultured melanocytes showed that CHD4 had huge melanosomes restricted to the perinuclear area, while melanosomes in CHD6 were much smaller, with a normal dendritic distribution. Both CHD4 and CHD6 melanocytes showed some focal pigment deposits in melanosomes. Fibroblasts stained with the lysosomal markers LAMP1, LAMP2 and CD63 showed a perinuclear distribution of a few large lysosomes in CHD4 cells, while CHD6 fibroblasts contained slightly enlarged lysosomes in the perinuclear area, and slightly less than the normal contingent in the periphery. The relatively mild findings in CHD6 might be attributable to the CHS1 missense mutation, with residual CHS protein expression. These studies illustrate that genotype-phenotype correlations span the clinical, molecular and cell biological spectra. These two melanocyte cultures also allow for comparative investigations into the possible role of the CHS1 protein in intracellular organelle trafficking.

Severe cystic fibrosis with R117H/DF508 and 7T/9T polymorphism : difficulties in genetic counselling after newborn screening. *C. Thauvin-Robinet[1], S. Pérez-Martin[2], A. Masurel-Paulet[1], A. Contrain[1], I. Sermet-Gaudelus[3], F. Chevalier-Porst[4], L. Faivre[1], F. Huet[2]* 1) Ctr de Genetique, Hosp d'Enfants, Dijon, France; 2) Service de Pédiatrie 1, Hosp d'Enfants, Dijon, France; 3) Service de Pédiatrie, Hosp Necker-Enfants Malades, Paris, France; 4) Laboratoire de Biochimie Pédiatrique, Hosp Debrousse, Lyon, France.

Over 1000 CFTR mutations have been reported with cystic fibrosis, congenital bilateral absence of the vas deferens (CBAVD), chronic pancreatic insufficiency, rhinosinusitis and allergic broncho-pulmonary aspergillosis. The presence of the R117H/DF508 on a background of 5T is associated with borderline or elevated sweat test, mild to moderate lung disease, pancreatic exocrine sufficiency and CBAVD. The genotype of R117H/DF508 in association with 7T/9T is associated with a normal, borderline and rarely elevated sweat test with a broad phenotype ranging from no symptoms, CBAVD to mild lung disease. Here, we report on a 1-year-old female case with R117H/DF508 and 7T/9T identified in the newborn screening. Sweat test showed normal chloride concentrations (19 mEq/l and 30 mEq/l). Follow-up revealed recurrent respiratory infections since 1 month of life, with Staphylococcus aureus and Haemophilus influenzae, and chronic diarrhoea secondary to pancreatic exocrine insufficiency, leading to the diagnosis of severe cystic fibrosis. Significant growth failure (-2.5 SD) required gastrostomy for hypercaloric enteral nutrition. Nasal potential difference was characteristic of classical cystic fibrosis. The possibility of the presence of another rare severe mutation that could better explain the severity of the clinical phenotype was ruled out by direct sequencing of the overall CFTR gene. This observation presents the very rare association of severe cystic fibrosis with R117H/DF508 and 7T/9T genotype, showing to difficulties in genetic counselling after R117H/DF508 carrier detection by newborn screening. A survey of the phenotype of such patients is needed on a large series of patients.

Identification of Na/K ATPase 1 subunit as an interacting partner of WFS1. *M. Zatyka[1], C. Ricketts[1], J.A.L. Minton[1], S. Fenton[1], G. daSilvaXavier[2], S. Hofmann[3], C. McConville[1], G.A. Rutter[2], T.G. Barrett[1]* 1) Med & Molec Genetics, University of Birmingham, Birmingham, UK; 2) Dept of Biochemistry, University of Bristol, UK; 3) Istitut fur Diabetesforschung, Muenchen, Germany.

Wolfram syndrome is a monogenic disorder characterised by the juvenile onset of diabetes mellitus, progressive optic atrophy, sensorineural deafness, diabetes insipidus, and psychiatric illness. The product of the gene responsible for this disorder (WFS1 or Wolframin) localises to the Endoplasmic Reticulum (ER) membrane and is important in the regulation of calcium homeostasis and the ER stress response. To gain insight into WFS1 function we performed a yeast two hybrid assay to identify WFS1 interacting partners. Among interacting clones we identified the 1 subunit of sodium pump (Na/K ATPase). This interaction was confirmed in Cos7 cells using Myc-tagged WFS1 and HA-tagged 1, and subsequently confirmed endogenously in placental JEG3 cells, neuroblastoma cell lines and fibroblasts expressing different levels of WFS1. Mapping of interacting domains revealed that WFS1 interacts with the 1 subunit via its C-terminal domain and another interacting domain present in the transmembrane region, whereas the WFS1 N-terminal domain does not interact with the 1 subunit. Our mapping data suggests that this interaction probably takes place in the ER lumen. The sodium pump is inserted into the plasma membrane but undergoes maturation and assembly in the ER, with the 1 subunit acting as a chaperone for the proper membrane insertion of the subunit. Our findings are consistent with the proposed WFS1 involvement in protein folding and processing. Experiments are underway to establish the role of this interaction.

Towards delineating cell types in the mouse central nervous system: a genome-scale high resolution expression map of the adult mouse brain. *S. Sunkin, C. Dang, M. Hawrylycz, J. Hohmann, E. Lein, P. Wohnoutka, A. Jones* Allen Institute for Brain Science, Seattle, WA.

Several techniques to study large-scale gene expression (microarrays and serial analysis of gene expression) have been applied to large brain structures; however, these techniques lack spatial specificity in the resulting expression profile. A high-throughput colorimetric in situ hybridization platform has been developed to generate cellular resolution gene expression maps of the adult mouse brain. The goal of the Allen Brain Atlas (ABA) is to map the expression of ~20,000 genes in C57BL/6J. Currently, over half a million high resolution images of expression patterns for 18,500 genes are in the ABA database (www.brainatlas.org), which contains ~500 terabytes of data. To assist in the search, visualization, and analysis of expression patterns, informatics tools allow the user to query the database by anatomic structure in combination with expression coverage, intensity, and pattern. Approximately 80% of the genes assayed are expressed in the adult brain. While many of the expressed genes have widespread or non-restricted expression, only ~25% display regional and/or cell type specificity. Of these genes, it is rare to find highly localized expression or expression in only one structure. Another striking finding is that gene expression is heterogeneous in known anatomical regions, such as the hippocampus, suggesting further functional divisions within defined cytoarchitectural units. In the cortex, gene expression subdivides both the laminar and functional organization. Major cell types in each brain region can be defined by panels of genes. Overall, the expression patterns show a tremendous diversity of cell types. It has been estimated that there are ~1,000 cell types in the brain; however, few of these have been defined transcriptionally. The ABA database is a resource that will assist in more global approaches to redefine functional cellular neuroanatomy in the mouse central nervous system. In addition, this high-throughput in situ hybridization platform can be applied to investigate gene expression in various models of nervous system disease.

Left-Sided Cardiac Defects and Genetic Variants of the Folate-Homocysteine Metabolic Axis. *P.C. Paluru[1], M. Mei[3], W. Huang[1], J. Garbarini[1], L.E. Mitchell[3], E. Goldmuntz[1,2]* 1) Division of Cardiology, Children's Hospital of Philadelphia, Philadelphia, PA; 2) University of Pennsylvania, School of Medicine, Philadelphia, PA; 3) Institute of Biosciences and Technology, Texas A&M University System Health Science Center, Houston, TX.

Congenital heart disease (CHD) is the most common major birth defect. Left-sided cardiac defects (LSCD) account for at least 10% of all CHD recognized at birth, and include valvar aortic stenosis, coarctation of aorta, and hypoplastic left heart syndrome. The etiology of LSCD is poorly understood but is likely to result from complex interactions between environmental and genetic factors. Several studies suggest that maternal periconceptional intake of folic acid decreases the risk of CHD, but relatively few studies have evaluated the contribution of common variants in genes in the folate-homocysteine metabolic axis to the etiologic risk of LSCD. We hypothesized that such variants would be associated with the risk of LSCD and that such variants could exert their effect through either the maternal or the embryonic genotype. Consequently, we performed family-based association studies to determine whether five common variants in four genes whose products participate in the folate-homocysteine methylation process are associated with the risk of LSCD. We genotyped 367 probands with LSCD and one or two parents for the C667T and A1298C variants of MTHFR (5,10-methylene tetrahydrofolate reductase), A2756G variant of MTR (methionine synthase), A66G variant of MTRR (methionine synthase reductase) and 844Ins68 variant of CBS (cystathionine -synthase). A log-linear approach was used to test for associations between LSCD and both the maternal and embryonic genotypes for each variant. Only one of these variants was found to be significantly associated with the LSCD. Specifically, the maternal MTR genotype was significantly associated with the risk of LSCD in offspring (uncorrected p-value = 0.01). This finding warrants further investigation in larger and independent samples. The association between LSCD and additional genetic variants of the folate-homocysteine metabolic axis is under further investigation.

A genealogical assessment of heritable predisposition to asthma mortality. *C.C. Teerlink, L.A. Cannon-Albright*
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Asthma is a multi-factorial disease; genetic factors are suggested but poorly defined. We have studied an extreme asthma phenotype (defined as death as a result of asthma) and describe the familiality of Utah asthma deaths observed. We examined evidence for a heritable component to asthma mortality using a unique data resource consisting of Utah death records (1904-present) linked to a Utah genealogy. Cases were defined as individuals with death records listing asthma as a cause of death (n = 1,436), who also had at least 3 generations of genealogy data. The genealogical index of familiality (GIF) statistic was used to compare the average relatedness of individuals dying from asthma to the expected relatedness in the population. Expected relatedness was estimated in 1000 sets of randomly selected, matched controls. We observed a significantly higher than expected average relatedness in individuals dying from asthma ($p < 0.001$). The excess relatedness had contributions from close relatives and from relatives as distant as second cousins. We also estimated the relative risk of asthma death in relatives of individuals dying from asthma. We observed a significantly increased risk of asthma death for first-degree relatives of cases (RR = 1.55, $p = 0.006$) and for second-degree relatives of cases (RR = 1.30, $p = 0.011$). These results suggest a heritable component to asthma mortality, in addition to an environmental component.

Comprehensive genetic study of an SRY-negative, XX male. *Y. Wang[1], J.E. Martinez[1], T.-L. Lee[2], R. Quimbo[3], C. Tuck-Muller[1], W.-Y. Chan[2], C. White[4], R. Del Fierro[5], W. Wertelecki[1], T.-J. Chen[1]* 1) Dept. Med. Genetics, University of South Alabama, Mobile, AL; 2) Laboratory of Clinical Genomics, NICHD, NIH; 3) Division of Pediatric Pathology, SUNY Downstate Med Center, NY; 4) Urology Associates of Mobile, Mobile, AL; 5) Mobile Diagnostic Center, Mobile, AL.

SRY is a well-known sex-determining factor but the pathway downstream from SRY remains largely mysterious. Several genes have been proposed for this function, including SOX9, SOX10, and DAX1. Comprehensive genetic analysis of the rare SRY-negative, XX male patients may yield clues to dissecting this pathway. The patient in this study is a 20 yr old WM who was born with hypospadias. Several surgical procedures were needed to repair this abnormality. Physical examination showed evidence of hypogonadism. The right testicle appeared small and the left had some degree of epididymal tenderness. A left testicular biopsy revealed malformed seminiferous tubule without spermatogenesis or germ cells. Ovarian-type stroma with a rare focus of luteinized stromal cells was also noted. No disproportions or dysmorphisms were noted. Chromosome analysis showed a 46, XX karyotype. A Y chromosome painting probe was used to detect the translocation of Y material to another chromosome. Fluorescent signals were only present on the distal Xp arms in pseudoautosomal regions, but not on other chromosomes. PCR analysis of SRY and AZF regions was negative from DNA samples isolated from blood and paraffin-embedded gonadal tissue. The combined results from FISH and PCR analysis confirmed the patient to be an SRY negative, XX male and ruled out gonadal mosaicism. X-chromosome inactivation studies on blood DNA showed a random inactivation pattern similar to normal female controls. No duplication was found on the 17q23-25 and 22q11-13 regions containing the SOX9 and SOX10 genes using STR markers. No mutation was detected in the entire coding region of DAX1 gene by direct sequencing. These results suggest that development of the male phenotype in this patient may result from defects in other as yet unknown genes in the sex-determining pathway. Further genetic analysis of the expression profiling is undergoing.

High-density QTL mapping for loci influencing gene expression patterns in entire biochemical pathways. *M.A. Zapala, J. Wessel, N.J. Schork* Polymorphism Research Laboratory, Department of Psychiatry, UCSD School of Medicine, La Jolla, CA.

Associating a set of SNPs to gene expression levels as phenotypes has been useful in identifying transcriptional regulatory networks. However, these studies suffer from two potential drawbacks. The first concerns the number of test statistics performed, since thousands of gene expression signals are being tested against thousands of SNP markers creating a potential for false-positive results. The second issue is that the biological meaning of a cis or trans association is difficult to discern. We have taken a multivariate approach to gene expression SNP associations in which the similarity/dissimilarity of the gene expression of a group of genes related to the same pathway are associated with the SNP markers. In this way, SNPs that perturb the expression of an entire pathway are identified. We have adapted and applied a novel analysis technique developed by us for use in other gene expression analysis contexts to whole genome association studies of pathway gene expression data. To showcase the method, we analyzed publicly-available gene expression data gathered on hematopoetic stems cells isolated from 22 recombinant inbred BXD mice on which 1093 unique SNPs from the Wellcome Trust CTC mouse strain SNP genotype set are available. Genes were grouped into KEGG pathways based on their Entrez ID. The pathway showing the lowest p-value ($p\text{-value} < 0.000001$) and largest proportion of variation explained (0.64) was the Complement and Coagulation Cascade. The association peak was located on Chromosome 13 at the Factor II receptors (*F2r* and *F2rl2*) locus, also known as the Thrombin receptors (PAR-1 and PAR-3), which play absolutely critical roles in the signal transduction of the coagulation cascade. This pathway-oriented approach to gene expression SNP associations reduces the number of test statistics and identifies genetic variation that may have effects throughout an entire pathway.

Mutation Positive and Mutation Negative Cowden and Bannayan-Riley-Ruvalcaba Syndrome Patients and Normal Controls Defined by Distinct 10q-Haplotypes. *M.G. Pezzolesi[1], Y. Li[2], X.P. Zhou[1,3], R. Pilarski[3], L. Shen[2], C. Eng[1]* 1) Cleveland Clinic Genomic Med Inst, Cleveland, OH; 2) Div of Biostats, The Ohio State Univ, Columbus, OH; 3) Hum Cancer Genetics, The Ohio State Univ, Columbus, OH.

PTEN is a tumor suppressor gene frequently mutated in sporadic and heritable human cancers. Germline mutations are associated with a number of clinically distinct heritable cancer syndromes referred to as the *PTEN* Hamartoma Tumor Syndrome (PHTS). Despite a lack of genetic heterogeneity, specific germline *PTEN* mutations have yet to be identified in 15% to 50% of PHTS patients. In order to better understand the genetic contribution of this locus, we set out to characterize its haplotype architecture utilizing a case-control haplotype-based approach in 94 normal control and 353 PHTS samples with various germline *PTEN* mutation status. We found the *PTEN* locus to be characterized by 3 distinct haplotype blocks of length 33kb, 65kb, and 43kb, respectively. Comparisons of the haplotype distributions for all blocks differed significantly among PHTS patient groups and controls ($P = 0.010$, <0.001, and <0.001, respectively). Rare haplotype blocks and extended haplotypes account for 2- to 3-fold more PHTS chromosomes compared to control chromosomes. *PTEN* mutation negative patients were strongly associated with blocks spanning upstream of *PTEN* and including the genes first intron ($P = 0.0066$). One rare block spanning this region was only observed in 8 patient samples, including 7 with previously undetected mutations. Additionally, the haplotype profiles of PHTS patients with known mutations/variations appear to contribute to the phenotypic complexity of this syndrome. Our efforts also enabled us to identify the first germline *PTEN* deletion in a patient with a clinical diagnosis of Cowden Syndrome (previously only observed in patients with Bannayan-Riley-Ruvalcaba Syndrome). Taken together, these data suggest that specific *PTEN* haplotypes and rare alleles can underlie the disease etiology in these patient populations, constitute low-penetrant, modifying loci, and may harbor pathogenic variant(s) which have escaped detection by standard *PTEN* mutation scanning methodologies.

Unequal Distribution Of DNA Methylation Within A DNA Repeat Array Linked To Facioscapulohumeral Muscular Dystrophy. *L. Qi, K. Jackson, M. Ehrlich* Department of Biochemistry, Tulane University, New Orleans, LA.

The D4Z4 array, which is associated with facioscapulohumeral muscular dystrophy (FSHD), consists of tandem 3.3-kb repeat units at 4q35 and 10q26. It usually contains 10-100 repeat units, and when an array at 4q35 has less than 10 units, FSHD almost always results. We studied methylation of three types of CpG-containing sites, *Hpy*CH4IV (6 sites per 3.3 kb), *Bst*UI (37 sites per 3.3 kb), and *Hpa*II (71 sites per 3.3 kb) within the whole array *vs*. at the proximal end of the array by blot hybridization. First, we used a D4Z4 hybridization probe under conditions that monitor all the D4Z4 repeat units, but not cross-hybridizing sequences. The examined sites were largely, but incompletely, methylated within the bulk of the array in normal somatic tissues and hypermethylated or hypomethylated in many ovarian carcinomas and Wilms tumors. We next tested methylation of these sites just at the proximal end (within the terminal 0.9 kb) of the D4Z4 array by using a probe from a sequence adjacent to the beginning of the array (p13E-11). Surprisingly, the end of the array exhibited much less methylation than the bulk of the array in somatic controls. Even cancers that were very hypermethylated at examined sites within the array (more than 97% of the sites methylated) displayed no hypermethylation at the proximal end of the array and were mostly unmethylated there. This D4Z4 position-dependent methylation cannot be attributed to differences in sequence because D4Z4 has an extremely conserved sequence throughout the array including at its proximal end. Therefore, the position-dependent differences in methylation in D4Z4 in both control tissues and cancers are likely to reflect an effect of chromatin conformation on the DNA methylation apparatus. These results give us new insight into the establishment of DNA methylation patterns in normal and malignant tissues. Our study may also help elucidate the relationship of D4Z4 chromatin structure to FSHD. (Supported in part by NIH grant R01 NS048859).

A Trans-Splicing strategy corrects the defective splicing of the Spinal Motor Neuron 2 (SMN2) transcripts. *M. Shababi[1], T.H. Coady[1], G. Tullis[2], C.L. Lorson[1,3]* 1) Veterinary pathobiology, University of Missouri, Columbia, MO; 2) Ophthalmology, Boston university; 3) Molecular Microbiology and Immunology, university of Missouri, Columbia.

Spinal Muscular Atrophy (SMA) is an autosomal neurodegenerative disorder characterized by loss of the motor neurons in the spinal cord. SMA is caused by the homozygous deletion or mutation of the Survival Motor Neuron 1 (SMN1) gene. A nearly identical homolog of SMN1 (SMN2) exists only in humans; however, it cannot compensate for the loss of SMN1 due to a single nucleotide exchange at the beginning of exon 7 that alters its splicing pattern. SMN1 predominantly produces full-length transcript resulting in functional SMN protein, whereas SMN2 expression gives rise to exon 7-deleted transcripts and therefore a truncated and unstable SMN protein. Since, SMN2 has the same protein coding capacity as SMN1 and is retained in all SMA patients, this disease is desirable for therapeutic strategies that target SMN2 and correct its splicing defect. Here, we have developed a trans-splicing strategy to increase incorporation of exon 7 in the SMN2-derived transcripts. Trans-splicing system involves exogenously delivering a trans-splicing RNA molecule that can interact with the defective pre-mRNA and alter its splicing. This molecule contains an anti-sense sequence binding to the target, a trans-splicing domain to interact with the mutant pre-mRNA, and intact coding sequence of exon 7 that will be incorporated in the SMN2 transcript. Several trans-splicing RNA molecules were initially delivered and expressed from a plasmid backbone. The introduction of these plasmids into the cells re-directed the splicing of SMN2 from a SMN2 mini-gene as well as the endogenous transcript. These plasmids were eventually developed into recombinant AAV vectors and introduced into SMA patient cells. Infection of the severe SMA patient cells with these vectors, expressing the trans-splicing RNA, increased the levels of the full length SMN transcript and elevated the level of the total SMN protein.

A new autosomal dominant distal arthrogryposis syndrome characterized by plantar tendon contractures in large Utah kindred maps to 2q. *D.A. Stevenson[1], R. Toydemir[2], K. Swoboda[1, 3], H. Coon[4], M. Bamshad[5]* 1) Dept of Pediatrics, University of Utah, Salt Lake City, UT; 2) Dept of Human Genetics, University of Utah, Salt Lake City, UT; 3) Dept of Neurology, Univeristy of Utah, Salt Lake City, UT; 4) Dept of Psychiatry, Neurodevelopmental Genetics Program, University of Utah, Salt Lake City, UT; 5) Dept of Pediatrics, University of Washington, Seattle, WA.

The distal arthrogryposis (DA) syndromes are a distinct group of disorders characterized by contractures of two or more different body areas. More than a decade ago, we revised the classification of DAs and distinguished several new syndromes. This classification facilitated the identification of nearly half a dozen genes (i.e., *TNNI2*, *TNNT3*, *MYH3*, *MYH8*, and *TPM2*) that encode components of the contractile apparatus of fast-twitch myofibers and when defective cause DA. We now report the characterization of a novel DA disorder in a large five-generation Utah family in which plantar tendon shortening was transmitted among 14 affected individuals in an autosomal dominant pattern. Contractures of hips, elbows, wrists, and fingers varied in severity among affected individuals. All affected individuals had normal neurological examinations; electromyography and creatinine kinase levels on selected individuals were normal. We have tentatively labeled this condition distal arthrogryposis type 10 (DA10). A genome-wide linkage scan showed a maximum LOD score of 3.96 at marker D2S364 on chromosome 2q near a region containing several genes that encode contractile proteins.

The effect of enzyme replacement therapy with imiglucerase on bone mineral density in type 1 Gaucher disease.

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Purpose: To determine the effect of enzyme replacement therapy (ERT) with imiglucerase on bone mineral density (BMD) in patients with type 1 Gaucher disease (GD) and assess fracture prevalence. **Methods:** Lumbar spine BMD data as assessed by dual energy x-ray absorptiometry (DXA) from all males 18-70y and females 18-50y with type 1 GD enrolled in the ICGG Gaucher Registry were analyzed; 160 untreated patients and 342 patients treated with ERT were included. Linear mixed effects models were used to analyze Z-scores over time and assess dose-response relationships. The reported prevalence of fractures and estimated prevalence of osteoporosis by age and sex were calculated. **Results:** DXA Z-scores in the untreated group were below normal at baseline (y intercept = -0.80 Z-score units, $p<0.001$) and remained about one standard deviation below normal over time (slope = -0.010 Z-score units/year, $p=0.68$). DXA Z-scores for patients on ERT were below normal at baseline (y-intercept = -1.17 Z-score units, $p<0.001$), but improved significantly over time (slope= +0.132 Z-score units/year for ERT 60U/kg/2wk, $p< 0.001$). A significant dose-response relationship was noted for ERT, with the slopes for 15, 30, and 60 U/kg/2wk of +0.064, +0.086, and +0.132 Z-score units/year, respectively. The BMD of patients with GD treated with ERT increased to within -0.12 (60U/kg/2wk), -0.48 (30U/kg/2wk) and -0.66 (15U/kg/2wk) standard deviations of the reference population after approximately 8 years. Estimated prevalence of osteoporosis of this GD population, if left untreated, ranged from about 10 to 30% in females, and 10 to 25% in males. Patients with GD had a much higher prevalence of fractures than expected, with >50% of women with GD at age 50 and men with GD at age 70 reporting the presence of vertebral fractures. **Conclusions:** ERT with imiglucerase significantly improves BMD in patients with GD. A normal (age- and sex-adjusted) BMD should be a therapeutic goal for patients with type 1 GD.

Identification of copy number variants in non-isolated congenital diaphragmatic hernia (CDH+). *D.A. Scott[1], M. Klaassens[3,4], A. Holder[1,6], K.P. Lally[5], C. Fernandes[2], A. de Klein[3], D. Tibboel[4], B. Lee[1,6]* 1) Dept Mol & Hum Genet, Baylor Col Med, Houston, TX; 2) Dept Peds, Baylor Col Med, Houston, TX; 3) Dept of Clin Genet, Erasmus Med Cent, Rotterdam, the Netherlands; 4) Dept of Paed Surg, Erasmus Med Cent, Rotterdam, the Netherlands; 5) Dept of Ped Surg, Univ of Texas Med School, Houston, TX; 6) Howard Hughes Medical Institute.

Non-isolated congenital diaphragmatic hernia (CDH+) is a common cause of severe newborn respiratory distress and is associated with high mortality and significant morbidity. The development of improved methods for identifying copy number variants in CDH+ is of particular importance since many chromosomal anomalies may not be detected using current screening methods and detection of a chromosome anomaly can impact prognosis, the selection of additional screening tests, treatment decisions, and recurrence risk counseling. Identifying more subtle copy number variation will also be an increasingly important approach to studying disease causality and susceptibility. To determine if the combination of oligonucleotide-based array comparative genome hybridization (aCGH) and quantitative real-time PCR is an effective method of identifying and confirming clinically relevant chromosomal deletions and duplications we screened a cohort of 26 CDH+ patients for de novo copy number variants. Critical breakpoints were also mapped using this combination of techniques. aCGH identified 105 putative copy number changes. Sixty-one of the 105 changes (58%) had been previously described in normal controls. Twenty of the remaining 44 changes (45%) were confirmed by real-time quantitative PCR or standard cytogenetic techniques. Seven de novo changes were identified in five patients, two of whom had previously normal G-banded chromosome analyses. We conclude that combination of oligonucleotide-based aCGH and quantitative real-time PCR is an effective method of identifying, confirming, and mapping clinically relevant copy number changes in patients with CDH+. This method is more sensitive than G-banded chromosome analysis and may find wide application in screening patients with congenital anomalies and/or mental retardation.

Unusual Presentation of Epidermal Nevus syndrome. *Y. Zarate, R. Hopkin* Div Hum Genetics, Cinn Children's Hosp Med Ctr, Cincinnati, OH.

A 35 year old female with a diagnosis of Neurofibromatosis type I since age 17 presented for a second opinion. At birth multiple café au lait spots were noted with lesions on the face and trunk. The skin lesions had evolved with increasing pigment and developing a raised verruciform surface. Multiple cosmetic surgeries resulted in little improvement as the lesions continued to progress. She developed pain in her hands and wrists eventually requiring surgery for carpel tunnel. She had also been diagnosed with spinal canal stenosis. Radiographs demonstrated multiple lucent lesions of the right ulna, and 3rd and 4th metacarpal bones. There was a 2 X 0.5 cm raised lesion on the anterior surface of the tongue. Skin examination in adulthood revealed multiple verruciform lesions with variable hyperpigmentation in addition to areas with multiple lentigines, but there were no typical café au lait spots. The lesions tended to follow the lines of Blaschko and were bilateral with some areas that crossed the midline. The final diagnosis is Epidermal Nevus Syndrome. There are no previous reports of carpel tunnel or spinal stenosis with this syndrome. Furthermore, we emphasize the overlap with Neurofibromatosis I, LEOPARD syndrome, and other phacomatosis especially early in disease progression.

Molecular Identification of Individuals with Spinal Muscular Atrophy through Newborn Screening to Benefit Clinical Trial Efficacy. *R.E. Pyatt, D.C. Mihal, T.W. Prior* Dept Pathology, Ohio State Univ, Columbus, OH.

Clinical trials are currently underway evaluating the first wave of potential therapeutics for Spinal Muscular Atrophy including phenylbutyrate and valproic acid. With the clinical course leading to the degeneration of anterior horn motor neurons, the window of time for effective administration of these agents may be short. Enrollment of children into these two trials is based on clinical diagnosis followed by confirmation of deletions status and determination of SMN2 copy number in our laboratory. However the time required for clinical and molecular diagnosis may exceed the lifespan of anterior horn cells particularly in individuals with Type I disease creating a cohort for therapeutic validation who may not be within that treatment window. We sought to examine then if new methodologies could be created for the detection of affected individuals in a more timely fashion. This would involve identification at birth and consequently necessitate that diagnostic testing be incorporated into newborn screening programs. We defined an assay to detect the 95% of affected individuals with homozygous deletions in SMN1 exon 7 using the Luminex micro-bead system and compared it to a similar assay previously developed by our lab on the Real-Time PCR platform using a series of 420 blood spots from affected, carrier, and normal individuals. Both chemistries displayed high sensitivity and specificity in this sample series, while the Real-Time platform processed 96 samples in 1.5 hours at a per sample cost of approximately $15 and the Luminex system took twice as long for the same number at a cost of $10. While both assays demonstrated similar effectiveness, the Luminex system was more cost effective compared to Real-Time PCR even with a longer protocol. The Luminex system also has greater expansion capabilities to incorporate the identification of small mutations seen in the 5% of affected who are compound heterozygotes or the addition of other disorders. We have shown that molecular technology exists for the effective and timely identification of newborns with Spinal Muscular Atrophy to hasten their enrollment in clinical trials and drug delivery.

Specific microRNA deregulation in follicular thyroid cancer & clinical implications. *F. Weber, R. E. Teresi, C. Eng*
Cleveland Clinic Genomic Medicine Inst, Cleveland, OH.

Follicular thyroid carcinoma (FTC) poses a diagnostic challenge due to its morphological and molecular similarities to the frequently occurring benign follicular adenoma (FA). In a pre-operative setting both are indistinguishable from each other and surgery is recommended. Micro-RNA's (miRNAs) are a new class of small, non-coding RNAs that may not only lend novel clues to FTC genesis but also help to identify novel diagnostic marker and molecular targets. For the latter process, a deregulated miRNA can orchestrate the aberrant expression of several hundred target genes. We performed a global miRNA and gene expression analysis in a total of 18 follicular thyroid carcinoma (FTC) and 12 benign follicular neoplasia (FA) using 2 high-density array platforms (custom miR-Chip and HG-U133A GeneChip) according to established protocols. Results were validated in independent sample sets by quantitative RT-PCR. In vitro experiments were performed to further characterize the functional effect of miRNA deregulation in thyroid cancer. 2 human miRNAs (miR-197, miR-346) were significantly overexpressed (1.6-2.0-fold, P0.005) in FTC than in FA. Potential miRNA target genes were cross-referenced with our data obtained from GeneChip experiments. A selected set of target genes was further validated, both in vivo and in vitro. Overexpression of miR-197 or miR-346 in HEK293T cells not only induced proliferation (~1.5-fold, p=0.003 and p=0.012) but also caused a ~2-fold transcriptional repression of their respective target genes. Silencing of endogenous miR-197 or miR-346 expression inhibited proliferation in thyroid cancer cell lines. In summary, we identified 2 miRNAs that are specifically overexpressed in FTC. Their deregulation directly affects the downstream transcriptome and cancer phenotype. These specific FTC-miR and downstream transcriptome profiles may be used as an accurate molecular diagnostic to differentiate FTC from FA preoperatively. In addition, interference with the deregulated miRNA expression in FTC will have a cascading effect on downstream target genes and could potentially reverse the malignant phenotype.

Rare case of vertical transmission of transposition of the great arteries. *C. Rigelsky, C. Eng* Genomic Medicine Institute, Cleveland Clinic, Cleveland, OH.

Current estimates suggest that transposition of the great arteries (TGA) has a low recurrence risk or is sporadic. Rare cases have been attributed to X-linked or autosomal genes. The majority of reports estimating recurrence risk for TGA investigate congenital heart defects (CHD) in siblings or other relatives of children born with TGA. These studies provide recurrence risks of between 2 and 10%. We report a father and son with TGA. The father, JB, is a 29 year old with a single functioning hypoplastic right ventricle, large VSD and TGA. JB has had numerous surgeries but remains cyanotic and has severe pulmonary stenosis. JBs condition has remained fairly stable but will probably require a heart transplant. His son, PB, was diagnosed prenatally. Deletion analysis for 22q11 was performed and was negative. After birth, he was found to have TGA with a small muscular VSD. An arterial switch procedure was performed and PB is doing well. Review by a medical geneticist revealed no dysmorphic features and his development has been normal. In an attempt to more accurately define recurrence risk for this family, a karyotype and microarray analysis were performed and both were normal. This case represents a rare occurrence of vertical transmission of TGA, and also provides a very difficult situation for genetic counseling. The current literature on this topic is potentially inadequate at estimating the risk of recurrence in this family. As more individuals with TGA and other complex CHD live into adult years, it is imperative for further studies to focus on their genetic etiology. It is likely that the number of cases of vertical transmission for complex heart defects will increase. This case highlights a growing area of need for further clinical and translational research as this area will likely become an increasing challenge for genetic counseling.

Satellite DNA Methylation Patterns by Hairpin Genomic Sequencing. *C. Shao, M. Ehrlich* Human Genetics, Tulane University School of Medicine, New Orleans, LA.

Both increased levels of DNA methylation in some parts of the genome and decreased levels in others are generally associated with human cancer. Most DNA methylation analyses do not address the issue of hemimethylation at CpG dyads because of technical difficulties. In addition, only genomic sequencing after DNA cloning reveals methylation patterns on individual DNA molecules. To better understand DNA methylation, including hemimethylation, in normal human tissues and cancers, we have used the new hairpin genomic sequencing method of C. Laird *et al*., which involves cleaving DNA with a restriction endonuclease, covalently linking DNA strands, denaturation, bisulfite treatment, PCR, cloning, and sequencing. We adapted this method to study a 0.2-kb subregion of satellite 2 from chromosome 1 after cleavage of DNA with *Bsm*I. First, we analyzed DNA from normal cerebellum, lung, and heart. We found symmetrical methylation and hemimethylation in 84 and 6.5% of CpG sites, respectively, in 56 DNA clones. The hemimethylation, which was seen in 50% of the clones, is not artifactual because its frequency was consistently higher than the frequency of bisulfite-resistant non-CpG Cs (overall, 2.1%). Most of the hemimethylated sites were dispersed in the examined region, and four clones had hemimethylated CpG dyads with opposite directionality. Although there was variety in the methylation patterns from clone to clone, 3 out of 13 CpG positions were symmetrically methylated in more than 90% of the clones. One CpG site, the least methylated one, was symmetrically methylated in only 41% of the clones. We characterized CpG methylation of satellite 2 DNA in ovarian epithelial tumors of different degrees of malignancy by Southern blotting, but the only suitable restriction site that was available was *Bst*BI. To better understand the nature of tumorigenesis-associated epigenetics, we will next use hairpin genomic sequencing to compare exact patterns of methylation in satellite 2 from ovarian carcinomas, low malignant potential tumors, and cystadenomas to that from somatic control tissues. (Supported in part by Louisiana Cancer Research Consortium).

Further confirmation of the polymorphism in a chromosome-specific interstitial telomere-like sequence (ITS) located at 22q11.2. *J. Yan, O. Samassekou, E. Bouchard, N. Bastien* Dept Pediatrics, Université de Sherbrooke, Sherbrooke, PQ, Canada.

Interstitial telomeric sequences (ITSs), telomere-like repeats at intrachromosomal sites, are common in mammals and consist of tandem repeats of the canonical telomeric repeat, TTAGGG, or a repeat similar to this. We previously revealed that the ITS in human chromosome region 22q11.2 is, in the sequenced genome database, 101 tandem repeats (909 bp) of the sequence TTAGGGAGG. Using the primed in situ labeling (PRINS) technique with primers against the canonical telomeric repeat (TTAGGG), we illuminated telomeric sites for all chromosomes and an ITS locus for chromosome 22 at q11.2. Using the TTAGGGAGG sequence, we designed PRINS primers that more efficiently and specifically illuminate the 22q11.2 ITS locus without illuminating telomeric and other ITS loci. We PCR-amplified DNA from 11 normal individuals using primers designed from sequences flanking the ITS locus. Our results demonstrated that the sizes of all PCR fragments are bigger than the 909 bp published in the genome database. A specific PCR pattern for each individual was also observed. In some individuals, the PCR fragments showed clear allelic differences and relations between the family members. Furthermore, DNA sequencing of some PCR fragments confirmed that the different PCR fragments indeed contain TTAGGGAGG repeats. As seen in many reports, the 22q11.2 region is associated with hot spots for disease-related chromosome breaks for multiple disorders, such as DiGeorge syndrome and chronic myeloid leukemia. We describe our findings that the ITS at 22q11.2 is in the same area of, and proximal to the common rearrangements region of multiple disorders. We suggest that the ITS might regulate DNA repair process in this area to protect the chromosome from more serious damage. The population polymorphic ITS locus at 22q11.2 could become a useful marker for linkage analysis, for forensic applications, and for the detection of genetic instability in tumors.

Analysis of the clinical relevance of intron variants in BRCA1 and BRCA2. *M.P.G. Vreeswijk[1], J.N. Kraan[1], H.v.d. Klift,[1], C.J.van Asperen[1], G.R. Vink[1], C.J. Cornelisse[2], P. Devilee[1,2]* 1) Dept. Human and Clinical Genetics, Center for Human and Clinical Genetics, LUMC, Leiden, Netherlands; 2) Dept. Pathology, LUMC, Leiden, the Netherlands.

Germ-line mutations in BRCA1 and BRCA2 confer a high risk to breast and/or ovarian cancer. Whereas mutations causing frameshifts and premature stop codons are unambiguously defined as pathogenic, an increasing number of variants has been identified which cannot be readily distinguished as either disease-associated mutations or benign polymorphisms. These socalled unclassified variants include variants that are located in the intronic sequences of BRCA1 and BRCA2. The purpose of this study was to identify the effect of six of these variants on RNA splicing in order to differentiate between pathogenic or neutral variants.

For the IVS2-6 T>A variant in BRCA1, sequence analysis of cDNA from fibroblasts derived from a carrier of the variant showed the introduction of four bases from intron 2, leading to an out-of-frame fusion of BRCA1 exon 2 and 3.

A coding polymorphism in exon 16 was used to show monoallelic BRCA1 expression in a carrier of the IVS16+5 G>T variant. This was confirmed with the use of a primer specific for the wildtype transcript. Inhibition of nonsense mediated decay (NMD) revealed an additional fragment and sequence analysis showed the insertion of 65 nucleotides from intron 16.

RNA analysis from a carrier of an intronic variant in BRCA2 (IVS21+5 G>A) revealed the absence of transcript from the variant allele. Analysis after inhibition of NMD showed an additional transcript, containing an insertion of 46 nucleotides from intron 21.

Since these intronic variants (IVS2-6 T>A; IVS16+5 G>T in BRCA1 and IVS21+5 G>A in BRCA2) result in aberrant splicing of the mRNA transcript, they are considered to be clinically significant. Two other variants in BRCA1 (113 G>A; IVS4-15delTTTC) and one variant in BRCA2 (IVS2-7 T>A) did not show any effect on RNA splicing and can therefore be considered to be neutral alterations.

Single Cell Whole Genome Amplification Method. *D. Vassar-Nieto, C. Brown, C. Brueck, E. Mueller* Research & Development, Genomics, Sigma-Aldrich Research Biotech, St. Louis, MO.

Analyzing the genomic material in a single cell has long been desirable but heretofore unachievable due to the miniscule amount of DNA available for analysis. We have used a new and sensitive method for whole genome amplification, GenomePlex Single Cell WGA kit, to perform genomic analysis on a single cell. The process amplifies the DNA from a single cell a million fold, allowing the genetic analysis of the ultimate biological unit and opening the secret to maturation, regeneration, and genetic diseases.

In this poster we show the utility of this method with a variety of single cell samples. We show that DNA generated by this process can be used for analysis in various downstream applications such as comparative genomic hybridization and SNP analysis. Additionally we demonstrate the detection limitations of this method by comparing our results to those from unamplified genomic DNA. Finally, we utilize this method to examine the genetic variance between individual cells in an originally clonal culture of the lymphoma cell line U937.

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Detection of Philadelphia chromosome (Ph) in a patient with no evidence of leukemia: Further evidence of a changing paradigm? *G. Velagaleti, J. Northup, K. Suleman, N. Panova, D. Hudnall* Dept Pathology, Univ Texas Medical Branch, Galveston, TX.

The consistency with which the Ph chromosome is seen in CML lead to two valid assumptions: (1) all bona fide CML patients have a BCR-ABL fusion gene in their malignant cells; (2) finding of a BCR-ABL fusion gene in hematopoietic cells from an individual is a sign of impending/overt CML. Here, we report evidence that mere presence of Ph chromosome and/or BCR-ABL fusion gene, even at high proportion hitherto unreported, does not result in malignant disease. A 33-year old, Caucasian with diagnosis of Hodgkin lymphoma (HL) was evaluated for bone marrow involvement. Bone marrow biopsy showed no evidence of malignancy. However, chromosome analysis showed the characteristic Ph chromosome in 70% of the marrow cell metaphases. FISH studies with dual fusion BCR-ABL probe showed 68% of the interphase nuclei with one fusion signal (1R/1G/1F). Metaphase FISH analysis showed that the fusion signal on der(9) is not present, thus indicating a concomitant deletion. Since, Ph chromosome is not associated with HL, unstimulated blood lymphocytes were karyotyped which showed 38% of the cells with Ph chromosome. FISH studies on frozen lymph node tissue from initial biopsy and the peripheral blood also showed only one fusion signal similar to the bone marrow in 17% and 31% of the cells, respectively. RT-PCR analysis with nested primers showed the typical p210 transcript in the peripheral blood. Since the initial diagnosis in 10/05, his white count has ranged from low to high, but with no morphologic evidence of CML. The occasional increase in his white count is attributed to treatment with GCSF to alleviate leucopenia resulting from chemotherapy for the HL. The patient showed favorable response to chemotherapy and is currently shows no evidence of HL. Low-level BCR-ABL fusion gene expression is seen in normal individuals. However,this is the first report of an individual without CML with a sustained high percentage of hematopoietic cells with BCR-ABL fusion gene. Our results suggest that additional oncogenic events are essential for the development of CML.

Ameloblastin (AMBN) is Associated with Low Caries Experience. *K. Deeley, E.K. Rose, C.A. Brandon, J.M. Resick, S. Wendell, M.L. Marazita, A.R. Vieira* Oral Biology and Center for Craniofacial and Dental Genetics, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA.

Twin studies and animal models have provided compelling evidence for the existence of a genetic component in caries susceptibility. However caries is a disease greatly influenced by environmental factors such as oral hygiene, diet and fluoride exposure. Controlling for these factors in humans is extremely difficult. One approach is to study populations with similar cultural habits and socio-economic status (i.e. with similar dietary habits, hygiene habits, access to the dentist, and fluoride exposure). For the present study, we used 217 DNA samples collected from individuals and their family members that sought medical treatment for orofacial clefts and other illnesses during the 2006 Children of the Americas medical mission in Tiquisate, Guatemala. All individuals received an oral exam and DMFT scores were obtained. Unrelated individuals with very low caries experience (DMFT scores 2) were selected as controls (N=40), while unrelated individuals with very high caries experience (DMFT 9) were selected as cases (N=40). TaqMan chemistry was used to genotype SNP markers in selected candidate genes that influence enamel formation. Genes influencing enamel development could influence both bacterial adherence and/or the resistance of enamel to acid pH. Six markers were typed in five candidate genes: AMBN (Ameloblastin; hCV496502), AMELX (Amelogenin; hCV2190967), ENAM (Enamelin; rs3796704), TUFT1 (Tuftelin-1; rs3790506 and rs2337360), and TFIP11 (tuftelin interacting protein 11; rs134136). Individuals with very low caries experience presented the less common AMBN hCV496502 T allele more often than individuals with very high caries experience ($p=0.04$). Our results suggest that variation in AMBN may be a protective factor against caries susceptibility. It appears that the approach of comparing individuals with extremely distinct caries experiences is valuable for decreasing the potential influence that different environmental factors may impose to genetic studies of caries. This research is supported by NIH Grants R01-DE016148, R21-DE016930, P50-DE016215, and R01-DE014899.

Modifier genes substantially influence variability in severity of CF lung disease, independent of CFTR genotype and variation in body mass index. *L.L. Vanscoy[2], S. Blackman[2], J.M. Collaco[2], A. Bowers[1], K. Naughton[1], G.R. Cutting[1]* 1) Institute of Genetic Medicine, Johns Hopkins School of Medicine, Baltimore, MD; 2) Department of Pediatrics, Johns Hopkins School of Medicine, Baltimore, MD.

To estimate the contribution of genetic factors other than CFTR genotype to variation in CF lung disease severity, we compared the degree of intra-pair similarity in longitudinal measures of FEV1 in MZ twins to DZ twins and siblings before and after adjusting for nutritional status. To minimize variation due to environmental factors, we analyzed pulmonary function tests (PFTs) obtained while subjects were living with an affected twin or sibling. FEV1 was converted into percent predicted values (FEV1%pred) and CF-specific percentiles for FEV1 (FEV1CF%). FEV1%pred at 20 years (BayesFEV1%pred) was predicted using mixed modeling and Bayes estimation (Schlucter, et al) and average FEV1CF% (AvgFEV1CF%) was calculated using the best quarterly FEV1 measurement from all available PFT data. Nutritional status was calculated using the average of all available body mass index (BMI) z-scores (AvgBMIZ) for each study subject. Intra-pair similarity was determined using correlation coefficients. 47 MZ twin pairs, 10 DZ twin pairs, and 231 sibling pairs with CF were analyzed. CFTR genotype distribution was 51.4% F508/F508, 39.3% F508/other, and 9.2% other/other. All pairs of twins and siblings had identical CFTR genotypes. BayesFEV1%pred and AvgFEV1CF% showed a high degree of correlation for MZ twins (0.82-0.91; $p<0.0001$) and moderate similarity for DZ twins and siblings (0.50-0.64; $p<0.001$). After adjusting for AvgBMIZ, intra-pair correlations for BayesFEV1%pred and for AvgFEV1CF% among MZ twins were unchanged (0.80-0.93; $p<0.0001$), but were decreased for DZ twins and siblings (0.39-0.58; $p<0.05$). Estimates of heritability ranged from 0.54 to 0.64 and increased to 0.70 to 0.82 after adjusting for nutritional status. Controlling for non-genetic variation by studying twins and siblings living together at home enables detection of substantial influence of modifier genes upon variation in CF lung function that is independent of variation in CFTR genotype and body mass index.

Follow-up linkage mapping and family-based association analyses of SNPs in *NTRK2* in the candidate region at 9q22 in the NIMH Alzheimers Disease Genetics Initiative cohort. *R.T. Perry[1], H. Wiener[1], L.E. Harrell[2], D. Blacker[3], R.E. Tanzi[4], L. Bertram[4], S.S. Bassett[5], R.C.P. Go[1]* 1) Dept Epidemiol and Internat Health, UAB, Birmingham, AL; 2) Dept Neurology, UAB, Birmingham, AL; 3) Dept Psychiatry, MGH, Boston, MA; 4) Genetics and Aging Unit, Harvard Medical School, Boston, MA; 5) Dept Psychiatry, JHU, Baltimore, MD.

Purpose: Other than the *APOE* peak at 19q13, the 9q22 region was identified in our original genomic scan as the candidate region with the highest multipoint lod score (MLS) in the subset of late onset Alzheimers Disease (AD) families (MLS = 2.9 at 101 cM) from the NIMH Genetics Initiative sample. We genotyped an additional 12 microsatellites in this region and multipoint analysis showed an increase in the peak MLS from 2.9 to 3.8 at 95 cM near marker D9S1815 with further narrowing of the region from 21.5 cM to 6.6 cM (92.2-98.8 cM). SNPs in the Ubiquilin1 gene (*UBQLN1*), located at 83.3 cM, have been reported to be significantly associated to AD and that some of the linkage signal may have been due to *UBQLN1* (Bertram et al, 2005). We performed additional analyses conditioned on *UBQLN1* genotype and reject the hypothesis that *UBQLN1* could account for all the linkage signal at 9q22. Therefore, additional AD succeptibility genes are likely to be located here. One possible candidate gene is the neurotropic tyrosine kinase receptor 2 (*NTRK2*) gene, located at 84.4 cM. It codes for the receptor for brain-derived neurotrophic factor (BDNF) and is involved with the regulation of both short-term synaptic functions and long-term potentiation of brain synapses. We genotyped 14 SNPs (13 tagSNPs) located in 8 of the 20 haploblocks covering the gene to test for association to AD. **Methods**: We used capillary electrophoresis, TaqMan assay, and family-based association testing (FBAT) to analyze these 14 SNPs in *NTRK2* in the NIMH AD sibling dataset (n=481 families). **Results**: A 3 SNP haplotype located in a functional area of *NTRK2* was significantly associated with AD (p=0.005). **Conclusion**: Follow-up linkage data and preliminary FBAT analyses of SNPs in *NTRK2* supports the presence of more than one AD succeptibility gene located in the 9q22 candidate region.

Combined phenotype of Wolf-Hirschhorn and Beckwith-Wiedemann Syndromes in a female with der(4)t(4;11)(pter;pter). *A. Sathienkijkanchai[1], N.H. Robin[1], S. Prucka[1], J. Sanford Biggerstaff[4], J. Komorowski[3], R. Andersson[3], C. Bruder[1,2], A. Piotrowski[1,2], T. Diaz de Stahl[2], J.P. Dumanski[1,2], A.J. Carroll[1], F.M. Mikhail[1]* 1) Dept. of Genetics, Univ. of Alabama at Birmingham, AL; 2) Dept. of Genetics and Pathology, Uppsala Univ., Sweden; 3) Linnaeus Centre for Bioinformatics, Uppsala Univ., Sweden; 4) Dept. of Lab. Medicine, Sacred Heart Medical Center, WA.

We report an 8-month-old female with a novel unbalanced chromosomal rearrangement, consisting of a terminal deletion of 4p and a paternal duplication of terminal 11p. Each of these is associated with the well known clinical phenotypes of Wolf-Hirschhorn syndrome (WHS) and Beckwith-Wiedemann syndrome (BWS), respectively. She presented for clinical evaluation of dysmorphic features, developmental delay, atrial septal defect, and left hydronephrosis. There was no family history of miscarriages or birth defects. A 500 band karyotype was normal, but subtelomeric analysis revealed a der(4)t(4;11)(pter;pter). Using the *WHSC1* FISH probe (4p16.3), the der(4) showed no hybridization signal, indicating deletion of the WHS critical region. Furthermore, an *IGF2* FISH probe (11p15.5) showed two signals on the chromosome 11 homologues and a third on the short arm of der(4), indicating duplication of at least BWS critical region imprinted domain (ID) 1. Parental karyotype revealed that her father carried a cryptic balanced t(4;11)(pter;pter). The patients final karyotype was: 46,XX.ish der(4)t(4;11)(pter-,*WHSC1*-,pter+,*IGF2*+)pat. A 32K BAC array CGH chip demonstrated that WHS critical regions 1 and 2 were deleted, and that BWS IDs 1 and 2 were duplicated on der(4). Our patient manifests findings of both WHS (a growth retardation syndrome) and BWS (an overgrowth syndrome). Features of WHS included poor weight gain, broad forehead, hypertelorism, and micrognathia, whereas features of BWS included facial hemihyperplasia, earlobe creases, and renal anomalies. However, many clinical features of both WHS and BWS were absent, possibly due to the opposing phenotypes of these two disorders. To our knowledge, this is the first report of a child with this combined phenotype of WHS and BWS.

Chromatin DNaseI Sensitivity of the Facioscapulohumeral Muscular Dystrophy-Linked D4Z4 Repeat Array and an Adjacent Sequence. *K. Tsumagari[1], S. Hauschka[2], M. Ehrlich[1]* 1) Human Genetics, Tulane Medical School, New Orleans, LA; 2) Biochemistry, Univ. of Washington, Seattle, WS.

Facioscapulohumeral muscular dystrophy (FSHD) is a unique dominant disorder involving shortening of an array of tandem 3.3-kb repeats called D4Z4. This size-polymorphic array (1-100 repeat units) is present at both 4q35 and 10q26, but only a short 4q35 array (with 1-10 repeat units) is linked to FSHD. It has been proposed that array lengths in the normal range for 4q35 (11-100 repeat units) are heterochromatic while shorter arrays are not. We used in vivo assays for chromatin DNaseI sensitivity to study the chromatin structure of D4Z4 and its adjacent, non-repeated sequence, p13E-11. For fetal myoblasts, control fibroblasts, and FSHD or control lymphoblasts, we found conditions for DNaseI treatment of nuclei or lysolecithin-permeabilized cells that give differential DNaseI digestion of DNA standards. The standards were constitutively expressed genes, *HMBS* and *B2M*; genes unexpressed in the examined cell populations, *CST5* and *GHRHR*; and satellite 2, the constitutive heterochromatin standard. D4Z4, p13E-11, and the DNA standards give fragments of 1-to-3 kb after exhaustive digestion in vitro by *Sty*I. Degradation of these sequences by partial DNaseI digestion in vivo was quantitated by the phosphorimager-determined decrease in intensity of the corresponding *Sty*I fragment in a single rehybridized Southern blot for each sample. Initial results indicate that normal-length D4Z4 has a DNaseI sensitivity intermediate to that of constitutive heterochromatin and unexpressed genes. In contrast, for four of five tested cell cultures, including myoblasts, p13E-11, which is only 0.1 kb from the beginning of the D4Z4 array, was more sensitive to DNaseI than unexpressed genes. The fifth culture, one of the two FSHD lymphoblast cultures, had similar sensitivities to DNaseI for p13E-11 and unexpressed genes. Therefore, the p13E-11 sequence seems to have a much looser chromatin structure than the array itself, which may be important in the proposed looping between D4Z4 arrays at 4q35 and the currently unknown FSHD gene in cis. (Supported in part by NIH grant R01 NS048859).

A PEX7 Hypomorphic Mouse Model For Plasmalogen Deficiency Affecting the Lens and Skeleton. *R. Zhang[1], L. Chen[1], S. Scheper[1], R. Chaudhury[1], S. Steinberg[2], A. Moser[2], N. Braverman[1]* 1) McKusick-Nathans Institute of Genetic Medicine; 2) Kennedy Krieger Institute, Johns Hopkins Med Ctr, Baltimore, MD.

RCDP1 is a peroxisome biogenesis disorder clinically described by rhizomelia, punctate calcifications in epiphyseal cartilage, congenital cataracts and profound growth and developmental delay. It is caused by defects in PEX7, which encodes the receptor for a subset of peroxisomal matrix enzymes. The pathophysiology involves deficient plasmalogen (i.e. ether phospholipid) synthesis, although the underlying mechanisms are unknown. A few patients have milder phenotypes, residual plasmalogens in tissues and less severe defects in PEX7 that include alleles manifesting reduced PEX7 transcript. Considering the early lethality in RCDP1, we sought to recapitulate a milder phenotype and engineered a hypomorphic allele to reduce PEX7 transcript by introducing an intronic neomycin cassette. The PEX7-neo homozygotes are born in expected ratios, are fertile and have a normal life span. However, they are 70-80% as large as their littermates and 30% have visible cataracts by 3 months of age. PEX7 transcript levels are 1-5% of wild type in several tissues. RBC, brain and heart plasmalogen levels are 30-40% of their heterozygote littermates as measured by gas-liquid chromatography. Immunoblots of fibroblast lysates show reduced amounts of unprocessed thiolase and absence of the plasmalogen biosynthetic enzyme, AGPS, consistent with a defect in the import of PEX7 ligands. Whole mount skeletal staining at P1 indicates a proportionately smaller skeleton with delay in ossification centers; x-rays of adult mice also show a skeleton smaller than their heterozygote littermates. Routine histology of the eye is normal except for the lens, showing posterior proliferation of epithelial cells and enlarged, pale lens cells in the cortex or throughout the lens. Thus, the skeleton and lens are sensitive markers for plasmalogen deficiency and these mice are now being investigated to determine how these lipid compounds function in these tissues. Furthermore, the progression of cataracts in regards to lens phospholipid deficiency is of interest as a potential factor in common age-related cataract formation.

RMRP Analysis in Patients with Variable Clinical Presentations of Cartilage Hair Hypoplasia (CHH). *S. Vidal-Cardenas[1], B. Loeys[2], V. McKusick[1], C. Francomano[3], N. Braverman[1]* 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins Medical Ctr, Baltimore, MD; 2) Ctr Medical Genetics, Ghent Univ Hosp, Belgium; 3) Harvey Institute of Human Genetics, Greater Baltimore Med Ctr, Baltimore, MD.

CHH is an autosomal recessive, panethnic, pleiotropic disorder characterized by short-limbed dwarfism, metaphyseal dysplasia, sparse hair, anemia, immune deficiency and predisposition to malignancy. CHH is caused by defects in the RMRP gene, which encodes the untranslated RNA component of the mitochondrial ribonucleoprotein (MRP) complex, involved in cleavage of RNA in mitochondrial DNA synthesis and nucleolar cleaving of pre-rRNA. Mutations in RMRP impair ribosome assembly and alter cell cycle regulation, leading to decreased cell growth. A founder mutation, 70 A>G, underlies CHH in the Finnish and Amish. Worldwide, a remarkable 60+ unique mutations and 15+ polymorphisms have been reported in the 400 bp stretch of RMRP genomic sequence, a remarkably high frequency of polymorphic residues compared with the genome average. We identified RMRP mutations in 5 North American probands with various presentations of CHH. We amplified RMRP genomic DNA, directly sequenced the products and cloned these to show their chromosomal arrangements. 3 had classic phenotypes and were (1) homozygous for the founder mutation, (2) a compound heterozygote for 70A>G and 195C>T, a previously reported mutation, and (3) a compound for a novel indel, 28_33delCTACACinsAGGATACAGGGAGGA and 195C>T. Twin brothers with 70A>G and 4C>T had isolated skeletal involvement. Finally, an adolescent with minimal metaphyseal changes had novel mutations in cis, 91G>C and 101C>T, located in a conserved region of RMRP, and 242 A>G on the other allele. ASO analysis of 100 RMRP genes from unrelated control individuals did not identify the novel mutations reported here. 70G occurred on the founder haplotype A, 195T occurred on haplotypes A and B consistent with recurrence, and all other mutations were on haplotype B. Review of our patients and those in the literature suggest that modifying factors play a critical role in the clinical expression of CHH.

The Chemokine (C-C-motif) receptor 3 (CCR3) Gene is Linked and Associated with Age at Menarche in Caucasian Females. *F. YANG[1,3*], D.H. XIONG[2,3*], Y. GUO[4], H. SHEN[2], P. XIAO[2,3], F. ZHANG[3,4], H.W. DENG[1,2,4], * The first two authors contribute equally to this work.* 1) Laboratory of Molecular and Statistical Genetics, College of Life Sciences, Hunan Normal University, Changsha, Hunan 410081, P R China; 2) Department of Orthopedic Surgery, School of Medicine, University of Missouri-Kansas City, 2411 Holmes Street, Kansas City, MO 64108, USA; 3) Osteoporosis Research Center and Department of Biomedical Sciences, Creighton University, Omaha, NE 68131, USA; 4) The Key Laboratory of Biomedical Information Engineering of Ministry of Education and Institute of Molecular Genetics, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an 710049, P R China.

Chemokine (C-C motif) receptor 3 (CCR3), playing an important role in endometrium related metabolic pathways, may influence the onset of menarche. To test linkage and/or association between CCR3 polymorphisms with the variation of age at menarche (AAM) in Caucasian females, we recruited a sample of 1048 females from 354 Caucasian nuclear families and genotyped 16 SNPs spanning the entire CCR3 gene. LD (linkage disequilibrium) and haplotype blocks were inferred. Both single markers and haplotypes were tested for linkage and/or association with AAM using QTDT (quantitative transmission disequilibrium test). We also tested the association between CCR3 polymorphisms and AAM in a selected random sample of daughters using ANOVA (analysis of variance). In block 2, significant within-family association of SNP9 (p=0.04) with AAM were detected. We also detected total associations of SNP7 (p=0.04), SNP9 (p=0.009) and SNP10 (p=0.03). SNP14 was linked to AAM (p=0.02). In addition, there was evidence of linkage and total association with AAM for the AGA (p=0.03 and p=0.04, respectively) haplotype reconstructed by SNP7, SNP9 and SNP13. ANOVA confirmed the results of association analyses by QTDT (p=0.008, 0.0008, 0.005, 0.02, 0.001 for SNP7, 9, 10, 13 and haplotype AGA, respectively). Our results reported for the first time that CCR3 may have certain effect on AAM variation in Caucasian women. However, further studies are necessary to substantiate our conclusions.

HDC Gene Polymorphisms Are Associated with Age at Natural Menopause in Caucasian Women. *F. ZHANG[1, 3*], D.H. XIONG[2, 3*], W. WANG[1], H. SHEN[3], P. XIAO[3], F. YANG[2, 3], H.W. DENG[1, 2, 4], * The first two authors contribute equally to this work* 1) The Key Laboratory of Biomedical Information Engineering of Ministry of Education, and Institute of Molecular Genetics, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an 710049, P.R.China;; 2) Laboratory of Molecular and Statistical Genetics, College of Life Sciences, Hunan Normal University, Changsha, Hunan 410081, P.R.China; 3) Osteoporosis Research Center, Creighton University Medical Center, Omaha, NE 68131, USA; 4) Department of Orthopedic Surgery, School of Medicine, University of Missouri-Kansas City, 2411 Holmes Street, Kansas City, MO 64108, USA.

Menopause status is one important anthropological variable influencing the overall health of women, especially those in advanced ages. Extensive researches carried out in human have demonstrated that age at natural menopause (AANM) was under strong genetic control. However, few candidate genes underlying AANM have ever been identified so far. Histidine decarboxylase gene (HDC) encodes histidine decarboxylase, which may physiologically influence menopause status of women. In this study, we aim to investigate whether HDC gene polymorphisms are associated with AANM in Caucasian women. Data were collected from 265 postmenopausal women in 131 nuclear families. All these subjects were genotyped with 14 single-nucleotide polymorphisms (SNP) spanning from Promoter to 3 UTR in the HDC gene. 11 SNPs and the related haplotype blocks of the HDC gene were tested for association with AANM variation by the program QTDT (Quantitative Transmission Disequilibrium Test). Significant within-family associations with AANM for SNP rs854163 and SNP rs854158 were observed (P values = 0.0018 and 0.0197, respectively). Consistently, we also detected a significant within-family association between block 2 defined by SNP rs854163 and SNP rs860526 and AANM in the haplotype analyses (P value = 0.0397). We found that the HDC gene polymorphisms were significantly associated with AANM in Caucasian women. Further molecular genetics studies are required to confirm our results and find the exact causal alleles influencing AANM variation within HDC gene.

Association between Low-density Lipoprotein Receptor-Related Protein 5 (LRP5) Gene and Bone Mineral Density in Caucasian and Chinese Populations. *D.H. XIONG[1], S.F. LEI[3], L. WANG[2], W. WANG[2], H. SHEN[1], H.W. DENG[1,2,3]* 1) Department of Orthopedic Surgery, School of Medicine, University of Missouri-Kansas City, 2411 Holmes Street, Kansas City, MO 64108, USA; 2) The Key Laboratory of Biomedical Information Engineering of Ministry of Education and Institute of Molecular Genetics, Xi'an Jiaotong University, Xi'an 710049, P R China; 3) Laboratory of Molecular and Statistical Genetics, Hunan Normal University, Changsha, Hunan 410081, P R China.

Low-density lipoprotein receptor-related protein 5 (LRP5) has been established as a high bone mass gene. However, it is still controversial that whether LRP5 is associated with normal BMD variation. This study is to explore the association of BMD phenotypes at three clinically important skeletal sites - spine, hip and ultradistal radius (UD) - with LRP5 in two independent samples of Caucasians and Chinese background, respectively. The Caucasian sample is made up of 1873 subjects from 405 nuclear families while the Chinese sample consists of 350 unrelated men. High density SNPs across the whole LRP5 gene were genotyped and analyzed in both samples. Linkage disequilibrium analyses showed that the haplotype structures of the LRP5 between Caucasians and Chinese were in excellent agreement. Association testes showed that polymorphisms in the block5 spanning intron7 to intron19 of LRP5 were significantly associated with spine BMD in both samples (the most significant empirical global P values = 0.005 and 0.007, respectively). Furthermore, in Caucasians, a new spine BMD associated SNP- rs7105218 in the 3-UTR of LRP5 was detected (P value = 0.01) and the LRP5 block1 polymorphisms representing the 5-promoter region were significant for hip BMD (P values = 0.03). In Chinese, we found that the polymorphisms in blocks 4 and 5 were significant for hip BMD (P values = 0.022 and 0.008). An independent intron7 polymorphism - rs4930573 was significantly associated with UD BMD (P value = 0.002) in Chinese, too. Our work suggests the presence of both shared and population-specific genetic causal variants within the LRP5 gene that influence the normal BMD variation in various human populations.

SUSCEPTIBILITY GENES FOR CHILDHOOD AND ADULT ASTHMA. *Y. Suzuki[1], Y. Mashimo[1], H. Inoue[1], K. Hatori[1], A. Hata[1], T. Hirota[2], F. Kamada[3], Y. Matsubara[3], M. Tamari[2]* 1) Department of Public Health, Chiba University Graduate School of Medicine, Chiba, Japan; 2) SNP Research Center, The Institute of Physical and Chemical Research (RIKEN), Yokohama, Japan; 3) Department of Medical Genetics, Tohoku University School of Medicine, Sendai , Japan.

Ober et al. reported that 25 genes (IL4, IL13, CD14, ADRB2, HLA-DRB1, HLA-DQB1, TNF, FCER1B, IL4RA, ADAM33, GSTM1, IL10, CTLA4, SPINK5, LTC4S, LTA, GRPA, NOD1, CC16, GSTP1, STAT6, NOS1, CCL5, TBXA2R, TGFB1) showed positive association with either atopy or asthma in 6 or more papers (Gene Immun 7: 95-100 (2006)). We investigated significance of these most promising candidate genes for asthma in our Japanese case-control samples.

SNPs in the above mentioned genes except HLA-DRB1 and HLA-DQB1 were genotyped in the 524 controls, 339 childhood asthma patients, and 508 adult asthma patients who were recruited in Osaka City area, Japan. In the IL4, IL4RA, CTLA4, CCL5, and TBXA2R, association of 2-SNP haplotype was investigated. In the rest of the genes, association of single SNP was investigated. *P* values for chi-square test less than 0.05 were considered significant in the single-SNP association tests with dominant or recessive model. In haplotype frequency comparisons, permutation *P* values less than 0.05 were considered significant. Odds ratios for the disease were calculated using logistic regression formulae.

Significant association with childhood asthma was found in IL4RA(I50V), ADRB2(R16G), ADAM33(13236T/C), GSTP1(I105V). That with the adult asthma was in IL4RA(I50V), IL4(-590C/T:+33C/T haplotype), ADAM33(12433T/C), TNF(-1037C/T), TBXA2R(795T/C:924T/C haplotype). Odds ratios for the childhood asthma were in the order ADAM33IL4RAADRB2GSTP1; those for the adult asthma were IL4ADAM33TNFTBXA2RIL4RA.

Out of 23 promising candidate genes, 7 genes were significantly associated with asthma. ADAM33 and IL4RA seemed common susceptibility genes for childhood and adult asthma in the Japanese population.

The Genetic Structure of Human Populations in Africa. *F.A. Reed[1], A. Froment[2], M.W. Smith[3], S.M. Williams[4], S.A. Omar[5], M.J. Kotze[6], G.S. Pretorius[6], M. Ibrahim[7], O. Doumbo[8], M. Thera[8], C. Wambebe[9], S.E. Dobrin[10], J.L. Weber[10], S.A. Tishkoff[1]* 1) Dept. Biology, Univ. of Maryland, College Park, MD; 2) UR 092,IRD,Orléans, France; 3) Lab of Genomic Diversity, SAIC-Frederick, NCI-Frederick, Frederick, MD; 4) Cen. for Hum. Genet. Res., Vanderbilt Univ., Nashville, TN; 5) KEMRI, Nairobi, Kenya; 6) Div. Human Genet., Univ. of Stellenbosch, Tygerberg, South Africa; 7) Dept. Mol. Biol., Univ. of Khartoum, Khartoum, Sudan; 8) Malaria Research and Training Center, Univ. Bamako, Bamako, Mali; 9) Int. Biomed. Res., Abuja, Nigeria; 10) Marshfield Clinic, Marshfield, WI.

Africa contains the greatest levels of human genetic variation and is the source of the worldwide range expansion of all modern humans. Knowledge of the genetic population boundaries within Africa has important implications for the design and implementation of genetic epidemiologic studies of Africans and African Americans, and for reconstructing modern human origins. A dataset consisting of ~3.7 million genotypes has been generated from the Marshfield panel of 773 microsatellites and 392 in-del polymorphic genetic markers. These markers were genotyped in ~3,200 individuals from >100 diverse ethnic populations across Africa as well as in 118 African Americans and in the CEPH Human Genome Diversity Panel, consisting of 1048 individuals from 51 globally diverse populations. Preliminary analysis of population structure using the program STRUCTURE[1] indicates considerably more substructure amongst global populations (estimate for the number of genetic clusters, K, is 12) and amongst African populations (K = 9) than had previously been recognized[2]. Population clusters are correlated with self-described ethnicity and shared cultural and/or linguistic properties (e.g. Pygmies, Khoisan-speakers, Bantu-speakers, etc). African Americans have predominantly West African Bantu (~80%) and European (~17%) ancestry, although individual admixture levels vary considerably. These results justify the need to include a broad range of geographically and ethnically diverse African populations in studies of human genetic variation. [1]Pritchard JK, et al. Genetics 155:945-59 (2000) [2]Rosenberg NA, et al. Science 298:2381-5 (2002).

Dissecting the role of the *IL1* Gene Cluster in Ankylosing Spondylitis. *A-M. Sims[1,2], J.J. Pointon[1], A.E. Timms[1], B.P. Wordsworth[1], M.A. Brown[1,2], The International Genetics of Ankylosing Spondylitis Consortium* 1) Botnar Research Centre, University of Oxford, Oxford, UK; 2) Centre for Immunology and Cancer Research, University of Queensland, Brisbane, Australia.

Background: The *IL1* gene cluster is known to be associated and linked with ankylosing spondylitis (AS). We sought to refine the association by further LD mapping in a British Caucasian (BC) population, and then by planned meta-analysis, to determine the contribution of this cluster to AS susceptibility in different populations. **Methods**: The *IL1RN-VNTR* and 64 SNPs in the *IL1* gene cluster were genotyped in 330 parent-case trios and 240 extended families of BC descent. Case-control analyses were undertaken using 1 affected individual per family (540) and AFBAC controls (1059). Within-family analyses were performed using conditional extended TDT to identify markers independently associated with AS. The meta-analysis was case-control in design. Nine markers in the *IL1* gene cluster previously associated with AS were studied in a total of 1900 cases and 1571 ethnically matched controls, including BCs, North American Caucasians, Hungarians, Taiwanese and Chinese. Genotype-known controls were provided to each centre for blind genotyping to confirm genotyping accuracy. Analysis was performed using the Mantel-Haenszel ^{2} test. **Results**: The BC case-control and within-family analyses each showed 6 markers individually associated with AS ($p<0.05$) spanning the cluster, but with the major region of disease susceptibility being in *IL1A* ($p<0.05$ at rs17561). Meta-analyses show significant association ($p<0.05$) in *IL1A* (rs2856836, OR=1.23; rs17561, OR= 0.84; rs1894399, OR=0.82), and in *IL1B* (rs16944, OR=1.16). Marginal association was seen with *IL1F10* (rs3811058, OR=1.2). No other association was seen in the combined dataset, although association was observed in at least one dataset for every marker. **Conclusions**: In the BC dataset the primary association of the *IL1* gene cluster with AS lies within *IL1A*. This observation was confirmed by the meta-analysis. *IL1A/B*, which lie in a single haplotype block in the p-telomeric end of the *IL1* gene cluster, are strongly associated with AS in genetically remote populations.

A truncating *DFNA5* mutation shows that only a very specific gain-of-function mutation leads to DFNA5-associated hearing loss. *L. Van Laer[1], N. Myer[2], F. Alasti[1,3], Y. Riazalhosseini[4], M. Malekpour[4], A. Vandevelde[1], M. Moghannibashi[4], K. Kahrizi[4], H. Najmabadi[4], G. Van Camp[1], R.J.H. Smith[2]* 1) Department of Medical Genetics, University of Antwerp, Antwerp, Belgium; 2) Molecular Otolaryngology Research Laboratories, University of Iowa, Iowa City, Iowa, USA; 3) National Research Center for Genetic Engineering and Biotechnology, Tehran, Iran; 4) Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran.

Mutations in *DFNA5* lead to an autosomal dominant type of nonsyndromic, sensorineural hearing loss starting at the high frequencies (Van Laer et al., 1998, Nat. Genet. 20, 194-197). Only three *DFNA5* mutations have been described hitherto: a complex deletion/insertion deleting 5 G-triplets in intron 7, a deletion of 3 nucleotides in the polypyrimidine tract of intron 7 and a nucleotide substitution in the splice-acceptor site of intron 7. Although different at the genomic DNA level, all lead to exon 8 skipping at the mRNA level. Here we describe a fourth *DFNA5* mutation: consisting of the insertion of a C at nucleotide position 640 (AF073308.1:c.640insC, AAC69324.1:p. Thr215HisfsX8). Unlike the previously described mutations, this frameshift mutation truncates the protein in exon 5 of the gene. Although the mutation was found in an extended Iranian family with hereditary hearing loss, it does not co-segregate with the hearing loss, and does not cause any obvious hearing loss. This remarkable fact provides further proof for the hypothesis that DFNA5-associated hearing loss is caused by a very specific gain-of-function mutation, involving skipping of exon 8, and not by haplo-insufficiency as initially thought. This hypothesis was previously supported by experimental evidence showing that mutant DFNA5 leads to cell death when transfected into yeast and into mammalian cells (Van Laer et al., 2004, J. Med. Genet. 41, 401-406).

***KIT, PDGFRA, VEGFR2* and *EGFR* gene amplifications in primary and recurrent astrocytic brain tumors.** *M. Puputti[1], H. Sihto[1], O. Tynninen[2], T. Blom[3], H. Mäenpää[1], J. Isola[4], A. Paetau[2], N. Nupponen[3], H. Joensuu[1]* 1) Department of Oncology, Helsinki University Central Hospital, Finland; 2) Department of Pathology, Helsinki University Central Hospital (HUSLAB) and University of Helsinki, Finland; 3) Molecular Cancer Biology Program, University of Helsinki, Biomedicum Helsinki, Finland; 4) Institute of Medical Technology, University of Tampere, Finland. MP and HS equally contributed to the work.

Histological biopsies taken from 87 primary brain tumors and their recurrent tumors were analyzed for the presence of *KIT*, *PDGFRA*, *VEGFR2* and *EGFR* gene amplifications using either chromogenic *in situ* hybridization (CISH) or fluorescence *in situ* hybridization (FISH). *KIT*, *PDGFRA* and *VEGFR2* receptor tyrosine kinase genes are located adjacent to each other on chromosome 4q12, whereas *EGFR* is located on chromosome 7p12. CISH analyses were performed from a representative tumor tissue microarray. The primary tumors consisted of oligodendroglioma grade II-III (n=20), oligoastrocytoma grade II-III (n=11), astrocytoma (n=38) and anaplastic astrocytoma (N=18). The histological diagnoses of the corresponding recurrent tumors were oligodendroglioma grade II-III (n=17), oligoastrocytoma grade II-III (n=16), astrocytoma (n=17), anaplastic astrocytoma (n=21) and glioblastoma (n=16). *KIT*, *PDGFRA*, *VEGFR2* and *EGFR* gene amplification were detected in 8 (10%), 6 (8%), 10 (12%) and 3 (4%) of the primary neoplasms, respectively. In the recurrent tumors the same genes were amplified in 19 (27%), 13 (18%), 10 (14%) and 6 (8%) of the cases, respectively. *KIT* amplifications were associated with *PDGFRA* and *VEGFR2* amplifications both in primary and secondary gliomas, and with *VEGFR2* amplifications in recurrent gliomas. Aneuploid (3 to 5) *KIT*, *PDGFRA*, *VEGFR2* and *EGFR* gene copy numbers were commonly present in both primary gliomas and in their recurrent tumors. Strong KIT, EGFR and VEGFR2 expression were infrequently found in immunohistochemistry of the tumor tissue. The results suggest that *KIT* and *PDGFRA* amplifications may have a role in the genesis of some human gliomas.

Mt-DNA deletions in muscle biopsies: interpretation and analysis of responsible genes. *M. Tesarova, T. Honzik, J. Buzkova, H. Hansikova, J. Zeman* Dept. of Paediatrics, Faculty of Medicine, Charles University, Prague 2, Czech Republic.

Within last two years, screening for large-scale deletions of mitochondrial DNA (mtDNA) was performed in muscle biopsies of more than 180 patients with myopathy or encephalomyopathy. The results were compared with histological investigations and analyses of the amount and activity of OXPHOS system. Based on these results, candidate genes were analyzed in selected patients. Two methods were used for the detection of deleted mtDNA molecules (-mtDNA): Southern blot and amplification of whole mtDNA molecule using two pairs of primers (LX-PCR). Using LX-PCR method, the presence of -mtDNA was identified in 88 samples of muscle biopsy; age of the patients was 6 - 73 years. Only in 10 samples, D-mtDNA was identified by Southern blot. These patients fulfil the diagnostic criteria of Kearns-Sayre syndrome. In remaining 78 samples, the -mtDNA (single or multiple) was identified only by LX-PCR. Immunohistochemistry revealed other than mitochondrial type of myopathy in 22 patients. In 13 samples of muscle biopsy, no histological changes were present. Based on the phenotype, enzyme and protein analyses of OXPHOS complexes combined with the results of histochemical investigations, the mitochondrial aetiology of the disease is supposed in 43 patients. The candidate genes (*POLG1*, *ANT1*, *TP* or *C10orf2*) were analysed in 19 patients so far. A homozygous mutation G145R in *TP* gene was found in one patient. A heterozygous mutation Y831C in *POLG1* gene was found in two unrelated patients (A,B) and in 5 out of 9 siblings of the patient A. No other pathogenic mutation was found in exons or adjacent intronic regions. The siblings are without clinical symptoms so far. The Y831C mutation wasnt found in 216 control alleles. A dominant mode of inheritance of Y831C mutation in *POLG1* gene has been already proposed in a family with neuropathy. Due to high sensitivity of the LX-PCR used for detection of -mtDNA, interpretation of obtained results and further molecular genetic analysis should be done with respect to biochemical, histological and clinical data. Supported by GAUK 41/2004/C and IGA NR8065-3.

Localisation of a Fifth Gene involved in Autosomal Dominant Hypercholesterolemia. *M. Varret, M. Abifadel, A. Marques, J. Bonneau, M. Devillers, D. Erlich, A. Munnich, J-P. Rabès, C. Boileau* INSERM U781, Hopital Necker-Enfants Malades, Paris, France.

Autosomal Dominant Hypercholesterolemia (ADH), a major risk factor for atherosclerosis, was initially associated with mutations in two genes : LDLR (encoding low-density lipoprotein receptor) or APOB (encoding apolipoprotein B). Our team has pioneered the claim that the disease is far more heterogeneous. We have 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 the HCHOLA4 gene at 16q22.1 in a 5.31 cM interval. Through the ADH French Research Network, we collected genetic material from a large french pedigree HC126). In this multiplex familly, 26 samples from 16 affected and 10 unaffected members were obtained. Linkage and sequencing analyses in familly HC126 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 HC126 family for linkage, 500 simulations were carried out using the SLINK software in which genotypes were simulated using parameters compatible with ADH. The average lod score was 2.08 and the maximum lod score was 3.96 indicating that the statistically-significant threshold of 3 could be reached in this single family. Genomewide scan is underway in collaboration with the French National Genotyping Center. Identification of the HCHOLA5 gene could reveal a new aspect of cholesterol metabolism and may lead to the development of new potent drugs.*LDLR*.

First Trimester Invasive Trophoblast Antigen (ITA) as a Down Syndrome Marker: Prospective Study with Fresh Serum. *P. Wyatt[1], C. Meier[2], L. Neveux[3], G. Palomaki[3]* 1) York Central, Toronto,Canada; 2) St.Michael's, Toronto; 3) Women & Infants, Providence, RI, USA.

Aim: Examine the screening performance of first trimester measurements of ITA, alone or in combination with other markers.
Methods: 12,610 women provided first trimester sera for routine Down syndrome screening. Most were part of an integrated Down syndrome test (both 1st and 2nd trimester sera). All sera were tested for pregnancy associated plasma protein-A (PAPP-A). ITA testing was performed on the fresh samples, but results were not used clinically. Results are reported in weight- and race-corrected multiples of the median (MoM). 30 affected pregnancies/births were identified.
Results: 10,606 women had both ITA and PAPP-A measured; 26 were affected with Down syndrome. At a 5% false positive rate (FPR), 8 cases (31%) were detected using either ITA or PAPP-A results alone. Median levels in affected cases varied by gestation (regressed medians of 2.01, 2.22, 2.47 for ITA at 11 12, and 13 weeks, and 0.23, 0.41, 0.75, for PAPP-A). Logarithmic standard deviations for ITA and PAPP-A in the unaffected were 0.2961 and 0.2526; and 0.2562 and 0.2905 in affected pregnancies. Correlations between markers were 0.1955 and -0.3463, for unaffected and affected pregnancies, respectively. At 12 weeks gestation, modeling indicates that maternal age with PAPP-A and ITA, detects 67% of cases at a 5% FPR; nuchal translucency (NT) measurements increases detection to 84%.
Conclusions: ITA and PAPP-A measurements in fresh samples closely match results from an earlier prospective study of 54 cases(Palomaki et al., Clin Chem 2005;51:1499-04)which found ITA levels in affected cases nearly as high at 11 weeks as 13 weeks. Combinations of maternal age, NT, PAPP-A and ITA also had similar detection when ITA was replaced with free- (FPR of 5%). This is true at each week from 11 through 13. Based on these two datasets, ITA is a suitable replacement for free- in first trimester screening, when combined with PAPP-A and NT.
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Chromosomal Abnormalities in Ependymal Tumors. *Th. Palm[1], C. Godfraind[2], M. Vikkula[1]* 1) Lab Human Molecular Genetics, Christian de Duve Institute & UCL, Brussels, Belgium; 2) Lab Neuropathology, Cliniques universitaires St-Luc & UCL, Brussels, Belgium.

Ependymal tumors are derived from ependymal cells covering cerebral ventricles and the central canal of the spinal cord. Their overall incidence is 0.28 cases per 100,000 individuals per year in the United States with a mean age at diagnosis of 35 years. Their clinico-pathological presentation varies in between patients. In cytogenetic studies, up to 66% of the ependymal tumors present a normal karyotype. The most frequent aberrations are losses of chromosome 22, 6q and 9p; gains are frequently observed on 1q and on chromosome 7. Additional molecular markers are needed to define prognostic and/or clinical subgroups. In the current study, we used Affymetrix GeneChip 50K Arrays to establish high-resolution profile in a group of 35 ependymal tumors. It included two WHO grade I tumors, 13 WHO grade II tumors, 15 WHO grade III tumors and 5 tumors of unknown WHO grade. For the genomic profiles, only anomalies larger than 10Mb were included. Such chromosomal aberrations were observed in 27/35 tumors (~77%). All grade II tumors exhibited anomalies. In addition, they were more numerous in grade II than in grade III tumors (6.8/case vs. 3.8/case). The most frequent aberrations were monosomy 22 (13/35 cases = 37%), deletion or amplification of 9p (34%), deletion of 6q (31%), amplification of chromosome 7 (25%), and amplification of chromosome 12 (22%). Our results using the Affymetrix SNP-Chips platform are in concordance with previously published data established by microsatellite and cytogenetic studies. The resolution of the arrays allows us to focus on finer genetic alterations. Furthermore, expression studies will be integrated into these results in order to identify molecular markers for diagnostic/prognostic outcomes. (catherine.godfraind@anpg.ucl.ac.be).

High frequency of SDHB mutations in a series of head and neck paraganglioma from Belgium. *A. Persu[1], V. Grégoire[2], P. Garin[3], H. Reychler[4], G. Chantrain[5], G. Mortier[6], J.-F. De Plaen[1], M. Hamoir[7], M. Vikkula[8]* 1) Nephrology Dept, Cliniques universitaires St Luc & UCL, Brussels, Belgium; 2) Radiotherapy Dept, Cliniques universitaires St Luc & UCL, Brussels, Belgium; 3) Otolaryngology Dept, Cliniques universitaires de Mont-Godinne & UCL, Yvoir, Belgium; 4) Oral and Maxillofacial Surgery, Cliniques universitaires St Luc & UCL, Brussels, Belgium; 5) Centre Hospitalier Universitaire Saint-Pierre & ULB, Brussels, Belgium; 6) Center for Medical Genetics, Gent University Hospital, Gent, Belgium; 7) Otolaryngology Dept, Cliniques universitaires St Luc & UCL, Brussels, Belgium; 8) Lab of Human Molecular Genetics, Christian de Duve Institute & UCL, Brussels, Belgium.

Mutations of SDH genes encoding subunits of complex II of the mitochondrial respiratory chain are involved in the pathogenesis of paraganglioma (PG) and pheochromocytoma. While SDHD is more frequently involved in the pathogenesis of head and neck PG, SDHB mutations are mainly associated with malignant and/or extra-adrenal pheochromocytoma. To look for the nature and frequency of SDH mutations as well as for possible genotype-phenotype correlations in head and neck PG from Belgium, genetic screening included 30 patients without familial history of PG and 6 families including 18 subjects with known PG were done. Three frameshift mutations leading to premature stop codon, 2 splice site mutations and one substitution were found in 7 different patients including 5 familial cases and 2 apparently sporadic cases. Four of the mutations were not described previously. Furthermore, 2 different SDHB mutations (substitutions) were found in 4 unrelated patients with apparently sporadic PG. One of them, found in 3 of the 4 subjects, had been already described in a family with malignant pheochromocytoma. Surprisingly, in this Belgian series, SDHB mutations were three times as frequent as SDHD mutations (23 vs. 7%) in sporadic head and neck PG without evidence of dissemination, mainly due to a single mutation previously associated with familial metastatic pheochromocytoma (alexandre.persu@nefr.ucl.ac.be).

Common genetic variants of the ion channel transient receptor potential membrane melastatin 6 and 7 (TRPM6 and TRPM7), magnesium intake, and risk of type 2 diabetes in women. *Y. Song[1], Y.H. Hsu[2], J.E. Manson[1,3], J.E. Buring[1,3,4], S. Liu[1,2]* 1) Div. of Preventive Medicine, Brigham & Women's Hospital, Boston, MA; 2) Dept. of Epidemiology, UCLA School of Public Health, Los Angeles, CA; 3) Dept. of Epidemiology, Harvard School of Public Health, Boston, MA; 4) Dept. of Ambulatory Care and Prevention, Harvard Medical School, Boston, MA.

Ion channel transient receptor potential membrane melastatin 6 and 7 (TRPM6 and TRPM7) play a central role in magnesium homeostasis critical for maintaining glucose and insulin metabolism. We prospectively investigated whether single nucleotide polymorphisms (SNPs) in TRPM6 and TRPM7 genes contribute to the risk of type 2 diabetes. We genotyped 21 haplotype tagging SNPs in TRPM6 and 4 common SNPs in TRPM7 in a nested case-control study of 359 cases and 359 controls in the Womens Health Study and examined their associations with type 2 diabetes. After adjustment for matching factors (age, race, and fasting status), BMI, physical activity, alcohol, smoking, and family history of diabetes, our allele-specific analyses showed no significant association between any single variant and diabetes risk. Although no overall association by haplotype-based analysis was observed, there was a suggestion of a significant interaction between magnesium intake and the haplotype of TRPM6 based on two nonsynomous SNPs (Ile1393Val in exon 29 [rs3750425] and Lys1584Glu in exon 30 [rs2274924]) on diabetes risk. The haplotype 1393Val-1584Glu was significantly associated with increased risk of diabetes (OR, 2.75, 95% CI, 1.04-7.31) only among women with low magnesium intake (in the lowest 25th percentile, 288 mg/day). A similar association was also observed in our sliding-window 2-SNP haplotype analysis after adjustment for multiple comparisons. In conclusion, our results suggest that the two common non-synonymous TRPM6 coding region variants, Ile1393Val and Lys1584Glu polymorphisms, may influence risk of type 2 diabetes in women with low magnesium intake. Our data support the hypothesis that the role of TRPM 6 in the regulation of intestinal magnesium absorption may be critical when passive magnesium transport is slowed by inadequate magnesium intake.

Bicuspid aortic valve and other cardiovascular malformations are genetically heterogeneous. *V. Ramachandran[1], L.J. Martin[2,3], L.H. Cripe[1,3], R.B. Hinton[1,3], M. Tabangin[2], K. Shooner[1], D.W. Benson[1,3]* 1) Department of Cardiology, MLC 7042, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 2) Center for Epidemiology and Statistics, Cincinnati Childrens Hospital Medical Center, Cincinnati, OH; 3) The University of Cincinnati School of Medicine, Cincinnati, OH.

Valvular heart disease remains a common and important problem. Among all forms of valvular heart disease, aortic valve disease is most common and aortic valve replacement is the second most common cardiac surgery in United States. In individuals of all ages aortic valve disease is linked to a common congenital valve malformation bicuspid aortic valve (BAV) with a prevalence of 1-2%. Heritability studies indicate that BAV determination is almost entirely genetic. The aim of the current study was to identify genes causing BAV and other cardiovascular malformations (CVMs) by linkage analysis. Thirty-eight families with BAV and other CVMs including dilated aortic root, coarctation of aorta, ventricular septal defect and hypoplastic left heart syndrome were enrolled in the study. Definitive diagnosis was made by standardized complete 2D and Doppler Transthoracic Echocardiograms. Genome-wide scan was performed using the microsatellite marker panels, ABI Prism Linkage Mapping Set. Phenotypic and genotypic data from 324 individuals belonging to 38 families were used for the genetic analysis. Both parametric and non-parametric linkage analysis was performed with the softwares GENEHUNTER and SOLAR respectively using 2% and 5% prevalence. The highest LOD score, 3.8, occurred on chromosome 18q between markers D18S68 and D18S1161. Other genetic locations 5q (between D5S644 and D5S2027) and 13q (between D13S1265 and 13qter) across the genome exhibited a LOD score of 2.7 suggestive of linkage (LOD greater than 2.0). In this genome-wide scan we demonstrate, for the first time, that BAV and other CVMs are genetically heterogeneous exhibiting linkage to 18q, 5q and 13q. Based on the biology of valve development, candidate genes may be valvulogenesis building block genes such as extracellular matrix proteins, signaling molecules and transcription factors.

Analysis of candidate genes for nonsyndromic cleft lip and/or palate in Brazilian trios with both a parent and offspring affected. *A.L. Silva[1,2,4], L.A. Ribeiro[2], M.E. Cooper[3], M.L. Marazita[3], D. Moretti-Ferreira[1], J.C. Murray[4]* 1) Universidade Estadual Paulista, UNESP, Botucatu, SP, Brazil; 2) Hospital de Reabilitação de Anomalias Craniofaciais, USP, Bauru, SP, Brazil; 3) Center for Craniofacial and Dental Genetics, University of Pittsburgh, Pittsburgh, PA, USA; 4) Pediatrics, University of Iowa, Iowa City, IA.

Cleft lip and/or palate (CL/P) is a major congenital anomaly with a birth prevalence from 1 in 500 to 1 in 2500 births, depending on geographic, ethnic, and socioeconomic factors. Approximately 2/3 of cases do not show any others anomalies and are classified as nonsyndromic (NS). Studies have suggested a handful of genes and/or loci in humans, as well as environmental disrupters appear to play a role in the genesis of such cases. In the present study we analyzed a total of 29 single nucleotide polymorphisms (SNPs) in 7 candidates the genes: IRF6 (7), TGFA (4), MSX1 (2), FGFR1 (4), FOXE1 (3), BMP4 (2) and TGFB3 (7) in 120 Brazilian trios with both a parent and offspring affected with NS-CL/P. We also performed direct sequencing of the MSX1 gene. TDT analysis for the SNPs was performed in the total group and also in subgroups dependent on the parent affection status. We found significant results for the candidate genes IRF6 (p-value=0.02), TGFA (p-value=0.02), MSX1 (p-value=0.006) and TGFB3 (p-value=0.006). All the genes analyzed in this study are strong candidate genes for nonsyndromic cleft lip and/or palate as previous studies had shown their expression in craniofacial tissues during the development and mutations in some of them are know to cause syndromic forms of oral clefts. This study suggests that cases with two generation family history may arise secondary to the interactive effects of currently recognized candidate genes and may have implications for genetic counseling in these high risk families.

Test Interaction between Two Unlinked Loci Using Composite Linkage Disequilibrium. *X. Wu*[1], *L. Jin*[1], *M. Xiong*[1,2] 1) Fudan University, Shanghai, China; 2) University of Texs Health Science Center at Houston.

Alternative methods to the traditional logistic model and Fishers formulation for testing interactions are to test interaction by measure of linkage disequilibrium (LD). The various measures of LD are based on haplotypes. The haplotype-based methods for detection of interaction have tow limitations. First, when Hardy-Weinberger equilibrium does not hold, it is difficult to estimate haplotypes. Second, the estimation of haplotype frequencies by statistical methods often has errors. It is recently noted that the errors of estimation of haplotype frequencies have big impact on the haplotype-based tests. To overcome these limitations in this report we propose to use composite measure of LD to test interaction between two unlinked loci which allow using genotypes. We develop a general theory for studying composite measure of LD in the disease population under two-locus disease models and explore using composite LD measure between two unlinked loci in the disease population as a function of the measure of interaction between two unlinked loci. We investigate that how interaction between two loci create LD in the disease population and derive the mathematical formulation of new definition of interaction between two loci based the composite LD measure, which motives us to present a composite measure of LD-based statistic to detect interaction between two unlinked loci. The null distribution and the type 1 error rates of the composite measure of LD-based statistic for testing interaction between two unlinked loci are validated using simulation studies. We compare the power of the developed test statistic with the traditional logistic models. Finally, we apply the developed statistic to three real data sets. Preliminary results show that the newly developed statistics have much smaller P-values than that of the traditional logistic models.

Disease Balanced Chromosomal Rearrangements and their relevance in genetic counselling. *A. Sánchez-Díaz[1], C. Morales-Peydro[1,2], E. Margarit[1], I. Madrigal[1], M. Milà[1], A. Soler[1]* 1) Servei Bioquímica i Genètica , Hospital Clínic, Barcelona, Spain; 2) Fundació Clínic, Hospital Clínic, Barcelona, Spain.

Introduction: Balanced Chromosomal Rearrangements (BCR) are the most frequent chromosomal abnormality and imply segmental chromosomal exchange without loss or gain of genetic material, and, essentially, are not associated to clinical features, especially those that are inherited. BCRs de novo are associated to abnormal phenotype in 6% of cases, due to gene truncation at the breakpoints, to positional effect, to uniparental disomy of imprinted chromosomes, or cryptic duplications and deletions. In the present work, we report the genetic study and the phenotypic implications of BCR in our patients. Material and methods: Forty-six BRCs cases diagnosed in our laboratory during the past 5 years were collected and re-analysed. High-resolution cytogenetic analysis was carried out in all cases. In 35 cases, parents were also karyotyped. Some cases were complemented using Fluorescence in Situ Hibridation (FISH) or Multiplex Ligation-Dependent Probe Amplification (MLPA) techniques. A physical examination and a detailed clinical history were done in all cases. Results: Nine out of the 46 cases were de novo and seven of them presented phenotypic affectation; 26 cases were inherited and in one of them the rearrangement co-segregated with the pathology. Eleven cases were of unknown origin. Two cases were complex BCRs involving 3 chromosomes, one of them de novo that was associated to phenotypic abnormalities. Conclusion: this study, according to others previously reported, discloses the high frequency of BCRs associated to abnormal phenotype, mainly when they are de novo. In these cases, the application of complementary molecular techniques is required to obtain an accurate characterization of the rearrangements, in order to establish a genotype-phenotype correlation. Moreover, this allows providing familial genetic counselling, the unique tool of prevention for genetic diseases.

Experiences of genetic counseling in eight families with Vietnamese wives in Taiwan. *H.P. Pan[1,4], S.J. Lin[2,4], M.C. Huang[1,3]* 1) Department of Nursing, National Cheng Kung University Hospital, Tainan, Taiwan; 2) Department of Pediatrics, National Cheng Kung University, Tainan, Taiwan; 3) Department of Nursing, National Cheng Kung University,Tainan,Taiwan; 4) Center of medical Genetic, National Cheng Kung University Hospital, Tainan, Taiwan.

According to report from Taiwan Ministry of Interior in 2005, one out of five newly married couples in Taiwan is international marriage. Among Southeast Asian brides, seventy percent are from Vietnam. For the family with a Vietnam wife, the purpose of marriage is to have a son to carry on the family name. Raising children is the major task in her life. Since couples in an international marriage usually have little time to know each other before wedding and have language obstacles and culture gaps, it will become a much more serious problem when they have to face a genetic disease. In order to improve the effectiveness of counseling, we investigated the genetic counseling process of eight families with Vietnamese spouse during 2004 to 2005 in Southern Taiwan. The process recordings of counseling were taken and analyzed to explore the psychosocial responses in these families. The result is divided into four parts: (1) the reasons for referral (2) the interaction and power structure among family members (3) the responses of family members during counseling (4) the impact of a genetic disease on the Vietnamese spouses. Our results show that reproductive planning in these families is decided by Taiwanese husbands and mothers-in-law. Due to language barrier and social isolation, Vietnamese wives husband and mother-in-law used to dominate the conversation. Genetic counselors could barely know whether Vietnamese wives understand the content of counseling. The result also found the key factors for effective genetic counseling (1) helping these international spouses express themselves well, (2) keeping good communication between their families and medical staff, (3) facilitate their adaptation to the environments in Taiwan.

Hypogonadotrophic hypogonadism associated with a subtelomeric deletion of 9p chromosome in a girl. *L. Telvi*[1], *C. Bouvattier*[2], *M. Minz*[1], *P. Bougneres*[2] 1) Cytogenetics Laboratory, Hospital St Vincent de Paul, Paris,; 2) Endocrinology Department, Hospital St Vincent de Paul, Paris, France.

We report a girl with a subterminal deletion of the short arm of chromosome 9, detected after classical R banding and high resolution banding and confirmed by in situ fluorescence hybridization techniques (FISH) in all analyzed metaphases. The patient showed a short stature (-4 SD), motor and mental retardation with dysmorphic features. Actually, the patient is 20 years-old, and presented a hypogonadotrophic hypogonadism. It was reported that the male patients with 9p deletion is associated with failure of normal testis development. However, there are no report of endocrinological anomalies associated with 9p deletion in female patients. In this report, we analyse the relationship between the endocrinological anomaly as a hypogonadotrophic hypogonadism associated with 9p deletion in female patients. We describe the phenotype of the patient and compare this case to the other female cases reported in the litterature with a subtelomeric deletion of 9p chromosome.

Association study aimed at identifying genes influencing the penetrance of DYT1 Dystonia. *G. Senthil[1], R. Saunders-Pullman[2], D. Raymond[2], S.B. Bressman[2], N. Risch[3], L.J. Ozelius[1]* 1) Department of Molecular Genetics, AECOM, Bronx, NY,United States; 2) Department of Neurology, Beth Israel Medical Center, New York, NY,United States; 3) Center for Human Genetics, University of California at San Francisco, San Francisco, CA, United States.

Dystonia is a neurological movement disorder characterized by sustained muscle contractions and abnormal postures. The DYT 1 locus on human chromosome 9q34 is responsible for the most severe form of hereditary dystonia-early onset, generalized torsin dystonia. This disorder is inherited as an autosomal dominant trait with reduced penetrance of only 30-40%. A single mutation, a 3-bp (GAG) deletion causing the loss of a glutamic acid residue from the encoded protein torsinA, accounts for about 50% of non-Jewish individual and over 90% of Ashkenazi Jewish individuals with this form of dystonia. In an effort to understand the reduced penetrance associated with the disease, we examined genetic variants within the DYT1 gene (3 SNPs) and its interacting partners, TOR1B (located adjacent to DYT1, 2 SNPs) and LAP1B (located on chromosome 1q25, 3 SNPs). The polymorphisms in these candidate genes were assessed in affected, non-manifesting DYT1-GAG deletion carriers and controls, to determine whether any were associated with penetrance. In addition, haplotypes were generated across these genes and also examined for association. Haplotypes across the DYT1 and TOR1B region of chromosome 9 are more frequent in non-manifesting carriers than affecteds suggesting a protective role contributing to the reduced penetrance.

Somatic Mosaicism for an *HRAS* mutation causes Costello Syndrome. *K. Sol-Church[1], D.L. Stabley[1], L. Nicholson[2], J.D. Hoffman[3], K.W. Gripp[2]* 1) Dept Biomedical Research, Alfred I duPont Hospital for Children, Wilmington, DE; 2) Division of Medical Genetics, Alfred I duPont Hospital for Children, Wilmington, DE; 3) Department of Pediatrics, Division of Genetics, Tufts-New England Medical Center, Boston, MA.

De novo heterozygous *HRAS* point mutations have been reported in more than 81 patients with Costello syndrome (CS), but genotype/phenotype correlation remains incomplete because the majority of patients share a common mutation, G12S, seen in 65/81 (80%). Diagnostic testing for *HRAS* mutations is now available and the identification of a mutation in a patient with consistent clinical findings confirms a diagnosis of CS. It is unclear at this time if the absence of an *HRAS* mutation precludes a clinical diagnosis of CS. One area of uncertainty remains in the differential diagnosis to cardio-facio-cutaneous syndrome (CFC).

We report here on a female with findings suggestive of CS in whom mutation analysis performed with standard techniques on white blood cell derived DNA did not show an *HRAS* mutation. However, analysis of DNA derived from three independently collected buccal swabs showed a sequence change qualitatively consistent with the G12S mutation. Allelic quantitation, performed using pyrosequencing, revealed the presence of the mutation in ~25-30% of the sampled buccal cells. Further analysis of the blood sample using restriction endonuclease analysis confirmed the presence of the G12S mutation.

In this patient, standard technology failed to identify the disease causing mutation on DNA derived from a blood sample, highlighting the potential pitfalls in the interpretation of negative mutation studies.

This is the first reported CS patient mosaic for the common *HRAS* mutation, likely due to a somatic mutation occurring early in fetal development.

FFIGdb - A Fast, Flexible, Integrated Genotype Database for storing very large genotype and phenotype data sets. *W. Rayner, E. Zeggini, M.I. McCarthy, and the International Type 2 Diabetes 1q Consortium* University of Oxford, Oxford, Oxfordshire, United Kingdom.

Many current genetic studies are generating increasingly massive data sets, both in terms of phenotypic and genotypic information. Efficient exploitation of these data requires dedicated data management systems with the capacity to store and manipulate these very large data sets.

FFIGdb is a high-throughput data management system initially developed for the International Type 2 Diabetes 1q Consortium. The system is capable of storing both phenotype and genotype information and is currently based on an Oracle database utilising a Perl client interface. The system is widely portable as the database schema utilises standard SQL and is therefore usable, with minor keyword changes, on any other relational database system that supports stored procedures.

Unlike most previous systems using Access or MySQL the system has been designed from the outset to utilise Enterprise level software to deal with multibillion point data sets: for example, on a low end server consisting of dual Xeon processors with a 1.4Tb RAID, it is possible to load in excess of 400 million genotypes/hour. Exports are similarly fast: data, and user-defined subsets of the data, can be exported in a wide variety of formats, including Stata and Helixtree, at a speed of 100 million genotypes/hour.

For flexibility, the database utilises an Entity-Attribute-Value (EAV) data model for storing phenotype, sample and marker data. The EAV model allows for user-defined data sets to be easily loaded thereby giving the system great flexibility and allowing it to be extended to any project. The system also incorporates many features for data management such as genotype and phenotype Quality Control (QC), duplicate sample- and marker-checking and audit trails.

To date, the system is handling multibillion point genome wide association data sets with ease. Development continues to ensure the speed, storage capability and flexibility all keep pace with ever-increasing data sizes.

Differential Gene Expression in Pediatric Embrionary Advanced Rhabdomyosarcomas. *O. Perez-Gonzalez[1], A. Hidalgo-Miranda[1], I. Silva-Zolezzi[1], R.M. Rivera-Luna[2], G. Jimenez-Sanchez[1]* 1) National Institute of Genomic Medicine, Mexico; 2) National Institute of Pediatrics, Mexico.

Rabdomyosarcoma (RMS) is the most frequent soft tissue malignant tumors and accounts for ~6% of all neoplasias in chilhood. The overall survival rate has significantly improved with the current therapy. The allocation of the neoadyuvant antineoplasic treatment is based on the Intergroup Rhabdomyosarcoma Study (IRS) classification system. However, therapeutic response is still inconsistent even in patients under similar classification. Thus, a classification system for a more individualized allocation of the antineoplasic treatment would result in significant benefit to patients with this disease. To explore the existence of a differential gene expression in patients with identical histopathological diagnosis and different responses to neoadjuvant antineoplastic treatment, we analyzed total RNA of nine RMS from pediatric patients from 9 to 12 years old with advanced stages using Affymetrix U133 Plus 2.0 gene expression arrays. Histopatological samples were obtained before administration of neoadyuvant treatment based on the IRS staging system, after which all patients were re-evaluated to classify response rate into good, intermediate or poor, using the same system. We obtained a probe-calling rate of 35-54% and are performing a supervised cluster analysis to find gene expression profiles associated to specific response rates. Results will be confirmed by independent expression analysis methods and the number of samples is continuously increasing. We are currently performing hierarchical clustering analysis using MacOS_Mev_3_1, version 10.2. Differential gene expression profiles in RMS are being analyzed as a mean to provide a more accurate system to allocate neoadyuvant antineoplasic treatment in patients with this disease. In addition, this will allow considering new therapies for those patients with expression profiles consistent with poor response rates.

A Novel X-Linked Locus for Metacarpal Synostosis. *A. Praggastis, Z. Yang, Y. Zhao, J. Baird, E. Pearson, Z. Tong, K. Zhang* Ophthalmology, University of Utah.

Purpose: The failure of the 4th and 5th metacarpal bones to differentiate into separate appendages is a condition known as Metacarpal synostosis. Depending on the severity, this condition can have significant effects on an individuals ability to perform everyday functions. Metacarpal synostosis is genetically heterogeneous and may present as an autosomal dominant, or X-linked recessive trait. We investigated a large Utah pedigree spanning 4 generations with 9 affected individuals. Methods: Clinical examination and testing were performed in all participants. Peripheral blood was taken from 8 members, including 3 affected males and 4 carriers. Genotyping using STR and SNP markers on the X chromosome for metacarpal synostosis was performed. Linkage and LOD scores were computed using Llinkage 5.1 Results: Hand x-ray radiography revealed fusion of the 4th and 5th metacarpal bones in affected individuals. Within this family, there were no females affected and no instances of male-to-male transmission, indicating X-linked recessive transmission. Linkage analysis mapped the disease gene to Xp11.22-Xq21.32 with maximal LOD score of .9053, at theta = 0, with DXS986. Haplotype analysis localized the disease gene in a 18 CM interval between DXS993 and DXS990. Conclusions: We mapped a disease gene for metacarpal synostosis in a Utah family to Xp11.22-Xq21.32. Further mapping and identification of the disease gene for metacarpal synostosis will increase our understanding of the condition and provide us with a more general knowledge of the mechanisms for hand development.

RASA1: an important gene for Parkes Weber syndrome. *N. Revencu[1, 2], L.M. Boon[1, 3], O. Enjolras[4], P.E. Burrows[5], M.R. Cordisco[6], E. Baselga[7], H.J. Paltiel[5], I. Quere[8], A. Delerue[9], C. Delerue[9], J.M. Caballos Quintal[10], R. Vanwijck[3], J.B. Mulliken[5], M. Vikkula[1]* 1) Lab Human Molecular Genetics, Christian de Duve Institute & UCL, Brussels, Belgium; 2) Centre for Human Genetics, Cliniques universitaires St-Luc, Brussels, Belgium; 3) Centre for Vascular Anomalies, Division of Plastic Surgery, Cliniques universitaires St-Luc, Brussels, Belgium; 4) Hôpital Lariboisière,Paris, France; 5) Children's Hospital, Boston, MA, USA; 6) Hospital Nacional de Pediatria, Buenos Aires, Argentina; 7) Hospital de la Sant Pau, Barcelona, Spain; 8) Centre Hospitalier Universitaire, Montpellier, France; 9) Lille, France; 10) Laboratorio de Genetica, Yucatan, Mexico.

Parkes Weber Syndrome (PWS; OMIM 608355) is characterized by a large cutaneous vascular stain in association with soft tissue and skeletal hypertrophy in an extremity, with underlying multiple arteriovenous micro-fistulas. Even if rather different, PWS has often been taken, in the literature, for two other conditions associated with limb hypertrophy: Klippel Trenaunay syndrome and capillary malformation with non-progressive congenital hypertrophy. Previously, we showed that an individual harbouring a germline mutation in the RASA1 gene, had PWS. In addition, this individual had multiple capillary malformations (CMs), which were transmitted as a dominant trait across his family. Therefore, we wanted to test if RASA1 is the causative gene of PWS in general. We screened 21 individuals with PWS for RASA1 mutations by DHPLC followed by sequencing and we identified mutations in 12 individuals (57%). Of these, 10 were familial and 2 were sporadic cases. These individuals and other 4 belonged to a phenotypically homogenous group in which PWS was associated with CMs. The remaining 5 individuals, in whom no RASA1 mutation was identified, constituted a phenotypically distinct group with less overgrowth in the affected limb and without CM lesions. In conclusion, RASA1 is an important gene for PWS with specific clinical characteristics. (vikkula@bchm.ucl.ac.be) (http://www.icp.ucl.ac.be/vikkula).

Evolution of interaction in disease-related, zinc-finger proteins: Stabilization to regulation in WT1, ZIC, and GLI proteins. *G.J. Wyckoff[1], A.K. Solidar[1], A. Bergen[1], J.H. Laity[2]* 1) Div Molec Biol & Biochemistry, Univ Missouri, Kansas City, Kansas City, MO; 2) Div Cell Biol. & Biophysics, Univ Missouri, Kansas City, Kansas City, MO.

Cys_2His_2 zinc finger domains found in transcription factor proteins have been described as beads connected by flexible consensus peptide strings, which become ordered upon binding DNA targets. The highly specific interactions involving conserved zinc finger residue positions and nucleic acid bases underlie canonical zinc finger biology. A novel evolutionary approach is used which identifies over 150 genes within this newly-identified two-finger subclass within the larger Cys_2His_2 superfamily. These genes, including the disease-related proteins GLI, ZIC, and WT1; involved in human brain disoriders and cancer, have functions dependent upon close interactions between N-terminal zinc finger domains mediated by invariant tryptophan residues in each finger. Our data suggest that the evolution of these two-finger motifs proceeded due to selection driving finger stability, eventually leading to interaction between fingers. This produced diverse biological functions including regulatory roles mediated by finger interaction. To assess whether positive, Darwinian selection has acted to drive the functional diversity in the two-finger class, we culled a large number of proteins from human and several other species. While two-finger proteins are relatively well-conserved across homologous proteins, Ka/Ks comparisons across types of domains was often higher than one-- a hallmark of adaptive selection (p-value < 0.0001 via T-test). This finding has implications for understanding disease causal mutation in proteins involved with developmental disorders and cancer mediated by zinc finger function.

The Jackson Laboratory Repository: New mouse models of human disease. *S. Rockwood, D. Bergstrom, B. Chang, L.R. Donahue, K.R. Johnson, C.M. Lutz, M. Sasner, M.T. Davisson, The Repository Team* The Jackson Laboratory, Bar Harbor ME.

The mouse continues in its role as one of the most persistent and utilitarian model organisms, evidenced by the ever increasing number and variety of models generated. To ensure ready access of these mice to the scientific community The Jackson Laboratory Repository serves as a centralized facility for the purpose of developing, rederiving, cryopreserving and distributing mouse models to the international biomedical research community. Approximately 200-300 new strains are added annually to the hundreds of unique mouse strains that comprise the largest collection of characterized mouse strains available. Models newly imported and/or developed for distribution have applications in studies focusing on Spinal Muscular Atrophy (SMA), Cystic Fibrosis and Alzheimers disease. Larger sets of mice originating from Deltagen (NIH-sponsored) and the Consortium for Functional Glycomics have been added to the Repository in the last year. Mutant mice useful in neurological (Grin 1 glutamate receptor KO, and a Tg (Gfap-Tk) 7.1Mvs/J transgenic) and immunological (Ccr7 and Cxcr3 chemokine receptor KOs) studies are also newly available. Supplementing these strains are a wide variety of cre-expressing strains (constitutive or inducible), a growing number of targeted mutants harboring loxP-flanked genes and tetracycline regulated transactivator-expressing mice. Two consomic and 6 Recombinant Inbred strain panels are maintained for complex trait analysis. An on-line resource (www.jax.org) allows researchers to retrieve information related to strains in the Repository. Donating a strain to the Repository fulfills the requirements for sharing of mice required by NIHs policy for the sharing of research reagents. Researchers wishing to have strains considered for inclusion in the Repository may use the submission form available at: http://www.jax.org/grc/index.html The Jackson Laboratory Repository is supported by the NCRR (RR09781, RR11083, RR16049), NIA, The Howard Hughes Medical Institute, The Ellison Medical Foundation and donations from several private charitable foundations.

X chromosome inactivation patterns in females with Prader-Willi syndrome. *M.F. Theodoro[1], Z. Talebizadeh[1], D.J. Driscoll[2], M.G. Butler[1]* 1) Children's Mercy Hospital and University of Missouri-Kansas City, Kansas City, MO; 2) University of Florida, Gainesville, FL.

Prader-Willi syndrome (PWS) is characterized by infantile hypotonia, feeding difficulties, hypogonadism, small hands and feet, mental deficiency, behavioral problems, and obesity in early childhood. This genomic imprinting disorder is caused by loss of paternally expressed genes from the 15q11-q13 region due to a paternally derived deletion of the region or maternal disomy 15 (UPD). Maternal disomy 15 is thought to occur by maternal meiosis I nondisjunction associated with advanced maternal age and after fertilization with a normal sperm leads to trisomy 15, a lethal condition unless trisomy rescue occurs in early pregnancy with loss of the paternal chromosome 15. If trisomy 15 rescue occurs in the early stages of pregnancy, an unequal X chromosome inactivation pattern could result in the embryo. In healthy females, X chromosome inactivation follows a Gaussian or bell-shaped distribution with highly skewed patterns being uncommon events. To further characterize the maternal disomy 15 process in PWS and how it occurred, the status of X chromosome inactivation was calculated to determine whether nonrandom skewing of X inactivation was present which would indicate its origin from a small pool of early embryonic cells. We studied X chromosome inactivation in peripheral blood DNA from 110 Caucasian females (25 with PWS-UPD, 35 with PWS-deletion, and 50 healthy, unrelated controls without PWS) with similar means, medians, and ranges of ages using the X-linked polymorphic androgen receptor (AR) gene assay. No correlation was detected with X chromosome inactivation and age for any subject group. However, a significantly increased level ($p=0.024$) of extreme X inactivation skewness (>90%) was detected in our PWS-UPD group (6 of 25 subjects, 24%) compared to controls (2 of 50 subjects, 4%). This observation would further support that trisomy 15 occurs at conception with poor growth of cells and trisomy rescue in early pregnancy. In addition, extreme X inactivation skewness may lead to expression of X-linked recessive disorders in PWS females with UPD and extreme X chromosome skewness.

Butyrate mediates decrease of histone acetylation centred on transcription start sites and downregulation of associated genes. *C. Wadelius[1], A. Rada-Iglesias[1], A. Ameur[2], S. Enroth[2], C. Koch[3], G. Clelland[3], P. Respuela-Alonso[1], S. Wolcox[3], O. Dovey[3], P. Ellis[3], C. Langford[3], I. Dunham[3], J. Komorowski[2]* 1) Department of Genetics and Pathology, Uppsala University, Sweden; 2) Linnaeus Centre for Bioinformatics, Uppsala, Sweden; 3) Wellcome Trust Sanger Institute, Cambridge, UK.

Butyrate is a histone deacetylase inhibitor (HDACi) with anti-neoplastic properties, which theoretically reactivates epigenetically silenced genes by increasing global histone acetylation. It is structurally similar to valproic acid and phenylbutyrate that are approved for treatment of epilepsy and cancer. However, recent studies indicate that more genes are down-regulated than up-regulated by this drug. We treated hepatocarcinoma HepG2 cells with butyrate and characterized the levels of acetylation at DNA-bound histones H3 and H4 by ChIP-chip, using arrays covering 1% of the genome as defined in the ENCODE project. Western blot showed a marked increase of histone acetylation after treatment. However, ChIP-chip showed that many genomic regions close to transcription start sites were deacetylated after butyrate exposure. Only a few regions showed and increase in histone acetylation. ChIP and locus specific PCR showed that both butyrate and trichostatin A treatment resulted in histone deacetylation at selected regions, while nucleosome loss or changes in histone H3 lysine 4 trimethylation (H3K4me3) did not occur in such locations. Histone deacetylation was observed when colon adenocarcinoma HT-29 cells were treated with butyrate. Genes with deacetylated promoters were downregulated by butyrate, and this was mediated at the transcriptional level by affecting RNA polymerase II (Pol2) initiation/elongation. Immunofluorescence showed that the global increase in acetylated histones was preferentially localized to the nuclear periphery, indicating that it might not be associated to euchromatin. Our results are significant for the evaluation of HDACi as anti-tumourogenic drugs, suggesting that previous models of action might need to be revised, and provides an explanation for the frequently observed repression of many genes during HDACi treatment.

Development of verified quality control materials from the Coriell Cell Repositories fulfills needs of genetic testing community. *T. Sellers*[1], *D. L. Coppock*[1], *L. Kalman*[2] 1) Coriell Institute for Medical Research, Camden, NJ; 2) Centers for Disease Control and Prevention, Atlanta, GA.

Background: The expansion of molecular genetic testing in clinical and public health practice has increased the need for appropriate, verified quality control (QC) materials. However, for many tests the necessary QC materials are not publicly available. **Methods:** The Coriell Cell Repositories (Coriell) routinely conducts surveys at the American and European Societies of Human Genetics meetings to gauge interest in samples with characterized mutations. Individuals citing interest in these samples were asked to indicate desired diseases. Based on the input from these surveys and other sources, Coriell, in collaboration with the CDC's Genetic Testing Quality Control Materials Program (GTQC) and the genetic testing community, is developing verified QC materials based on cell lines from Coriells NIGMS Human Genetic Cell Repository. Cell lines containing clinically important alleles for Huntington disease (HD), Fragile X syndrome (FX) and 9 disorders common in the Ashkenazi Jewish (AJ) population were chosen for QC material development based on input from the genetic testing community. DNA prepared from each cell line was sent to multiple volunteer laboratories that verified the mutation using a variety of testing methods, including DNA sequence analysis. **Results:** Surveys were administered to 633 individuals at 7 meetings from 2001 to 2005. Of the 378 respondents (59.7%) who indicated interest in samples with characterized mutations, FX (45.1%) and cystic fibrosis (36.8%) were most commonly suggested. HD (20.9%) and the AJ disorders were also commonly requested. To date, GTQC, Coriell, and the genetic testing community have verified the alleles in 57 cell lines from 11 disorders and additional verifications are being planned. These materials are publicly available through Coriells NIGMS Repository. **Conclusions:** The development of verified QC materials by this collaborative effort will fulfill needs expressed by the genetics community and will help improve both the quality and accuracy of genetic testing.

Genetic analysis for ordinal traits. *H. Zhang, Y. Ye, X. Wang* Dept Epidemiology/Public Hlth, Yale Univ Sch Medicine, New Haven, CT.

For complex diseases, especially mental health conditions including nicotine dependence and substance use, the outcome variables are often recorded in an ordinal rather than quantitative scale. The naturally recorded ordinal traits are commonly analyzed either as quantitative traits or being dichotomized. We demonstrate that these commonly used approaches to dealing with ordinal traits are inadequate and result in loss of power. We propose a new framework and powerful statistical tests to conduct linkage and association analyses for any complex traits that include ordinal traits. Through simulation studies, we compare the type I error and power of our new methods with existing tests. The empirical result suggests that our methods produce reasonable type I errors and have power far exceeding (sometimes doubling) those of existing tests. We applied our proposed methods to identify genes for complex diseases. For example, using a data set on alcohol dependence, we found that six single nucleotide polymorphisms (SNPs) are significantly associated with alcohol dependence after adjusting for gender and age, and three of them were identified for the first time, underscoring the powerful of our new methods.

Townes-Brocks Syndrome and Renal Disease. *W. Reardon[1], R. Birkenhaeger[2], L. Casserly[3], J. Kohlhase[4]* 1) National Centre for Medical Ge, Our Lady's Hospital for Sick Children, Dublin, Ireland; 2) Dept of Otorhinolaryngology, University Clinics Freiburg, Freiburg, Germany; 3) Regional Hospital, Doordoyle, Limerick, Ireland; 4) Institute of Human Genetics and Anthropology, University of Freiburg, Freiburg, Germany.

Having presented at age 43 with unexplained chronic renal failure, an unrecognised case of Townes-Brocks syndrome was subsequently successfully treated by renal transplantation. First seen in the genetics clinic at age 49, the diagnosis was established clinically and a novel heterozygous mutation, resulting in a premature stop codon (Q507X), identified in the SALL1 gene, the locus for Townes-Brocks syndrome. Some clinical features in the patient, in particular myopia and hypothyroidism, were atypical of Townes-Brocks syndrome, leading us to exclude possible alternative pathologies by radiological and molecular approaches. Renal agenesis is established in mouse models of Sall1 deficiency but affected animals die in the neonatal period. Our observation of renal failure in this adult patient with Townes-Brocks syndrome led us to review the experience of renal malformation and physiological renal disease in this syndrome, establishing that both structural and functional abnormalities of the kidney are associated with SALL1 mutation. We suggest that longitudinal screening for renal disease may be justified in all patients with Townes-Brocks syndrome.

27-hydroxy-7-dehydrocholesterol is an endogenous teratogen in Smith-Lemli-Opitz syndrome that decreases cholesterol levels in patients and increases phenotypic severity in mice. *F.D. Porter[1], N. Javitt[2], M.F. Starost[4], M. Lyman[1], E. Leitersdorf[3], C.A. Wassif[1]* 1) HDB, NICHD, NIH Bethesda, MD; 2) NYU, New York, NY; 3) Hadassah Hebrew Univ Med Center, Jerusalem; 4) DVR, OD, NIH Bethesda, MD.

Smith-Lemli-Opitz syndrome (SLOS) is a malformation, mental retardation syndrome with a variable phenotype. SLOS results from a deficiency of 7-dehydrocholesterol reductase (DHCR7) activity. DHCR7 catalyzes the reduction of 7-dehydrocholesterol (7DHC) to cholesterol. 27-hydroxycholesterol (27-OH-C) which is synthesized from cholesterol by CYP27, may play a role in cholesterol homeostasis. In SLOS an atypical oxysterol, 27-hydroxy-7-dehydrocholesterol (27-7DHC) is present. We thus investigated whether 27-7DHC contributes to the pathology of SLOS. A negative correlation ($r2=0.79$, $p<0.001$) between plasma 27-7DHC and cholesterol levels was found in SLOS patients. No correlation was found between cholesterol and 27-OH-C or 7DHC. Previous work showed that hedgehog signaling is impaired by low cholesterol levels. We thus hypothesized that increased 27-7DHC levels would have detrimental effects during development due to suppression of cholesterol levels. To test this hypothesis we produced SLOS mice (*Dhcr7-/-*) expressing a *CYP27* transgene. *CYP27Tg* mice have elevated *CYP27* expression and 27-C levels, but normal cholesterol levels. *Dhcr7-/-* mice are growth retarded, have a low incidence of cleft palate (9%), and die during the first day of life. *Dhcr7-/-:CYP27Tg* embryos are stillborn and have multiple malformations. These include marked growth retardation, micrognathia, cleft palate (77%), lingual and dental hypoplasia, ankyloglossia, umbilical hernia, cardiac defects, cloacae, curled tails, and limb defects. Autopod defects (polydactyly, syndactyly and oligodactyly) were observed in 77%. Brain defects include abnormalities of the corpus callosum and cortical plate. The *Dhcr7-/-:CYP27Tg* model more closely resembles severe SLOS. As hypothesized, sterol levels were decreased 2-fold in liver and 20-fold in brain tissue in *Dhcr7-/-:CYP27Tg* compared to *Dhcr7-/-* embryos. Recognition of the role of 27-7DHC in SLOS may explain some of the phenotypic variability and may lead to development of novel therapies.

Pancreatic islet cell tumor in a germline CDH1 mutation carrier. *S.M. Weissman[1], W.S. Rubinstein[1,2]* 1) Ctr Medical Genetics, Evanston NW Healthcare, Evanston, IL; 2) Northwestern University Feinberg School of Medicine.

Hereditary diffuse gastric cancer (HDGC), a highly penetrant, autosomal dominant hereditary cancer syndrome caused by mutations in the CDH1 (E-Cadherin) gene, accounts for about 1-3% of all gastric cancers. Female germline CDH1 carriers face an 83% lifetime risk of diffuse gastric cancer (DGC) and a 39% lifetime risk of breast cancer with a predilection towards a lobular histology. Male carriers face a 67% lifetime risk of DGC. Some reports suggest that signet ring colon carcinoma is a feature of HDGC. We report for the first time an individual with a CDH1 germline mutation who developed a pancreatic islet cell tumor. The proband was a 66-year-old Caucasian female referred for a history of bilateral breast cancer (infiltrating lobular at age 58; contralateral infiltrating ductal at age 66) and a family history of breast cancer in her mother (age 77) and ovarian cancer in her maternal grandmother. Expansion of the family history revealed a history of gastric cancer in her sister (age 31) and father (age 50) and breast cancer in her paternal grandmother (age 75) and only paternal aunt (age 66). The proband was tested for BRCA1 and BRCA2 and found to have a previously unreported BRCA2 variant of uncertain significance (E2175Q). Four months after receiving her gene test results while being treated for breast cancer, she was diagnosed with stage IV DGC. During her total gastrectomy, significant adenopathy was noted in the tail of the pancreas and a subtotal distal pancreatectomy was performed. Final pathology revealed a well differentiated, pancreatic islet cell tumor metastatic to 7 of 9 peripancreatic lymph nodes. CDH1 genetic testing performed on research protocol revealed a germline CDH1 mutation (2195G>A) which was confirmed in a clinical laboratory. Further testing in the family revealed her sister with gastric cancer to have been an obligate carrier. This case may be an example of delayed recognition of component tumors of lower penetrance in HDGC. IHC staining of the probands pancreatic islet cell tumor for e-cadherin will be provided as evidence regarding whether the germline CDH1 mutation played an etiologic role in the development of this tumor.

Physical Mapping of 7q22 Deletions and Identification of a New Candidate Gene in Uterine Leiomyoma. *T. Ptacek[1], C. Song[2], C.L. Walker[3], S.M. Sell[2]* 1) Dept Biol, Univ Alabama at Birmingham, Birmingham, AL; 2) Division of Nutritional Genomics, Department of Nutrition Sciences, Univ Alabama at Birmingham, Birmingham, AL; 3) Department of Carcinogenesis, University of Texas and MD Anderson Cancer Center, Smithville, TX.

Uterine leiomyoma (UL) is a common, benign smooth muscle tumor of the uterus that can cause serious health problems. Recently, we reported a 7q22 microdeletion interval containing two known genes: origin recognition complex subunit 5-like (*ORC5L*) (previously studied in UL) and lipoma HMGIC fusion partner-like 3 (*LHFPL3*) (unstudied in UL). *ORC5L* codes for a subunit of the ORC. It has been proposed that hemizygosity of *ORC5L* contributes to tumorigenesis by disrupting ORC regulation. *LHFPL3* is a member of the tetraspanin superfamily of transmembrane proteins; tetraspanins have roles in proliferation, motility, differentiation and extracellular matrix formation, and have been implicated in other cancers. Although our laboratory is the first to study *LHFPL3* in UL, *LHFPL3* may have been missed previously because it was not annotated until recently. For this study we examined *LHFPL3* expression in human and Eker rat UL, and performed bioinformatics analyses of previously described 7q22 deletions from six reports. We present data for two patterns of *LHFPL3* expression in UL (reduced and elevated expression). Our expression data are consistent with reports of multiple expression patterns, at the mRNA and/or protein levels, for other genes in UL. We present data for four distinct deletion intervals at 7q22. Previous studies used the smallest region of overlap (SRO) method to define two 7q22 deletion intervals, and relied on genetic marker order as opposed to the physical placement of the markers. This is the first non-SRO comparison of single sample UL 7q22 deletions using complete human genome sequence information. Our findings support a role for *LHFPL3* in UL, do not rule out a role for *ORC5L*, and suggest the presence of at least four distinct deletion intervals in UL with 7q22 deletions. Future studies will examine the expression of *LHFPL3* in UL using a larger sample size, with tumors typed for UL chromosomal aberrations.

Genetic variation in the paraoxonase 3 (PON3) gene is associated with serum PON activity. *D.K. Sanghera[1], S. Manzi[2], P. Shaw[2], A. Kao[2], F. Bontempo[3], C. Kammerer[1], M.I. Kamboh[1]* 1) Human Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Division of Rheumatology and Clinical Immunology, Lupus Center of Excellence, University of Pittsburgh, Pittsburgh, PA; 3) 3Department of Medicine, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA.

Low serum PON activity determined by paraoxon substrate is associated with coronary heart disease and diabetes. Recently we have reported that low PON activity is also associated with SLE risk. The known genetic factors that influence PON activity are two SNPs in the PON1 gene, including Q192R and L55M. PON3 is linked to PON1 but, to our knowledge, the role of genetic variation in the PON3 gene in relation to PON activity has not been evaluated. In this investigation, we have examined the role of genetic variation in the PON3 gene in relation to PON activity and SLE risk in a biracial sample comprising 377 SLE patients (336 White, 41 Black) and 482 controls (442 White, 40 Black). We genotyped 6 PON3 tagging single nucleotide polymorphisms (tagSNPs) and examined their associations with PON activity, SLE risk, antiphopholipid autoantibodies (APA), lupus nephritis, carotid vascular disease and lipid profile. With the exception of the PON activity, no other significant associations were found with PON3 SNPs. Multiple regression analysis including all 6 PON3 tagSNPs and PON1/Q192R and L55M SNPs revealed significant associations with 4 SNPs: PON3/rs 740264 ($p<0.0001$), PON3/rs1053275 ($p=0.014$), PON 1/L55M ($p< 0.000$) and PON1/Q192R ($p< 0.000$). These four SNPs explained 3%, 2%, 8% and 19% of the variation in PON activity, respectively. In summary, our new data indicate that genetic variation in the PON3 gene influences serum PON activity independently of the known effect of PON1 genetic variation. The role of combined genetic variation at these linked loci may provide a better picture of the overall genetic control of PON.

Trends in the mortality of black children with sickle cell disease, United States, 1983-2002. *E.A. Yanni, R.S. Olney, S.D. Grosse, Q. Yang, J. Xing* NCBDDD, Centers for Disease Control and Prevention, Atlanta, GA.

BACKGROUND: Sickle cell disease (SCD) is one of the most common disorders identified by newborn screening in the United States. This study aimed to describe national trends in SCD mortality among black children from 1983 through 2002.

METHODS: We used U.S. death certificate data (1983-2002) to calculate the number of SCD-related deaths among black children aged 0-14 years and U.S. census data on the number of black children in each year to calculate SCD mortality rates in three age groups (0-3, 4-9, 10-14 years). We divided the study period into five intervals (1983-1987, 1988-1992, 1993-1996, 1997-2000, and 2001-2002) to analyze the possible effects of public health interventions on SCD mortality trends. We excluded deaths among children with SCD presumably caused by perinatal conditions, congenital anomalies in the early age group, autoimmune diseases, and different types of traumas.

RESULTS: The SCD mortality rate among black children decreased by 77% from 1983 through 2002 for 0- to 3-year-olds and by 51% for 4- to 9-year-olds. Statistically significant changes in mortality rates were also found for these age groups over specific, key time intervals. The SCD mortality rate decreased by 44% for 0- to 3-year-olds and by 34% for 4- to 9-year-olds for the period 1997-2000 versus 1983-1987 and by 36% for 0- to 3-year-olds for the period 1992-1996 versus 1983-1987. From 1997-2000 and 2001-2002, larger decreases in SCD mortality were observed at both 0-3 and 4-9 years of age. No significant changes in mortality rates for 10- to 14-year-olds were observed.

CONCLUSIONS: There was a significant decline in the mortality rates of black children with SCD younger than 10 years of age during the period of nationwide expansion of newborn screening for hemoglobinopathies. The substantial further decline in SCD mortality in recent years among younger children coincides with the approval of the 7-valent pneumococcal conjugate vaccine for infants in 2000. The 10- to 14-year age group might require different health care interventions to reduce SCD-related mortality.

Did phenylketonuria (PKU) arise after the Out-of-Africa migration? *C.R. Scriver[1], P. Hardelid[2], M. Cortina-Borja[2], A. Munro[3], H. Jones[4], Y. Foo[3], M. Cleary[3], M. Champion[4], C. Dezateux[2]* 1) Montreal Child Hosp Res Inst, McGill Univ, Montreal, PQ, Canada; 2) UCL Institute of Child Health, London, U.K; 3) Great Ormond Street Hospital, London, U.K; 4) Evelina Children's Hospital, London, U.K.

Prevalence rates of PKU in White Europeans and Chinese are similar (~ 1/10,000). While rare *PAH* mutation profiles are different, *PAH*-locus polymorphic haplotypes describe an "Out-of-Africa" human migration ~ 100,000 years ago (http://www.pahdb.mcgill.ca). There are no primary data on PKU prevalence for populations in Africa. Because anecdotal data known to us suggest that prevalence rates for PKU are low in populations of African descent, we studied prevalence rates in the multiethnic population of London (UK), where PKU screening and follow up are free and universal. We collected ethnic group data for all children screened for and diagnosed with PKU in the North and South East Thames areas (1994-2004 inclusive) as part of an audit of screening services. The denominator population was estimated by applying 2001 Census estimates of the ethnic group distribution of children aged <1yr to the number of live births in these areas, since direct estimates of births by ethnic group are not available. We obtained score-test-based confidence intervals for prevalence rates using Wilson's method. To date, complete data are available for North Thames. Among 121 children with PKU, 96 (79%) were of White British, White Irish or other White background; 23 (19%) were of Asian or other ethnic background and two were of unknown ethnic origin. No Black Caribbean, Black African or Other Black children with PKU were identified. The estimated proportions of White and Black births were 70 % and 9% respectively yielding birth prevalence rates for PKU in White children of 1.19/10,000 (95% CI 0.97-1.45/10,000), and 0/10,000 (0-0.39/10,000) in Black children. The findings support our hypothesis that the PKU phenotype is rare in African populations and arose after the Out-of-Africa migration of H. sapiens. Acknowledgements: Pia Hardelid is funded by the Medical Research Council (UK) and Department of Health.

Identification of Kalirin gene as a novel coronary artery disease gene through peak-wide association mapping on chromosome 3q13-21. *L. Wang[1], E.R. Hauser[1], S.H. Shah[1,2], C. Haynes[1], M. Harris[1], J. Rombaut[1], D. Crosslin[1], S. Nelson[1], A.B. Hale[1], S.G. Gregory[1], W.E. Kraus[2], M. Pericak-Vance[1], P.J. Goldschmidt-Clermont[2,3], J.M. Vance[1], GENECARD. Investigators[1,4,5,6]* 1) Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Dept of Med and Division of Cardiology, Duke Univ Medical Ctr, Durham, NC; 3) Miller School of Med, Univ of Miami, Miami, FL; 4) Vanderbilt Univ, Nashville, TN; 5) Univ of Wales College of Med, Cardiff, UK; 6) Univ of Sheffield, Sheffield, UK.

A susceptibility locus for coronary artery disease (CAD) has been mapped to chromosome 3q13-21 in a linkage study. Previously, we reported that one gene (LSAMP) underneath the linkage peak marker was associated with severe CAD. Herein, we completed a peak-wide association mapping using two independent Caucasian case-control datasets (CATHGEN, initial and validation) to see if additional loci were contributing to this region. Single nucleotide polymorphisms (SNPs) evenly spaced at 100 Kb intervals were screened in the initial dataset (N=372). Promising SNPs ($p<0.1$) were then examined in the validation dataset (N=383). Significant findings ($p<0.05$) were evaluated in additional datasets, including a family-based dataset (N=2954), an African-American case-control set (N=220), and a population-based Caucasian control set (N=255). The association between SNP and atherosclerosis was examined in 140 human aortas. Peak-wide survey found 3 SNPs in Kalirin gene to be associated with early-onset CAD (age-at-onset<55) in both CATHGEN datasets ($p<0.05$). Further genotyping in the gene found that rs9289231 was associated with early-onset CAD in all datasets ($p<0.05$). In the joint analysis of all Caucasian early-onset CAD cases (N=461) and controls (N=619), rs9289231 was highly significant ($p=0.0001$) with an odds ratio estimate of 2.2. In addition, the risk allele of this SNP was associated with atherosclerosis burden ($p=0.03$). Kalirin gene was associated with CAD in multiple independent datasets (N>4000). Kalirin is a protein with many functions, including the inhibition of inducible nitric oxide-synthase. Our data suggest that Kalirin is a novel candidate gene for CAD susceptibility.

Decreased expression of *Abcg5/Abcg8* is associated with increased tissue incorporation of dietary plant sterols and stanols in diabetic BB rats. *K.A. Scoggan[1,2], H. Gruber[1], L.J. Plouffe[1], J.M. Lefebvre[1,3], H. Rocheleau[1], B. Wang[1], J. Bertinato[1], M.R. L'Abbé[1], W.N.M. Ratnayake[1]* 1) Nutrition Research Division, Health Canada, Ottawa, ON, Canada; 2) Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, ON, Canada; 3) Department of Biochemistry, Carleton University, Ottawa, ON, Canada.

Type 1 diabetes is associated with low synthesis and high absorption of cholesterol which also characterizes the rare autosomal recessive disorder, sitosterolemia. Mutations in either ABCG5 or ABCG8 cause sitosterolemia and result in increased retention of dietary plant sterols. The objectives of this study were to determine if insulin-treated diabetic BB rats (BBdt), a model for type 1 diabetes, incorporated elevated levels of dietary plant sterols and stanols (PSS) in tissues and blood and to determine if sterol incorporation was associated with alterations in *Abcg5* and *Abcg8* transporters. BBdt and non-diabetic control (BBc) rats were fed a control diet or diets supplemented with plant sterols or stanols (5 mg/g diet) for four weeks. Tissue levels of PSS were measured by gas chromatography. Expression of *Abcg5/8* in liver and intestine was assessed by Western blot and/or RT-qPCR. The coding region of *Abcg5* and *Abcg8* genes were also investigated for mutations. BBdt rats showed increased accumulation of PSS, especially plant sterols, in the aorta, red blood cells, and liver compared to control rats when fed diets supplemented with plant sterols or stanols, respectively. Liver and intestinal *Abcg5/8* mRNA expression was lower in BBdt versus BBc rats fed the control diet. *Abcg8* protein was also decreased in liver of BBdt rats. Several SNPs were identified in *Abcg5* and *Abcg8* genes, however, none of these sequence variations resulted in amino acid changes. In conclusion, lower steady-state levels of *Abcg5/8* in BBdt rats may account for the increased accumulation of plant sterols in tissues of these rats. The lower levels of *Abcg5/8* in BBdt rats were not due to mutations in the coding region of either *Abcg5* or *Abcg8* genes. These results warrant studies to investigate the cause of increased incorporation of PSS in diabetic individuals.

Profiling Breast Cancer Cell Lines with High-density Oligonucleotide Array CGH. *S. Yu[1], M. Jorda[2], C. Gomez[2], Y.S. Fan[1]* 1) Dr. John T. Macdonald Foundation Center for Medical Genetics Leonard Miller School of Medicine, Miami FL University of Miami Medicine, Miami , FL; 2) Department of Pathology, University of Miami Miller School of Medicine, Miami, FL.

Genome-wide screening for DNA copy number changes in breast cancer cells will facilitate the identification of tumor suppressor genes and oncogenes. In this study, we have analyzed 4 epithelial breast cancer cell lines by microarray based comparative genomic hybridization (Array CGH) using 60-mer oligo microarrays (Agilent) containing 44 k probes for the whole human genome with a resolution of 35 ~75 kb. We have identified novel DNA segment alterations in addition to those occurring in previously reported regions. In brief, we found 80 gains of DNA segments ranging from 20 kb to 245 Mb in size (40 Mb in average) and 90 losses of DNA segments ranging from 35 kb to 138 Mb (25 Mb in average). 17 DNA segments showed multiple DNA copy amplifications. Very interestingly, we noted an over 30-fold amplifications of a DNA segment that contains only the cadherin genes, CDH1 and CDH3, in one cell line although E-cadherin has been considered as a tumor suppressor. There were 41 homologous DNA losses with a size ranging from 11kb to 55 Mb (average 26 Mb). Among them, common homologous DNA losses in at least two cell lines were observed on chromosomes 2p23.2(1.5Mb),3q28(7Mb), 8q21.13pter(58Mb), 17q11.1(2.9Mb), 17q12.1(200Kb), 17q2.32(4.5Mb), Xp11.21(3.8Mb). Complex alterations with multiple levels of changes occurred frequently on chromosome arms 3q, 8q, 17p, 17q, 22q and Xq. Novel DNA alterations identified by the array CGH at this time are being verified by fluorescence in situ hybridization (FISH) and Real-Time PCR. Further, FISH and real-time PCR will be performed on pure tumor cells isolated from breast cancer specimens by laser captured microdissection. Differential presentation of genomic alterations in breast cancer cells with different grades may also provide useful information for guiding treatment and predicting outcome of the patients.

HEM Dysplasia and Mouse Ichthyosis are not due to Sterol 14-Reductase Deficiency. *C.A. Wassif[1], K.E. Brownson[1], W.K. Wilson[2], M.F. Starost[3], F.D. Porter[1]* 1) Heritable Disorders Branch, NICHD, NIH, Bethesda, MD; 2) Rice University, Houston, TX; 3) DVR, OD, Bethesda, MD.

Mutations of the lamin B receptor (LBR) have been shown to cause HEM dysplasia in humans and Ichthyosis in mice. LBR has both a lamin B binding and a sterol 14-reductase domain. Although only a minor sterol abnormality has been observed, it has been proposed that LBR is the primary sterol 14-reductase, and that impaired sterol 14-reduction underlies HEM dysplasia. However, *DHCR14* also encodes a sterol 14-reductase. To test the hypothesis that LBR and DHCR14 are redundant sterol 14-reductases, we obtained Ichthyosis mice (Lbr-/-) and disrupted *Dhcr14*. *Dhcr14-/-* mice are phenotypically normal. No sterol abnormalities were found in either *Lbr-/-* or *Dhcr14-/-* tissues at 1 and 21 days of age. We then bred these mice to obtain compound mutant mice. *Lbr-/-:Dhcr14-/-* and *Lbr-/-:Dhcr14+/-* die *in utero*. *Lbr+/-:Dhcr14-/-* mice appear normal at birth, but by 10 days of age they are growth retarded and neurologically abnormal (ataxia and tremors). Pathological evaluation demonstrated vacuolation of and swelling of the myelin sheaths in the spinal cord consistent with a demyelinating process. This was not observed in either *Lbr-/-* or *Dhcr14-/-* mice. In contrast to *Lbr-/-* mice, *Lbr+/-:Dhcr14-/-* mice had normal skin and did not have Pelger-Huët anomaly. Peripheral tissue sterols were normal in all three mutant mice. However, significantly elevated levels (50% of total sterols) of cholesta-8,14-dien-3-ol and cholesta-8,14,24-trien-3-ol were found in brain tissue from 10 day-old *Lbr+/-:Dhcr14-/-* mice. Sterols were identified by GC/MS and NMR. These sterols were undetectable in *Lbr-/-* brain and only present at trace levels in *Dhcr14-/-* brain. Analysis of LBR and DHCR14 in human control and HEM dysplasia fibroblasts supports the redundant nature of these two proteins with respect to sterol 14-reduction. Our data supports the idea that HEM dysplasia and Ichthyosis are due to impaired lamin B receptor function rather than impaired sterol 14-reduction. Impaired sterol 14-reduction gives rise to a novel murine phenotype for which a corresponding human disorder has yet to be identified.

Developing genetic competency in undergraduate nursing students through the context of disease and the constructivist framework. *L. Tribble* Education Div, Greenwood Genetic Ctr, Greenwood, SC.

Background: The largest group of healthcare providers is registered nurses whose work allows a unique and holistic view of patients, often caring for patients throughout the life span. Nurses need to understand basic genetic concepts including the role of genes in common diseases, to identify individuals at risk through the collection of detailed family histories, to provide information about genetic testing and informed consent, and to know when and how to suggest referrals for genetic services.

Objective: To assess student opinion regarding constructivist methods of teaching in a semester-long course in human genetics for undergraduate nurses.

Methods: The study design was a single group, pretest-posttest of 35 3rd year nursing students in a semester course in human genetics. Students completed a 51 item objective assessment tool at the beginning and end of the course. Instruction utilized 3 constructivist approaches including case studies, family history analyses, and "shrink wrapped" lectures in addition to traditional lecture.

Results: Group analysis of pre-course and post-course objective assessments indicated statistically significant improvement in content knowledge. A post-course subjective assessment of teaching approaches showed preference to all 3 constructivist styles over traditional lecture and student presentations. Participants indicated confidence in their ability to obtain current genetic information and educational resources as well as an enhanced view of the role of genetics in common, complex diseases.

Conclusion: Based on the results of student preferences, it is suggested that curriculum design for nurses' training programs incorporate characteristics of constructivism, using a variety of teaching approaches. The curriculum should emphasize relevancy and application of material to nurses' work. Future studies should be conducted to determine if and how this instruction is utilized in actual clinical practice.

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Long-term improvement of bone disease and quality of life scores following treatment with Cerezyme in patients with skeletal manifestations of Type I Gaucher disease: Results of a 48-month single-arm, open label, clinical trial. *K.B. Sims[1], J. Barranger[2], P. Kaplan[3], H. Mankin[1], S. Packman[4], G.M. Pastores[5], B. Rosenbloom[6], D. Rosenthal[1], J. Angell[7], M.A. Fitzpatrick[7], N. Weinreb[8]* 1) Mass General Hosp, MA; 2) Univ of Pittsburgh, PA; 3) Children's Hosp of Philadelphia, PA; 4) Univ of California, San Francisco, CA; 5) NYU School Med, NY; 6) Tower Hematology/Oncology, CA; 7) Genzyme Corp, MA; 8) Univ Res Foundation, FL.

Objective: To evaluate the long-term effects of Cerezyme (CZ) on the skeletal and biochemical manifestations of Type I Gaucher disease (GD) and patients health-related quality of life (QOL). **Methods:** 33 CZ-naïve patients (age 12-70y) with skeletal manifestations of GD, and not treated with bisphosphonates, received CZ 60U/kg every 2 weeks for up to 48 months. Assessments included changes in bone mineral density (BMD) using dual energy x-ray absorptiometry (DEXA); incidence of bone pain (BP), bone crises (BC), and skeletal events; bone remodeling biomarker levels; and QOL (SF-36). **Results:** The mean change in BMD from baseline in lumbar spine and femoral neck DEXA Z-scores at 36 mos were +0.40 and +0.19, respectively ($p<0.05$). The incidence of BP decreased from 72% at baseline to 36% at 36 mos. No further episodes of BC were noted in 85% of patients with a history of BC. Bone formation biomarkers (osteocalcin and bone alkaline phosphatase) showed an increase from baseline by 6 mos and remained elevated through 48 mos ($p<0.05$). Osteoclast activity and bone resorption biomarkers (D-PYD and N-telopeptide crosslinks) decreased from baseline. New incidents of medullary infarction, avascular necrosis, lytic lesions and fractures were few and limited to the first 24 mos of CZ. QOL mean physical and mental component summary SF-36 scores increased by 24 mos ($p<0.01$) and achieved normal scores by 48 months compared with the reference population. **Conclusions:** CZ over 48 months resulted in meaningful improvements in the skeletal manifestations of GD that were associated with favorable changes in biomarker profile. These observations may account, in part, for the improvements in QOL.

The homogeneous mutant collagen in Brtl/Mov compound mice is associated with a milder osteogenesis imperfecta phenotype than occurs in heterozygous Brtl/+ mice, despite increased matrix insufficiency due to the mov13 null allele. *T.E. Uveges[1], J.A. Megnack[2], E.L.H. Daley[2], S.A. Goldstein[2], J.C. Marini[1]* 1) BEMB, NICHD/NIH, Bethesda, MD; 2) Ortho Res Labs, U. Mich, Ann Arbor MI.

The Brtl mouse (Brtl/+), a model for dominant-negative type IV osteogenesis imperfecta (OI), has a glycine substitution (G349C) in one col1a1 allele and a heterogeneous population of collagen forms, with 0, 1 or 2 mutant 1(I) chains. Surprisingly, mice homozygous (Brtl/Brtl) for this dominant mutation have a milder phenotype, with growth, BMD, and femoral geometry and mechanical properties intermediate between wt and Brtl/+. Brtl/Brtl synthesize homogeneous collagen with only mutant 1(I) chains (Forlino, 2005 EJHG 13(S1):61-65:C12), suggesting that heterogeneity of type I collagen may be an essential factor in OI severity. We generated mice with homogeneous mutant collagen by an alternative route; we crossed Brtl/+ with Mov13/+ mice, which have a null col1a1 allele. Analysis of the offspring indicate that Brtl/Mov mice also have a milder phenotype than Brtl/+ mice. Brtl/Mov, like Brtl/Brtl, has normal perinatal survival vs 30% lethality in Brtl/+. On skeletal staining, Brtl/Mov and Mov/+ do not have the in utero rib fractures of Brtl/+. Growth curves of Brtl/Mov and Mov/+ are intermediate between wt and Brtl/+. At 2 months of age, Brtl/Mov and Mov/+ dermal collagen fibril diameters were significantly smaller than Brtl/+ or wt fibrils. Brtl/Mov femurs have normal BMD and the same geometric adaptations found in Mov/+ (increased cross-sectional area and cortical thickness) rather than the improved material properties found in Brtl/Brtl. Brtl/Mov femurs also have improved mechanical properties (energy to failure and brittleness) compared to Brtl/+. Since Brtl/Mov femoral geometry and mechanics resemble Mov/+, we cannot rule out the possibility that the type I collagen insufficiency associated with the mov13 allele is disorganizing the matrix in a way that masks the weakening effect of the structurally abnormal collagen. However, the data on Brtl/Mov and Brtl/Brtl are most consistent with the interpretation that a homogeneous population of mutant collagen is associated with a milder OI phenotype.

Concurrent but apparently unrelated structural rearrangements of the band p13 of both chromosome 16 homologues in a case of renal angiomyolipoma. *S. Wei[1], C. Abbud-Mendez[2], M.W. Lee[2], A. Adeyinka[1]* 1) Dept Medical Genetics, Henry Ford Health System, Detroit, MI; 2) Dept of Pathology, Henry Ford Health System, Detroit, MI.

Angiomyolipoma is a benign neoplasm arising from the mesenchymal tissues of the kidney and exhibiting a collection of blood vessels, smooth muscle, and mature adipose tissue. It is the most common mesenchymal tumor of the kidney and accounts for 0.7-2% of all kidney tumors. About 80% of renal angiomyolipoma are sporadic and are not associated with Tuberous Sclerosis Complex (TSC), whereas 20% are associated with tuberous sclerosis. TSC-associated angiomyolipomas are often multiple and bilateral, but histologically indistinguishable from sporadic renal angiomyolipoma. Clonal chromosome aberrations have been reported in 7 cases of sporadic renal angiomyolipoma including trisomy 7 as a sole abnormality in 3 tumors, trisomy 8 as a sole abnormality in one case, monosomy 8 with deletions in 11q23 and Xq26 in one tumor, a t(7;12) in one tumor and a complex rearrangement involving chromosomes 12 and 21 in one tumor. Loss of heterozygosity (LOH) in the chromosomal region for the TSC2 gene (16p13) has been documented in up to 60% of TSC-associated angiomyolipomas, whereas only few cases of sporadic angiomyolipoma have had LOH of 16p13. We report the cytogenetic findings in a renal angiomyolipoma from a 47-year-old woman without clinical history of tuberous sclerosis. Metaphase cells from primary cultures of the renal mass were 46,XX,der(7)t(7;16)(q11.2;p13.3),der(16)t(7;16)(q11.2;p13.3)t(16;17)(q13;q22),inv(16)(p13q22),add(17)(q11.2). Both chromosome 16 homologues had structural rearrangement of band p13 the chromosomal location of the TSC2 tumor suppressor gene. Since two events are required to inactivate both copies of a tumor suppressor gene, the present findings suggest that concurrent structural changes involving both chromosomal bands 16p13 may be a mechanism of tumorigenesis in sporadic renal angiomyolipoma.

Gross Rearrangement in the MID1 gene in a patient with familial Opitz/GBBB syndrome: Implications for Molecular Diagnosis of Opitz/GBBB Syndrome. *A.B. Santani[1], E. Frackelton[1], C. Mulcahy[1], E. Zackai[2,4], D.M. McDonald-McGinn[2], T. Shaikh[2,4], D. Driscoll[3], C.A. Stolle[1]* 1) Dept of Path & Lab Med; 2) Div of Hum Genet, Children's Hospital of Philadelphia, Philadelphia, PA; 3) Dept of Ob & Gyn; 4) Dept of Pediatrics, UPenn School of Med, Philadelphia, PA.

Opitz G/BBB syndrome (OS), is a multiple congenital anomaly disorder that primarily affects midline structures. Clinical findings may include hypertelorism, cleft lip and palate, tracheo-esophageal clefts, heart defects, hypospadias, and developmental delay. Mutations in the MID1 gene have been identified in about 15-45% of males with the X-linked form of OS. This low mutation detection rate may reflect genetic heterogeneity, presence of intron/promoter mutations, or inability of current methodologies (SSCP, DHPLC, sequencing) to detect gross rearrangements. Three previous reports have identified genomic rearrangements of MID1 in patients with OS. Using Southern Blot analysis we identified a novel deletion encompassing the last two exons of the MID1 gene in a patient with familial OS. Characterization of the junction fragments by primer walking and long range PCR, confirmed the presence of a 13.8-kb deletion in the proband. Until recently, no rapid and reliable method was available to detect gross rearrangements. Assays such as Southern blot and FISH have obvious limitations. We have developed a real-time quantitative PCR assay for rapid identification of rearrangements in the MID1 gene. RQ-PCR primer/probe sets were designed for exons 8 and 9 that were deleted in the proband. The copy number of each exon was accurately determined for ten normal males and females demonstrating the high sensitivity and specificity of this assay. Analysis of the probands mother by RQ PCR confirmed her status as a carrier for the same deletion. Our results, along with previous studies, underline the importance of screening for major genomic rearrangements in the MID1 gene. The RQ-PCR assay is a simple, sensitive technique for rapid detection of exon dosage and we suggest that mutation screening in MID1 should include quantitative gene analysis in addition to DHPLC.

Attitudes Toward Genetic Testing and African Ancestry: How is Genetic Research Valued? *E.R. Santos[1], C. Glover[2], C. Bonilla[1], W. Hernandez[1], S.E. Hooker[1], P. Payne[2], R.A. Kittles[1], C. Royal[2]* 1) Ohio State University, Columbus, OH; 2) Howard University, Washington, DC.

Previous research has shown that African Americans distrust research; genetic research in particular. Yet genetic medicine has the potential to do more than just predict. It may ultimately be used at a personalized level to prevent and treat disease. There is a need to better understand the perceptions and attitudes toward genetic research that focuses beyond rare disorders. Insight into the knowledge, attitude, and behavior of African Americans is needed to better tailor prevention and treatment for individuals with high risk for cancer. Triangulation of quantitative and qualitative data helps elucidate associations between the knowledge, attitudes, and behaviors of two comparable populations. A volunteer sample of 415 adult participants was surveyed using Likert scale and open-ended questions that assessed knowledge, attitude, and behavior towards genetic testing. Triangulation of concepts combined quantitative (contingency tests) and qualitative data (content analyses) to investigate reported perceptions with behavior relevant to genetic testing. African Americans (n=415) from four communities from Oklahoma City, OK; Cincinnati, OH; Harlem, NY; and Washington, DC were surveyed. Baseline demographics of the four communities were comparable. Quantitative analysis revealed a strong association between different levels of knowledge regarding DNA testing and education (P0.005). Content analysis of the free-response survey questions showed recurring themes of prevention and family as important in seeking genetic testing. In comparison, a deterrent to seeking genetic testing was commonly stated to be the suspicion of abuse of information. Insight and understanding of knowledge, attitudes, and behaviors toward genetic research could possibly help maximize the impact of biomedical advances on communities most affected by health disparities. Proper utilization of genetic research could lead to more than diagnoses but also improving the odds through prevention and possible treatment; thereby fine tuning clinical treatment.

Gender-Related Clinical Features in a Large Cohort of Cowden Syndrome (CS) and Bannayan-Riley-Ruvalcaba Syndrome (BRRS) Patients with *PTEN* Mutations. *R. Pilarski[1], J. Stephens[2], H. Hampel[1], XP. Zhou[1], C. Eng[3]* 1) Human Cancer Genetics Program, and; 2) Center for Biostatistics, Ohio State University, Columbus, OH; 3) Genomic Medicine Institute, Cleveland Clinic, Cleveland, OH.

CS is a multisystem disease involving hamartomatous tissue overgrowth and increased risks for thyroid, breast and uterine cancer. Benign breast, thyroid, uterine and skin lesions are also common. BRRS is an allelic disorder characterized by macrocephaly, intestinal polyps, lipomas and pigmented penis macules. 80-85% of patients with CS and 60-65% of patients with BRRS have germline mutations in *PTEN*. Because of the rarity of these disorders, prior data on the frequencies of component clinical features have been based on small numbers of patients. METHODS: 2205 patients with at least one major and two minor or three minor CS/BRRS diagnostic criteria were accepted for research *PTEN* analysis. Clinical features present at the time of molecular testing were ascertained through the referring clinician using a checklist. When available, medical records were also reviewed. Age of onset was obtained for all cancer diagnoses. RESULTS: 206 patients (107 female, 99 male) were found to have probable deleterious mutations in *PTEN*. Age at molecular diagnosis was significantly earlier (23.8 vs 34.3 yo; $p<0.001$) for male vs. female patients. The expected clinical features of CS/BRRS were seen at increased frequency over the general population. Macrocephaly was almost twice as common as previously estimated (80% vs. ~40%). Aside from gender-specific features, female mutation carriers had more hemangiomas ($p=0.037$), thyroid cancers ($p=0.004$) and thyroid goiters ($p=0.003$) in contrast to MR/DD ($p=0.012$) favoring males. Borderline differences were seen for skin papules ($p=0.052$) and trichilemommas ($p=0.066$). DISCUSSION: This is the largest cohort of *PTEN* mutation-positive patients on whom clinical features have been reported. Significant differences were noted between genders in the frequency of several clinical features. In addition, macrocephaly and hemangiomas/AVMs were reported more commonly than expected from the literature. Projected lifetime risks for the component cancers will be presented.

Association of a functional SNP in the Interferon Regulatory Factor 5 (IRF5) Gene with Childhood-onset Systemic Lupus Erythematosus in Mexican Population. *R. Velazquez[1, 2], V. Baca[3], F. Espinosa-Rosales[4], M. Morales-Marin[1], G. Jimenez-Sanchez[1], L. Orozco[1]* 1) Laboratory of Research, National Institute of Genomic Medicine, Mexico City, Mexico; 2) PhD Biomedical Sciences-UNAM; 3) Department of Pediatric Rheumatology, CMN-Siglo XXI, IMSS. Mexico City, Mexico; 4) Department of Immunology, INP,SS. Mexico City, Mexico.

Introduction: Recently several studies have identified a functional single nucleotide polymorphism (SNP) rs2004640 C/T in the Interferon Regulatory Factor 5 (IRF5) gene as a susceptibility allele in Systemic Lupus Erythematosus (SLE) and Rheumatoid Arthritis. These findings and the observation that several autoimmune diseases tend to cluster in the same families suggest that different autoimmune diseases may share common susceptibility genes. The rs2004640 T allele creates a 5' donor splice site in an alternative exon 1 of IRF5, allowing expression of several unique IRF5 isoforms. Objetive: To asses the possible association between the rs2004640 SNP in the IRF5 gene and SLE in Mexican population. Methods. We performed a case control association study in 250 pediatric patients with SLE and 282 unrelated healthy Mexican controls. Genotyping of the rs2004640 SNP was carried out by the 5'exonuclease assay (TaqMan). Association with the SNP was analyzed by Chi square test. Results: The T allele frequency of the rs2004640 SNP was more frequent in SLE patients than in controls (63% vs 46%, P=1.33 x 10-7; OR=1.93, 95%CI= 1.5-2.5). We found a single copy of the rs2004640 T allele in 43% of cases, which conferred modest risk (OR=1.97, P=0.003), whereas the 41% of cases homozygous for the T allele were at a greater risk for SLE (OR= 3.31, P= 6.87 x 10-7). A family-based association was carried out in 130 families to rule out the possibility of stratification, (P=0.02). Conclusion: Our results confirm the association of IRF5 rs2004640 SNP with SLE and suggest that IRF5 gene could play an important role in childhood onset SLE susceptibility in Mexican population.

Albinism and Developmental Delay in an African patient: Emphasizing the need to test for 15q11-q13 deletion. *R. Saadeh, MD[1], E. C. Lisi, MS[1], D. Riegert-Johnson, MD[2], D.A. Batista, PhD[3,4], I. McIntosh, PhD[1,5], J.E. Hoover-Fong, MD[1]* 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Dept. of Gastroenterology, Mayo Clinic, Rochester, MN; 3) Dept. of Pathology, Johns Hopkins University, Baltimore, MD; 4) Cytogenetics Laboratory, Kennedy Krieger Institute, Baltimore, MD; 5) Dept. of Molecular and Cell Biology, American University of the Caribbean, St. Maarten.

Oculocutaneous albinism type 2 (OCA2) is the most common form of albinism worldwide and particularly common in Africans and African-Americans. OCA2 is an autosomal recessive disorder characterized by decreased pigmentation of the skin, hair, retina and iris with nystagmus, foveal hypoplasia, decreased visual acuity and optic nerve misrouting. Mutations in the P gene, located within the Angelman syndrome (AS) and Prader-Willi syndrome (PWS) critical region at 15q11-q13, cause OCA2. Patients with albinism should not have features found in AS (severe speech impairment, seizures, gait/movement disorder and happy demeanor) or PWS (psychomotor retardation, infantile hypotonicity, hyperphagia, obesity, hypogonadism and short stature). We report a 17-month old African female with cutaneous and ophthalmologic features of OCA2, as well as microcephaly, absent speech and tremulous somatic movement. Suspected co-morbid OCA2 and AS were confirmed by a cytogenetically visible deletion on chromosome 15q and absence of fluorescence in situ hybridization of the SNRPN probe for AS/PWS at 15q11.2. Molecular analysis revealed she inherited the common African 2.7-kb deletion in exon 7 of the P gene from her father and acquired a large de novo deletion of the AS critical region, including the P gene, from her mother. Query of 30,000+ patient pedigrees in the Johns Hopkins Genetic Clinic database revealed 49 albino patients, 8 with phenotypic features suggestive of AS or PWS. Further evaluation of these patients is underway to evaluate for these co-morbid conditions. Therefore, phenotypic features of AS or PWS in a patient with albinism should prompt investigation for these disorders.

An Analysis Pipeline for Large SNP Genotype Scans. *S. Stefanov, J. Lautenberger, M. Dean, B. Gold* LGD, NCI-Frederick, Frederick, MD.

Single nucleotide polymorphism (SNP) genotyping arrays are gaining in popularity. We developed an efficient pipeline for analysis of genotyping scan results. PC-Linux or Macintosh computers are advantageous because they avoid high performance hardware and most proprietary statistical software costs. Therefore, we developed an analytic platform in which extensive General Public License (GNU) software was coupled with Perl scripting. This permits efficient assembly of genotype calls and confidence scores from genotype data. We analyzed Affymetrix microarrays using both Dynamic Modeling (DM) and Bayesian Robust Linear Modeling using Mahalanobis Distance (BRLMM) algorithms. Calls are assembled into a single Prettybase file because it provides as an economic representation of SNP typing data that lends itself to analytic transportability. SAS software converts the prettybase files into SAS data sets and performs further analyses. For each marker and population, summary statistics including measures of Hardy-Weinberg equilibrium, Fis, allele and genotype frequencies are computed. We then calculate population differentiation statistics including informativeness for assignment (In), Kullback-Leibler divergence, entropy, Fst, and Ds. Pairwise linkage disequilbrium statistics D, D and R2 are elaborated using the command line version of HaploView. We have also used an R-program, provided by C. Carlson, to detect selection signatures via the Tajima's D statistic. The path of our pipeline consists of a contingency table analysis that calculates the likelihood of genetic association by comparing marker allele and genotype frequencies in affected versus unaffected individuals. Significant associations may indicate the proximity of a disease gene to the marker. Chi-square tests permit computation of a P-value for a genotype test, an allele test, and an Armitage linear trend test. In addition, we perform exact tests and compute odds ratios for both trend and allele statistics. Subsequent Perl scripts permit loading the SAS output into the Generic Genome Browser (gbrowse) for comprehensive visualization.

Polymorphisms in the PDCD1 are associated with childhood-onset Systemic Lupus Erythematosus in Mexican population. *Y. Saldaña[1], V. Baca[2], R. Velazquez[1,3], F. Espinosa-Rosales[4], M. Morales-Marin[1], S. Jimenez-Morales[1], G. Jimenez-Sanchez[1], L. Orozco[1]* 1) Laboratory Research, National Institute of Genomic Medicine, Mexico City, Mexico; 2) Department of Pediatric Rheumatology, CMN-Siglo XXI, IMSS, Mexico City, Mexico; 3) PhD Biomedical Sciences Program, UNAM; 4) Department of Immunology, INP, SS. Mexico City, Mexico.

Systemic Lupus Erythematosus (SLE) is a complex autoimmune disorder that predominantly affects women during their childbearing age. It is estimated that 15-17% of all SLE patients present in children younger than age 16. Initial manifestations of childhood-onset SLE are diverse and often more severe than in adults. Susceptibility to SLE has been attributed to complex interaction between genetics and environmental factors. Moreover, it has been suggested that genetics may play a greater role in children than adult onset SLE. An intronic polymorphism (PD1.3A) on the programmed cell death gene (PDCD1) has been associated with SLE susceptibility in several large case-control studies on adult patients. We hypothesized that the associated allele PD1.3A may also play a role in childhood onset SLE in Mexican patients. We performed a case-control association study in 261 unrelated childhood-onset SLE Mexican patients and 355 healthy individuals as control. We genotyped PD-1.3G/A polymorphism and three more single nucleotide polymorphisms (SNPs) within the PDCD1 gene (PD-1.1 G/A, PD-1.5C/T and PD-1.6A/G). Genotyping was carried out by 5'nuclease assay (TaqMan). The frequency of PD-1.3A allele was more frequent in SLE patients when compared with healthy controls (5.2% vs 2%, P=0.003; OR= 2.71; 95% CI = 1.35-5.50). Nevertheless, the frequencies of the PD1.1 G/A, PD1.5C/T and PD1.6A/G alleles were similar between the childhood-onset SLE patients and controls (30% vs 33%, 63% vs 64% and 54% vs 56% respectively). On the other hand in the haplotype analysis we observed that one haplotype characterized by the presence of PD1.3A allele, showed association with SLE susceptibility. Our results shown that PD1.3A is also a risk allele in childhood-onset SLE in Mexican population.

1p36 interstitial deletion and CVS mosaicism for add(1)(p36.1) in a child with asymmetry and multiple anomalies. *J. Wagstaff[1], L. Shaffer[2,3], D. Boles[4], A. Baughman[1], F. Grass[1]* 1) Clinical Genetics Program, Carolinas Medical Ctr, Charlotte, NC; 2) Health Research and Education Center, Washington State University Spokane; 3) Signature Genomic Laboratories, Spokane, WA; 4) Cytogenetics Laboratory, Presbyterian Hospital, Charlotte, NC.

Cytogenetic analysis of CVS direct preparation from a 32-year-old woman with history of a previous fetus with trisomy 21 revealed 8 cells with a structural abnormality involving additional material translocated onto 1p36 and 1 cell with normal female karyotype. Cultured chorionic villi and amniocytes each showed 20 cells with apparently normal female karyotype, 46,XX. The child was born at term with birthweight at the 5th percentile, length less than 3rd percentile, and head circumference at the 5th percentile. Her left ear was smaller than her right ear, and she had hypoplasia of the right fourth and fifth fingers and limb asymmetry with right upper extremity shorter than left and left lower extremity shorter than right. She was diagnosed with coarctation of the aorta at one week of age. Lymphocyte chromosome analysis showed a possible deletion of 1p36 in all cells. FISH with a subtelomeric probe from 1p36 detected no deletion; however FISH with a 1p36 probe containing D1S1347 and D1S1310 showed heterozygous deletion in all cells. Microarray CGH of lymphoblastoid cell line DNA confirmed a heterozygous interstitial deletion extending from 2.75 Mb to 9.25 Mb in 1p36. FISH analysis of 1p36 in lymphocytes from both parents gave normal results. The cytogenetic and molecular analysis of this patient suggests that she is mosaic for 2 cell lines: one cell line contains a 6.5 Mb interstitial deletion of 1p36; the other cell line contains translocation of unidentified chromosome material onto 1p36. We hypothesize that this mosaicism arose from a 1p36 break in an unreplicated chromosome, either in a gamete or in the zygote, followed by chromosome replication, then by repair of the break by different mechanisms on the two sister chromatids: on one sister chromatid by interstitial deletion, and on the other sister chromatid by translocation.

Mutations in the Novel Mitochondrial Protein REEP1 Cause Hereditary Spastic Paraplegia Type 31. *G. Wang[1], K. Viet[1], M. Nance[2], P. Gaskell[1], A. Ashley-Koch[1], M. Pericak-Vance[1], S. Züchner[1, 3]* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Park Nicollet Clinic, Minneapolis, MN; 3) Department of Psychiatry and Behavioral Science, Duke University Medical Center, Durham, NC.

Hereditary spastic paraplegia (HSP) comprises a group of clinically and genetically heterogeneous diseases that affect the upper motor neurons and their axonal projections. HSP is characterized by progressive lower limb spasticity and paresis of varying severity. So far, 11 genes have been identified for autosomal dominant HSP with most of them being a rare cause. In two multigenerational families we have found a novel HSP locus on chromosome 2p12. The combined two-point LOD score was 4.7 at marker D2S2951. By direct sequencing of candidate genes in the chromosomal area we isolated two deletions in the gene receptor expression enhancing protein 1 (REEP1). We screened index patients from 92 unselected HSP families and found out a total of six unique mutations which includes one missense mutation, a splice site mutation, and two small deletions leading to frame shifts and alternative stop codons. Two other changes occurred in a conserved predicted micro RNA target sites in the 3`-UTR of REEP1. We did not detect these mutations in 360 ethnicity matched controls. Using a REEP1 specific antibody we showed in different cell lines that the endogenous REEP1 protein targeted mitochondria. We conclude that the novel mitochondrial protein REEP1 accounts for about 6.5% of all HSP.

Evidence for paternal allele-specific chromatin extension and looping of the *SNRPN* to *UBE3A* locus in mature neurons by fluorescence in situ hybridization. *K.N. Thatcher, R.O. Vallero, J.M. LaSalle* Dept Med Micro & Immuno, Univ California, Davis, Davis, CA.

The imprinted region 15q11-13 contains a cluster of imprinted genes whose complex developmental and tissue specific expression is regulated by small imprinting control regions (ICR) within the cluster. Several neurodevelopmental disorders are associated with deletions, mutations or aberrant gene expression in this region including Angelman syndrome, Prader-Willi syndrome, autism, and Rett syndrome. It has been proposed that chromatin looping in this region may allow the paternal ICR to bi-directionally regulate gene expression over several Mb. *SNRPN* is a paternally expressed gene within the cluster whose gene products are involved in splicing and are highly expressed in brain. The brain-specific *SNRPN* transcription unit also includes multiple small nucleolar RNAs and the antisense to *UBE3A*. *UBE3A* is oppositely oriented to *SNRPN* and is paternally silenced in neurons, presumably by the *UBE3A* antisense. Utilizing DNA FISH we have found visual evidence for allele-specific chromatin extension and looping of the locus from *SNRPN* to *UBE3A* in human post-mortem brain. In mature neurons, the paternal allele was highly extended and looped into the euchromatin while the maternal allele was observed as a single spot found primarily in the heterochromatin. A similar pattern was found using a probe to the *Snrpn* locus in mouse brain samples of multiple ages. The paternal allele-specific chromatin extension increased with brain age and neuronal maturity (~0.5m at P1d to ~4m at P70d), while the maternal allele remained small and compact (~0.5m at all ages). High concentrations of RNAse A used pre- or post-hybridization did not affect the size of the chromatin structures, demonstrating that the extended signal was due to DNA not RNA. Extended paternal chromatin loops were specific to neurons as they were not found in glial cells, thymus, kidney, liver or spleen. These results suggest dynamic changes in chromatin conformation of the paternal *SNRPN* to *UBE3A* locus during neuronal maturation that may serve to regulate neuronal specific expression and imprinting in this region.

Tissue Specific Expression Of Type X Collagen In Hypertrophic Chondrocytes Is Specified By A 150 BP *Col10a1* Distal Promoter Element. *Q. Zheng[1], B. Keller[1], G. Zhou[1], D. Napierala[1], Y. Chen[2], A. Parker[3], B. Lee[1, 2]* 1) Molec & Human Genetics, Baylor College Med, Houston, TX; 2) Howard Hughes Med Inst. Houston, TX; 3) Respiratory and Inflammation Res. Area, Astrazeneca, Cheshire U.K.

Type X collagen gene (*Col10a1*) is a specific molecular marker of hypertrophic chondrocytes, a stage of late endochondral ossification during cartilage development. Multiple cis-elements and trans-acting factors have been shown to be involved in the regulation of type X collagen expression and therefore contribute to chondrocyte maturation. We have recently generated a series of transgenic mouse lines harboring various *Col10a1* promoter/intronic fragment (**Tg-10kb**) or distal *Col10a1* promoter elements (**Tg-6kb** and **Tg-4.6kb**) each driving a *LacZ* reporter. High-level tissue-specific reporter expression was observed in these transgenic lines. These data together with previous observations suggest that the *Col10a1* distal promoter element (-4.6 to -3.9 kb) harbors a critical tissue-specific enhancer that mediates its high-level expression in hypertrophic cartilage *in vivo*. (**Tg-4kb**, Zheng et al., 2003; Gebhard et al., 2004). To further localize the tissue-specific enhancer element within this region, three additional reporter constructs have been generated for similar transgenic studies. These constructs contain different *Col10a1* distal promoter fragments upstream of the *Col10a1* basal promoter each driving a *LacZ* gene (**Tg-2x700**, **Tg-4x300** and **Tg-4x150**). All three transgenic lines showed high-level tissue-specific reporter expression. This demonstrated that the 150 bp (-4.3 to -4.15 kb) *Col10a1* promoter element is sufficient to direct its high-level tissue-specific expression *in vivo*. Lastly, we have also generated a transgenic reporter construct in which Cre-expressing cassette is placed under the control of the 4x300 bp element and the *Col10a1* basal promoter. The tissue specificity of transgenic founders is being tested upon breeding onto the RosA26R genetic background. These potential hypertrophic chondrocyte-specific Cre-expressing transgenic lines and the **Tg-4x150** reporter lines enable us to study genes of interest that involve molecular pathogenesis of skeletal disorders and/or osteoarthritis.

Efficient multipoint analysis of association studies. *B. Servin, P. Scheet, M. Stephens* Statistics, University of Washington, Seattle, WA.

We present a new statistical method for analysing genetic association studies. The method improves on standard approaches in two ways. First, it exploits the availability of dense genotype data in a "panel" of unrelated individuals (eg the HapMap) to effectively increase the number of markers typed in the phenotyped individuals, and hence to increase both the power to detect association in a given region, and the ability to accurately identify the most plausible functional variants within a region. Second, instead of using a p value to measure the strength of the evidence for association between each SNP and the phenotype, we instead use a Bayesian measure of the strength of the evidence, a Bayes Factor (BF). Our presentation will include a description and illustration of some of the advantages of BFs over p values, including i) BFs naturally weight different SNPs according to how informative they are (eg their minor allele frequency), effectively reducing the burden of multiple testing; and ii) BFs provide a natural and efficient way to combine information over multiple SNPs in a region. For those who prefer p values to Bayesian approaches, we will also describe how the BFs for multiple individual SNPs can be turned into p values for assessing the overall strength of the evidence for association between the phenotype and the group of SNPs, and illustrate how this approach has increased power compared with the natural alternative of performing multiple single-SNP tests and correcting for multiple comparisons. We argue that single-SNP BFs, rather than single-SNP p values, should therefore become the standard choice for initially assessing associations in genomewide association studies.

Development of TaqMan snoRNA Assays for Endogenous Controls. *L. Wong, D. Ridzon, Y. Liang, P. Sali, C. Barbacioru, R. Samaha, E. Spier, D. Ginzinger, K. Livak, K. Guegler, C. Chen* Arrays and SDS R&D, Applied Biosystems, Foster City, CA.

Quantitation of microRNA (miRNA) genes has become a critical step in determining the function and identifying the biomarkers related to cell differentiation and disease states. A total of 236 TaqMan miRNA assays were used to profile the level of miRNA expression in 40 normal human tissues and 59 NCI-60 cell lines. Two types of endogenous controls were examined for normalizing the expression data. House-keeping genes including -actin, GAPDH, 18S rRNA, and 2M were first examined: of the four genes, 18S rRNA showed the least variation. Non-coding genes including snoRNAs and tRNAs were then considered as a new class of endogenous controls for normalizing miRNA expression data. Genes were selected based on the size, abundance, and ubiquitous nature of the small RNAs; and assays were designed using similar chemistry as the TaqMan miRNA assay. Eight tRNA and fifteen snoRNA candidates, ranging from 45 to 200 nucleotides, were evaluated. Expression of these small RNAs showed that they were relatively abundant and constant across all 40 human tissues or 59 NCI-60 cell lines. In particular, snoRNAs showed similar expression pattern to 18s rRNA as well as the least variable miRNA controls, indicating that snoRNAs are better controls for normalizing TaqMan miRNA expression data. A total of ten snoRNA assays including RNU24, RNU66, RNU19, RNU38B, RNU49, Z30, RNU6B, RNU48, RNU44, and RNU43 have been successfully released worldwide. Further, fourteen of the same snoRNA genes used in the TaqMan miRNA assays were subjected to conventional TaqMan assay design. Conventional TaqMan assays were then performed and data compared to the TaqMan miRNA assays. Similar to the TaqMan miRNA assays, the expression levels for these assays were constant across 38 normal human tissues. In addition, similar expression patterns for these snoRNAs were observed from both conventional TaqMan and TaqMan miRNA-like designs, suggesting that the small non-coding genes may also be better endogenous controls than the typical coding genes for normalizing conventional TaqMan assay expression data.

Penetrance of craniofacial anomalies in mouse models of Smith-Magenis syndrome is modified *in trans* by genomic sequence surrounding *Rai1*. *J. Yan*[1], *W. Bi*[1], *J.R. Lupski*[1, 2, 3] 1) Dept Molecular & Human Gen; 2) Dept Pediatrics, Baylor Col Medicine, Houston, TX; 3) Texas Childrens hospital, Houston, TX.

A characteristic craniofacial phenotype is presented in many genetic syndromes, which can enable an initial diagnostic impression to an experienced clinical geneticist and dysmorphologist. Craniofacial abnormality is one of the major clinical manifestations of the Smith-Magenis syndrome (SMS) and distinct enough to enable a clinical diagnosis. We constructed several SMS mouse models with chromosome engineered deletions of the mouse genomic region that maintains conserved synteny to the human SMS interval. The models include *Df(11)17* (a 2 Mb deletion) and *Df(11)17-1* (a 590 kb deletion), as well as *Rai1* targeted disruption. Analyses of these mutations in a mixed N2 genetic background revealed that the penetrance of the craniofacial phenotype is reduced with decreased deletion size. We have generated an additional deletion strain, *Df(11)17-4*, which contains a 1 Mb deletion. Remarkably, the penetrance of its craniofacial anomalies in an N2 background was between those of *Df(11)17* and *Df(11)17-1*. We further analyzed the deletion mutations and *Rai1*-/+ allele in a pure C57BL/6 background to examine isogenic littermates and to control for nonlinked modifier loci. The penetrance of the craniofacial anomalies was markedly increased for all the strains in comparison to the N2 background. Mice with *Df(11)17* and *Df(11)17-1* deletions had a similar penetrance for the craniofacial abnormalities, whereas that of *Rai1*-/+ mice was significantly reduced. Our results suggest that a *trans* regulator(s), that resides within the 590 kb genomic interval surrounding *Rai1*, is the major modifying genetic element(s) affecting the craniofacial penetrance. Moreover, we confirmed the influence of genetic background and different deletion sizes on the phenotype. The complicated control of the penetrance for one phenotype in SMS mouse models provides tools to elucidate molecular mechanisms for penetrance and clearly shows that null alleles caused by deletion can have different phenotypic consequences than null alleles due to gene inactivation.

Impact of haplotype frequency estimation and use of molecularly phased haplotypes in linkage analysis of a candidate gene region with linkage disequilibrium. *W. Sieh[1], C.-E. Yu[2], T.D. Bird[1,3], G.D. Schellenberg[2,3], E.M. Wijsman[1,4]* 1) Medical Genetics, University of Washington, Seattle, WA; 2) Gerontology and Geriatric Medicine, University of Washington, Seattle, WA; 3) Neurology, University of Washington, Seattle, WA; 4) Biostatistics, University of Washington, Seattle, WA.

Most current linkage analysis programs assume linkage equilibrium (LE) between markers. Violation of this assumption by the use of densely spaced SNPs in investigations of candidate genes can lead to inflated evidence for linkage. Linkage disequilibrium (LD) between SNPs can be accommodated by using multi-SNP haplotype frequencies, if known, rather than the component SNP allele frequencies. Phased haplotypes in candidate genes can provide gold standard haplotype frequency estimates, and are also of inherent interest as markers in linkage analysis.

We evaluate the impact of using haplotype frequencies estimated by molecular phase determination, maximum likelihood estimation, or by assuming LE in LOD score analysis. We also compare the effects of using directly observed haplotypes rather than unphased SNP markers for linkage analysis of a cluster of five SNPs in a candidate region for Alzheimers disease (AD) on chromosome 19p. Strong LD (r^2 = 0.88-0.95) was observed between two SNP pairs, while moderate LD (r^2 = 0.29-0.67) was found among the other SNP pairs. We show that ignoring LD between markers can lead to substantial (79%) inflation of the LOD score (from the referent value of 1.05 to 1.88), but that the method of haplotype estimation makes little difference provided that LD is allowed. Use of phased haplotypes produces a modest (8%) increase in the LOD score over unphased SNPs, under the same set of haplotype frequencies. Gains from using phase information may be important in studies where the costs of family recruitment and phenotyping greatly exceed the costs of marker phase resolution. Thus, molecular haplotyping may be a cost-effective approach in studies of AD, where the cost for recruiting and phenotyping each affected person is more than ten-fold higher than for a genome scan.

Strong genetic evidence for DYX1C1 as a susceptibility factor for dyslexia. *M. Zucchelli[1], F. Dahdouh[2], H. Anthoni[1], M. Peyrard-Janvid[1], I. Tapia Paez[1], J. Schumacher[2], G. Schulte-Körne[4], J. Kere[1,3], M.M. Nöthen[5]* 1) Department of Biosciences and Nutrition at Novum and Clinical Research Center, Karolinska Institute, Stockholm, Sweden; 2) Institute of Human Genetics, University of Bonn, Bonn, Germany; 3) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 4) Department of Child and Adolescent Psychiatry and Psychotherapy, University of Marburg, Marburg, Germany; 5) Dept of Genomics, Life & Brain Center, University of Bonn, Germany.

The DYX1C1 gene on chromosome 15 was the first candidate gene associated to Developmental Dyslexia based on cloning of a translocation breakpoint cosegregating with dyslexia and association of SNPs in a Finnish cohort of unrelated patients (Taipale et al. 2003). Until present, five groups have published replication studies on DYX1C1. One study that found significant association, though to a different haplotype, was considered positive replication, whereas four were interpreted as negative. However some of them reported nominally significant p-values to the same haplotype as the one in the positive replication. Thus, the role of DYX1C1 as a common susceptibility gene for dyslexia remains unsettled. To address this question, we resequenced the gene and found two new SNPs. Adding these and two more polymorphic sites from the database to the SNPs reported earlier, a refined haplotype structure was disclosed, splitting the most common haplotype into two. We show that the new most common haplotype is associated to dyslexia in a population of German descent (p-value = 0.008) and a similar trend is present in a small sample of Finnish descent (p-value = 0.27). A meta-analysis of our data and all the previous studies shows a global haplotype association p-value of 0.003. Finally, one the new SNPs that tags the haplotype division is found to regulate transcription factor binding, suggesting a mechanism for DYX1C1 susceptibility effect.

Study of the untranslated region of CFTR (ABCC7) by 5'-RACE in the human tissue. *X. Pepermans[1], V. Janssens[1], A. Bosmans[1], N. Bletard[2], C. Sempoux[2], Ch. Verellen-Dumoulin[1], P. Lebecque[3], K. Dahan[1]* 1) Center for Human Genetics, Cliniques Universitaires Saint-Luc, Bruxelles, Belgium; 2) Pathology, Cliniques Universitaires Saint-Luc, Bruxelles, Belgium; 3) Pediatric, Cliniques Universitaires Saint-Luc, Bruxelles, Belgium.

CFTR chloride channels are encoded by the gene mutated in patients with cystic fibrosis (CF), a recessive condition affecting all exocrine epithelia. Our goal was to validate in human tissues a recent publication showing strong evidence for additional CFTR exons 1 in cardiac rabbit (Davies et al., J Biol. Chem. 279, 2004:15877-15887). Indeed, they have identified two non-coding mRNA (AY256886 and AY256887) and one new coding mRNA (AY256888). We then examined the 5 untranslated region of CFTR mRNA in human pancreas, human colon and human liver selected to their high CFTR Transcriptional activity. Rapid amplification of 5cDNA and PCR (5RACE-PCR) revealed no novel form of exon 1 in a variety of human tissues suggesting that if alternative promoters of CFTR exist, they have no efficient transcriptional activity. Interestingly, we have observed the appearance of two RNA molecules from the colonic biopsy, one normal (r.-) and one containing an insertion of an 74bp intronic sequence between exon 2 and exon 3 (r.296_297ins74pb), responsible for a new reading frame ending in a stop codon at position +4 (p.Arg55ArgfsX59). This additional exon 2b never reported so far caused at the genomic level by a T-to-C transition within intron 2 (c.296+685T>C). The causal effect of this splice defect need to be evaluted in a control and affected population.

Increased efficiency of case-control association analysis by using allele-sharing and covariate information. *S. Schmidt, M. Schmidt, X. Qin, E.R. Martin, E.R. Hauser* Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC.

It has been reported previously that the efficiency of a case-control association analysis, which relies on linkage disequilibrium (LD) between marker and disease susceptibility alleles, can be improved by using linkage information to select cases for follow-up genotyping. Specifically, a linked best design that includes only cases from multiplex families with non-negative NPL scores was shown to provide power similar to a design including all family probands at a fraction of the genotyping cost (Fingerlin et al. 2004). The previous study did not examine the effect of disease-associated covariates, which may include environmental risk factors or measured endophenotypes, and may partially account for the genetic heterogeneity of many complex human diseases. Here, we examine the efficiency of several case and control selection strategies under three plausible covariate models: a QTL underlying a a trait that is a measured risk factor for the disease, a multiplicative gene-environment interaction (GxE) model and a heterogeneity model in which covariate distributions differ between linked and unlinked families. Results of our simulation study show that the linked best design provides very similar power as an analysis of all familial cases (all) under all three covariate models, even though linked best does not use covariate information. Under the QTL and heterogeneity models, an analysis of cases identified by the ordered subset analysis (OSA) can be substantially more powerful than either the linked best or all design. The extent of the power difference depends on the covariate model, the proportion of linked families in the dataset (), the susceptibility allele frequency and the strength of LD. For example, for a recessive QTL model with p=0.35, r^2=0.1, =0.5, the linked best and all designs both have power of 65%, compared to 87% for OSA, and for a recessive heterogeneity model with =0.2, the respective numbers are 41% vs. 84%.

Chronic renal insufficiency among South Indians with Type 2 diabetes mellitus: Role of oxidative stress pathway genes. *B. K. Thelma[1], ArunK. Tiwari[1], Pushplata. Prasad[1], K. M. Prasanna Kumar[2], A. C. Ammini[3], Arvind. Gupta[4], Rajeev. Gupta[5]* 1) Dept.Genetics,Univ.Delhi South Campus,New Delhi; 2) Dept.Endocrinology & Metabolism,MSR Medical College,Bangalore; 3) Dept.Endocrinology,Dept.Nephrology, AIIMS,New Delhi; 4) Jaipur Diabetes & Research Centre, Jaipur; 5) Monilek Hospital and Research Centre, Jaipur,India.

Chronic Renal Insufficiency(CRI), a microvascular complication, developing in ~20% patients with type2 diabetes mellitus(DM) is a leading cause of end stage renal disease worldwide. Genesis of CRI involves myriad of factors, hyperglycaemia and ethnicity being major ones along with variable genetic susceptibility. Hyperglycaemia induced oxidative stress plays a central role in the development of diabetic complications. Using CRI as a phenotype, we investigated the contribution of 26 SNPs from 13 commonly investigated genes, involved in maintenance of cellular redox balance, among patients with type2 DM with CRI (n=106)and without(n=148). CRI patients were those with duration of diabetes was 2yrs, serum creatinine 3mg/dl,UAER200mg/l and presence of diabetic retinopathy. PCR-RFLP based genotyping of the SNPs and test of allelic/genotypic association was carried out. Polymorphisms tested in SOD1, SOD3, VEGF, CYBA, PON1, PON2, GSTM1 & T1 genes were not associated with CRI. The wt genotype of SOD2 Ala9Val was found to be in excess in the CRI group [OR 1.92,95%;CI(1.15-3.21);p=0.01 for Ala/Ala]. Similar association and excess of the wt genotype was observed for UCP1 -112T>G [OR 2.07 (1.18-3.62); p=0.009 for TT]; UCP1Ala64Thr [OR 2.58,(1.31-5.07); p=0.005 for Ala/Ala]; eNOSGlu298Asp [OR 2.10,(1.19-3.69); p=0.009 for Glu/Glu] & GSTPI Ile105Val [OR 2.41,(1.42-4.06);p=0.0008 for Ile/Ile] SNPs. Unlike the other markers mentioned above, in UCP2 -866G>A SNP, the wt allele (p=0.03)& genotype GG was protective [OR 0.56,(0.34-0.93);p=0.02 for GG]. The notable observation in this study was the excess of wt allele in CRI patients for several associated markers. Thus, we may conclude that oxidative stress genes are an important predictor of development of complications and carriers of wt allele are probably more likely to survive the comorbid complications.

Comparative analysis of Copy Number Variation polymorphisms between Mexican Mestizos and European population. *L. Uribe-Figueroa, A. Hidalgo-Miranda, I. Silva-Zolezzi, J. Estrada-Gil, M. Arrieta, A. Contreras, G. Jimenez-Sanchez* National Institute of Genomic Medicine, Mexico.

Copy number variation (CNV) has been recently identified as a major source of human genomic diversity. These polymorphisms have been identified in several chromosomal regions. However, there is limited information about potential differences in CNV within and between populations. To identify CNV in the Mexican Mestizo population and compare them with European population, we used the Affymetrix 100K SNP Mapping Array and the DChip software to infer CNV in 104 samples from Mexican Mestizos. We compared our results with those obtained from the 60 parental samples of the CEU population analyzed in the International HapMap Project that were generated by the same technological platform. Results from each population were loaded into DChip and fluorescence intensity was normalized using the Invariant Set method. Signal values from each microarray set were calculated using the Perfect Match/Mismatch (PM/MM) Difference Model. To analyze CNV in the Mexican population, we performed a 1% trimmed analysis using a 3 SNP median smoothed window. For comparative analysis between populations, we calculated copy number in the Mexicans and compared it with 60 European samples used as a normal diploid control. Trimmed analysis in the Mexican samples showed several regions with 1 copy in 20% of the cases, while regions with 5 copies were detected in 15% of the samples. *In silico* comparative analysis with the European sample set showed a higher number of CNV sites, both gains and losses, present in more than 50% of the Mexican population. Our results suggest that the Mexican Mestizo population is, in general, homogeneous for CNV. However, they show important differences when compared with this European set of samples. CNVs have been reported in association with different pathologies. Thus, characterization of these variants in different populations will contribute to better understand their molecular contribution to human disease.

Cosegregation of the ND4 G11696A mutation with the LHON-associated ND4 G11778A mutation in a four generation Chinese family--Lebers hereditary optic neuropathy in a Chinese family. *J. Qu[1], X. Zhou[1], Y. Tong[1], L. Yang[1], F. Zhao[1], C. Lu[1], F. Lu[1], M.X. Guan[2]* 1) Dept Ophthalmology/Optometry, Wenzhou Medical Col, Wenzhou, Zhejiang, China; 2) Division of Human Genetics, Cincinnati Childrens Hospital Medical Center, Cincinnati, Ohio 45229, USA.

Lebers hereditary optic neuropathy (LHON) is a maternally inherited disorder leading to the rapid, painless, bilateral loss of central vision. Recently, a systematic and extended mutational screening of mtDNA has been initiated in the large clinical population of Ophthalmology Clinic at the Wenzhou Medical College, China We report here the characterization of a four-generation Han Chinese family with LHON. This Chinese family exhibited a variable severity and age-at-onset of visual impairment. Notably, the average-age-at-onset of vision impairment changed from 26 years (generation III) to 14 years (generation IV), with the average of 18 years in this family. In addition, 30% and 50% of matrilineal relatives in generation III and IV of this family developed visual loss with a variability of severity, ranging from blindness to normal vision. Sequence analysis of the complete mitochondrial DNA in this pedigree revealed the presence of the homoplasmic ND4 G11778A mutation and 33 other variants, belonging to the Asian haplogroup D4. Of other variants, the homoplasmic G11696A mutation in the ND4 gene is of special interest as it was implicated to be associated with LHON in a large Dutch family and five Chinese pedigrees with extremely penetrance of visual loss. In fact, the G11696A mutation caused the substitution of an isoleucine for valine at amino acid position 312, located in predicted transmembrane region of ND4. These imply that the G11696A mutation may act in synergy with the primary LHON-associated G11778A mutation in this Chinese pedigree.

Energy Balance Analysis of Metabolic Networks. *Q. Zhou[1], L. Jin[1], M. Xiong[1,2]* 1) Genetics, Fudan University, Shanghai; 2) Human Genetics Center, University of Texas Health Science Center at Houston.

Abstract The purpose of metabolism is to produce metabolites and proteins, which in turn to generate the energy required by the cell and materials to construct the cells and tissues. The organisms have evolved and developed control structures to ensure optimal growth in response to environmental changes and maximizing their chance for survival. Although kinetic model provides detailed results, it is difficult to use them for modeling real metabolic networks because of limited availability of kinetic parameters for most metabolic networks. However, for many organisms the structural information of metabolic networks is available. Therefore, many researchers focus on flux balance analysis (FBA) approach. FBA studies how the metabolic networks operate at steady state and determine distribution of metabolic flux under optimal growth. Although FBA has been successfully applied to optimization of the metabolic networks, its limitation is that FBA does not consider energy balance whether including energy balance equation constraints has big impact on the optimization of the metabolic networks has not been systematically investigated. In this report, we performed optimization of metabolic networks in E. Coli under both flux and energy balance to investigate the impact of the energy balance on the behavior of metabolic networks. However, our preliminary results showed the impact of posing energy balance constraints on the optimization of metabolic networks is limited.

Acute megakaryoblastic leukemia in an infant with t(1;22)(p13;q13) and complex secondary chromosomal aberrations including hyperdiploidy. *D. Wei[1], K.H. Ramesh[1], D.T. Walsh[1], C. Johnson[1], V.R. Pulijaal[1], H. Ratech[1], E.A. Kolb[2], L.A. Cannizzaro[1]* 1) Dept Pathology, Montefiore Medical Ctr. Bronx, NY 10467; 2) Dept Pediatrics, Albert Einstein Col Med. Children's Hospital at Montefiore Bronx, NY 10467.

Acute megakaryoblastic leukemia (AMKL) is an uncommon acute leukemia. It is classified as acute myeloid leukemia-M7 (AML-M7) in the FAB system, and as AMKL in the WHO system. AMKL occurs at all ages. The t(1;22)(p13;q13), characteristic of AMKL, is restricted to infants and children. It has been reported as the sole chromosomal aberration (CA) in about 60% of AMKL cases below 6 months of age and above the age of 6 months, the t(1;22) is often associated with complex secondary CA including hyperdiploidy. In both cases, the prognosis appears to be poor. We report a 2 years old infant with a history of anemia, thrombocytopenia and peripheral blasts. Bone marrow (BM) biopsy showed normocellular marrow, diffusely replaced by blasts and essentially no normal hematopoiesis except for a few scattered megakaryocytes. Immunohistochemical stains revealed blasts that expressed CD117+, CD15-, CD34-, CD68-, bcl-2- (protein). Flow cytometry revealed CD7+, CD13+, CD33+, CD34-, CD38+, CD64+, CD117+, MPO-, CD56-, TdT-, HLA-DR-, and 5% CD61+. The differential diagnoses included acute promyelocytic leukemia, acute monoblastic leukemia and AMKL. Cytogenetic analysis of BM showed a 57,XY,+Y,+1,der(1)t(1;22)(p13;q13)x2,+2,+add(4)(q31),+6,der(7)t(1;7)(p36;q36),+8, +9,+10,+13,+19,+21,-22,+der(22)t(1;22)(p13;q13)t(1;?)(p36;?)[17]/46,XY[3] karyotype. The t(1;22) was questionable by G-banding, as the der(22) had evolved with additional unidentifiable changes. FISH analyses however, confirmed the der(1) and the der(22) chromosomes to be products of the t(1;22). The final cytogenetic results were strongly in favor of AMKL in this infant. The diagnosis of AMKL, particularly in infants and children is difficult to establish in spite of all the hematological parameters pointing to acute leukemia. Therefore, chromosome analyses and FISH tests are highly significant in the final diagnosis of AMKL.

De Novo 4q Partial Duplication with Congenital Cataract, Hypothyroidism and no Urogenital or Preaxial Limb Defects. *A. Singer[1], A. Polishchuk[2], A. Aviram[3], C. Vinkler[4]* 1) Clinical Genetics, Barzilai Medical Center, Ashkelon, Israel; 2) Cytogenetics,Barzilai Medical Center, Ashkelon, Israel; 3) Cytogenetics, Shiba Medical Center, Tel-Hashomer,Israel; 4) Clinical Genetics, Wolfson Medical Center, Holon, Israel.

De novo partial duplication of 4q, has been described in less than 20 patients so far. In an attempt to further delineate the clinical findings we present a patient with some characteristics which were not previously described. The patient is a three and a half years old girl, born at term to healthy unrelated parents. Right after her birth she was found to have a complicated heart defect (Tetralogy of Fallot). She also has a cataract in one eye and hypothyroidism. She has growth and psychomotor retardation. Head circumference is in the 2nd percentile. Brain MRI demonstrated delayed myelination and enlarged ventricles. On examination she has, a prominent metopic suture, low hairline, hypertelorism, epicantal folds, short philtrum, carp mouth, retromicrognathia, small cupped ears, and rockerbottom feet. Cytogenetic and CGH analysis demonstrated an abnormal karyotype 46XX,dup(4),(q26;q34) Urogenital anomalies were previously associated with the 4q25q31 duplication. However our patient does not have urogenital malformation. Thumb anomalies were previously assigned, by karyotype/phenotype relationship, to duplication site encompassing the 4q27q28 location. Our patient does not have a preaxial anomaly . It is hence suggested that both preaxial limb anomaly and urogenital malformations be assigned to a location proximal to q26. While congenital cataract has not been described in association with duplication of 4q, hypothyroidism is a frequent endocrine problem in neonates and could be accidental.

Tuning Relief for Genome-Wide Association Analysis of Epistasis. *B.C. White, J.H. Moore* Computational Genetics Laboratory, Department of Genetics, Dartmouth Medical School, Lebanon, NH.

Detecting epistasis in the absence of marginal effects is both a statistical and a computational challenge. This is particularly true in genome-wide association studies where it is not computationally feasible to exhaustively evaluate all combinations of SNPs. One strategy to address this problem is to statistically select or filter a subset of SNPs that can then be exhaustively evaluated for interactions. Our goal was to develop a powerful and efficient algorithm for filtering SNPs that have a high likelihood of interacting. To accomplish this goal, we selected the Relief algorithm (Kira and Rendell 1992) as a starting point. Relief determines the quality of a SNP by repeatedly sampling subjects and considering the relevance of the SNP for the nearest subject of the same and different class. We modified this algorithm by introducing a tuning parameter that iteratively removes noisy SNPs. This new Tuned Relief (TuRF) algorithm was evaluated using simulated epistatic interactions of varying heritability (0.01, 0.025, 0.05, 0.1, 0.2, 0.3, 0.4) and minor allele frequency (0.2, 0.4) that were embedded in genome-wide datasets (100 replicates each) with varying numbers of SNPs (1000, 10000, 100000) and varying sample sizes (200, 400, 800, 1600, 3200, 6400). We found that the TuRF algorithm significantly outperformed both ReliefF and a myopic chi-square test of independence for selecting epistatic SNPs from among thousands of candidates. There are three important advantages of the TuRF algorithm. First, TuRF is able to assign a single measure of relevance to each SNP in a dataset without ignoring the context of the other measured variations. Second, the TuRF algorithm is computationally efficient and can be applied to a single genome-wide dataset with 100000 or more SNPs in a matter of minutes. Finally, we have made TuRF available in an open-source software package for anyone to use. This study demonstrates that it is possible to filter epistatic SNPs from a genome-wide association study. (Funded by NIH R01s LM009012 and AI59694, PI-Moore).

Hearing loss in biotinidase deficiency: Preliminary results indicate genotype-phenotype correlation. *B. Wolf[1], H.S. Sivri Kalkanoglu[2], G.A. Genc[3], L. Sennaroglu[4], H.I. Aydin[2], A. Dursun[2], A. Tokatli[2], T. Coskun[2]* 1) Dept. Medical Genetics, Henry Ford Hospital, Detroit, MI; 2) Hacettepe University, Dept. of Pediatrics, Nutrition and Metabolism Unit, Ankara, Turkey; 3) Hacettepe University, Dept. ENT, Audiology Unit, Ankara, Turkey; 4) Hacetteppe University, Dept. ENT, Ankara, Turkey.

Biotinidase deficiency (BD) is an autosomal recessively inherited disorder that is due to an inability to recycle the vitamin biotin. If not treated with biotin, the clinical features of BD usually include cutaneous and neurological symptoms, including hearing loss. Newborn screening for BD is not conducted in Turkey. We studied 18 Turkish children with profound BD (less than 10 % of mean normal serum activity); 9 symptomatic children (homozygous with null mutations), 6 symptomatic children (homozygous with missense mutations), and 3 asymptomatic children who were diagnosed and treated immediately after birth because an older sibling was affected (homozygous with null mutations). Audiologic, impedancemetric, auditory brainstem response and otoacoustic emissions measurements were performed on all patients. Hearing loss of varying degree occurred in all symptomatic children with null mutations, whereas all symptomatic children with missense mutations had normal hearing. The three children with null mutations who were treated since birth did not develop hearing loss. These preliminary results indicate that symptomatic children with null mutations are likely at risk of developing hearing loss, whereas those with missense mutations may not develop hearing loss even if they are not diagnosed or treated for a prolonged period of time. In addition, once hearing loss occurs, it appears to be irreversible in spite of biotin treatment, although treatment may prevent progression of the hearing loss. Biotin treatment immediately after birth appears to prevent hearing loss in children with null mutations. Our study further supports that BD should be considered in all children with sensorineural hearing loss and all newborns should be screened for this readily treatable disease.

Gene expression profiling of archival specimens distinguishes *BRCA1*-mutated from sporadic breast cancers.

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Purpose To refine gene expression profiling (GEP) of *BRCA1* germline-mutated (*BRCA1+*) breast cancers and to adapt GEP for analysis of paraffin-embedded formalin-fixed (FFPE) specimens. Methods We performed genome-wide GEP on 7 *BRCA1+* and 15 mutation negative fresh-frozen breast tumors using Affymetrix HG-U133 Plus 2.0 arrays. We used these data, along with published GEP data on 25 *BRCA1+* tumors and *BRCA1* interacting genes, to create a 128-gene custom Illumina DASL array with 3 probes per transcript. 64 of 68 FFPE samples qualified for analysis (94% had ≥50% probe detection): 35 *BRCA1+* of which 8 were estrogen receptor (ER) positive, 28 sporadic, and 1 *BRCA2+*. Sporadic tumors were *BRCA* mutation negative; age-, pathology-, receptor-, and grade-matched; or both. Student *t*-test was applied to a 41-sample training dataset to select *BRCA1+* associated genes. Since ER status markedly affects global gene expression, genes more strongly associated with ER were discarded. Hierarchical clustering and k-nearest neighbor (kNN) were used for unsupervised and supervised classification, respectively. Results We selected 18 genes that distinguish *BRCA1+* from sporadic breast tumors and 25 genes associated with ER status. Among 18 independent test samples, kNN predicted *BRCA1* status with 91% sensitivity and 83% specificity. Accurate *BRCA1+* classification was accomplished for archival tumors as old as 39 years. Conclusions We show for the first time that GEP of FFPE specimens can reliably distinguish *BRCA1+* from sporadic breast tumors, independently of ER status. False positives may be due to an imprecise classifier, *BRCA1* promoter methylation, or undiscerned mutations, possibilities which require exploration. Discriminating genes may lend insight into *BRCA1* biological functions. GEP may be useful for prescreening breast cancers given the high false negative rate of family history; analyzing archival specimens from deceased relatives; as a functional assay to define *BRCA* variants as benign or deleterious in combination with established methods; and as an adjunct for genetic counseling and testing.

Weekly enzyme replacement therapy may slow decline in renal function in a subset of Fabry patients after long-term biweekly dosing. *R. Schiffmann, H. Askari, M. Timmons, C. Robinson, M. Ries* DMNB/NINDS, National Institutes of Health, Bethesda, MD.

Agalsidase alfa (Replagal, Shire Human Genetic Therapies, Inc.) infused at a dose of 0.2 mg/kg every other week (EOW) is approved in 35 countries for the treatment of Fabry disease. This dosing regimen maximally reduces plasma Gb_3 and stabilizes renal function in most patients with mild-to-moderate chronic kidney disease. This study was performed to determine if adult male patients who demonstrate a continuing decline in renal function despite two or more years of conventionally dosed agalsidase alfa therapy show an improved clinical response with weekly administration of the same 0.2 mg/kg dose. Eleven of 41 (27%) adult male Fabry patients who participated in long-term clinical trials and who had demonstrated a continuing decline in estimated glomerular filtration rate (MDRD, eGFR) of ? 5 mL/min/1.73m^2 per year during long-term treatment with agalsidase alfa at 0.2 mg/kg, EOW, were enrolled in this open-label prospective study. Patients were switched to weekly infusion of 0.2 mg/kg and followed for an additional 24 months. The primary outcome measure was the slope (linear regression) of the change in eGFR over 24 to 54 months before compared to the slope over 24 months after switching dosing regimens. Mean baseline eGFR was 81.6 8.1 mL/min/1.73m^2 (mean SEM) and mean rate of change in eGFR was -7.9 0.9 mL/min/1.73m^2 per year with EOW dosing. During the 24-month follow-up after increasing the dosing frequency from EOW to weekly, the mean rate of change in eGFR had slowed to -3.2 2.0 mL/min/1.73m^2 per year (P=0.01). After switching to weekly dosing, the slope of the change in eGFR became positive or less negative in all but 3 patients. A multiple regression model confirmed the weekly infusion regimen as the strongest explanatory variable for the change in eGFR (P=0.009) with a weaker contribution from the use of ACE-I/ARB medication (P=0.03). These preliminary results suggest that weekly infusions of agalsidase alfa may be considered for patients whose kidney function continues to decline with EOW dosing and warrants further study. *Not approved in the US.

Homozygosity Mapping of Autosomal Recessive Disease Gene in Patient of a Consanguineous Family by High Density Oligonucleotide Arrays. *J.Y. Wu[1,2], H.H. Wang[1], C.F. Chang[1,3], F.J. Tsai[2], Y.T. Chen[1,4]* 1) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) Department of Medical Research, China Medical University Hospital, Taichung, Taiwan; 3) Molecular Medicine Program, Institute of Microbiology and Immunology, National Yang Ming University, Taipei, Taiwan; 4) Department of Pediatrics, Duke University Medical Center, Durham, NC 27710, USA.

Acromesomelic dysplasia, type Maroteaux (AMDM [MIM 602875]), is one form of skeletal dysplasia that involves shortenings of the limbs (Maroteaux et al. 1971). This skeletal dysplasia is identified of autosomal recessive inheritance and therefore mostly found in consanguineous families. In fact, 17 of the 21 families of different ethnic backgrounds are consanguineous in the mapping of NPR2 as the gene responsible for causing AMDM (Bartels et al. 2004). We have identified a small nucleus Han Chinese family with one patient diagnosed of ADMD whose parents are cousins. While linkage analysis and positional candidate approach were successfully adopted to zoom in the interval to 9p12-q12 (Kant et al. 1998) and subsequently concluding on the mutations of NPR2 as the cause of AMDM, and high density single nucledotide polymorphism (SNP) genotyping assays have been utilized as the substitute of genomewide linkage analysis of complex disease with their higher information content (Middleton et al. 2004, John et al. 2004), there has not yet an instance of using such approach in mapping the homozygosity region and thereafter the disease gene of autosomal recessive disease in a small consanguineous family. We report here the use of high density SNP genotyping array for filtering the homozygosity regions from the whole genome and then locating the mutation that leads to AMDM in a family with only one affected member in a total of six members.

Association between a matrix metalloproteinase 3 promoter polymorphism and functional severity in a Japanese rheumatoid arthritis cohort. *S. Tsukahara, K. Ikari, E. Inoue, S. Momohara, T. Tomatsu, M. Hara, H. Yamanaka, N. Kamatani* Institute of Rheumatology, Tokyo Women's Medical University, Japan.

Rheumatoid arthritis (RA) is a systemic, chronic disease that causes progressive joint destruction, thereby leads to impaired functioning and a poor quality of life. Matrix metalloproteinase 3 (MMP-3) plays a pivotal role in joint destruction seen in RA. A biallelic polymorphism on promoter region, one allele has a run of six adenosines (6A) and the other has five (5A), was found to influence the production of MMP-3. A recent prospective study indicated that this promoter polymorphism is associated with the progression of radiographic joint destruction in European patients with early RA. The aim of the present study was to determine whether this MMP-3 promoter polymorphism is associated with long-term functional impairment in Japanese RA patients.

The study was approved by the Tokyo Women's Medical University Genome Ethics Committee. It was a part of a Japanese RA cohort project that included over 4000 Japanese RA patients (IORRA: Institute of Rheumatology RA cohort). DNA samples were available from 1284 patients, of whom, 1128 were randomly selected for this study. Genotyping of the 5A/6A polymorphism (rs3025058) was performed using TaqMan SNP genotyping assay (Applied Biosystems). The Health Assessment Questionnaire (HAQ) was used to assess functional severity of the patients. A cross-sectional analysis using the HAQ data in the cohort study in April 2003 was performed on 1077 patients (patients with missing data for HAQ were excluded). Differences in HAQ score among genotypes of 5A/6A polymorphism were analyzed by regression analysis, which was implemented in the R software package. Significant differences were found among MMP-3 genotypes for HAQ ($p = 0.01$; mean HAQ were 0.95, 0.75 and 0.66, respectively for genotype 5A/5A, 5A/6A and 6A/6A). We conclude that MMP-3 gene is associated with the functional severity of RA in a Japanese population.

A novel ORF15 frameshift mutation of RPGR in a Chinese family with Dominant X-linked retinitis pigmentosa.

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Retinitis pigmentosa (RP) denotes a group of hereditary retinal dystrophies, characterized by the early onset of night blindness followed by a progressive loss of the visual field. This paper aim is to found the genetic basic for a Chinese family with X-linked dominant retinitis pigmentosa. Peripheral blood was taken from all the members of the retinitis pigentosa family. Linkage analysis was performed using a panel of microsatellite markers flanking candidate genetic loci for known retinitis pigentosa genes, and linkage analysis results excluded all autosomal dominant retinitis pigentosa (ADRP) loci as causative of the dominannt RP family, but show positive link with markers DXS993 and DXS1068, where RPGR is located. For mutation analysis, direct sequence of whole coding region of RPGR with DNA from the proband revealed a hemizygous 1166A deletion of ORF15 in affected male, this deletion results in a frameshift leading to altered amino acid structure and early termination of RPGR. The 1166delA mutation co-segregated with all affected individuals, but didnt present in normal members in the family, the clinical feather of the mutant carriers show high intrafamilic variability in the family.

Testicular expressed genes are missing in familial X-linked Kallman syndrome due to Two large different deletions around the KAL1 gene. *R. Parvari[1], N. Loewenthal[2], A. Peretz[2], E. Hershkovitz[2]* 1) Dept Genetics& Virology, Ben Gurion Univ, Beer Sheva, Israel; 2) Pediatric Endocrinology & Metabolic Unit, Soroka Medical University Center, Beer Sheva 84101, Israel.

X-linked Kallman syndrome is caused mainly by point mutations in the KAL1 gene. Large deletions of more then 1Mb are rare events in the human population and commonly result in contiguous gene syndromes. We report on two large deletions on Xp22.3 causing isolated Kallman syndrome in two pairs of cousins, sons of two sisters. The deletions were identified by the failure to PCR amplify the Kallamn genes exons using patients DNA. The definition of the deletions' borders was done by STS analysis. The definition of the genes in the deletions and the elucidation of significant sequences at the deletion borders was done by in-silico analysis. The identification of two different, apparently independent deletions larger then 1 Mb, in two pairs of first degree cousins with Kallman syndrome (KS) of two sisters was unexpected. One of the events causing the deletion appears to have been mediated by an L1 transposition, the other by non homologous end joining. The deleted sequences encompass the KAL1 gene and four known additional genes exclusively expressed in testis. Although our patients presented clinically as an isolated Kallman syndrome, the findings of contiguous gene deletions which involves the deletion of testis specific genes would be probably of clinical relevance. The absence of these genes may impair future fertility despite replacement therapy. The demonstration of an additional mechanism creating deletions in the Kallman region may hint that deletions in Kallman patients involving testicular expressed genes are more frequent then previously recognized.

Familial colorectal neoplasia is not associated with the *TGFBR1*6A* allele. *G.L. Wiesner[1,2,5], W. Morgan[1], J. Willis[3,5], S. Lewis[1], S.D. Markowitz[2,5], R. Elston[4,5], D. Daley[4,6]* 1) Depts of Genetics; 2) Medicine; 3) Pathology; 4) Epidemiology; 5) and Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, OH; 6) University of British Columbia, Vancouver, BC, Canada.

Case-control studies have reported an association between TGFBR1*6A, a common variant in the type 1 transforming growth factor beta receptor, and risk for human colorectal cancer. TGFBR1 co-localizes to 9q22.2-31.2, which was first identified by the Colon Neoplasia Sibling Study as a region containing a putative susceptibility locus for colorectal neoplasia (PNAS 100:12961, 2003). In order to determine whether the *6A allele is responsible for the observed linkage at 9q22.2-31.2, 53 kindreds having two or more siblings affected with colon cancer or advanced colon adenomas were genotyped for the presence of the *6A or *9A allele. The percentage of affected individuals with one or more *6A alleles in kindreds linked to the region was less than the percentage of affected individuals in unlinked kindreds (11% and 25%, respectively). Further, the distribution of the *6A allele did not differ between affected or unaffected siblings. The TGFBR1 genotypes were then analyzed along with the17 polymorphic markers first used in the linkage analysis of this region. Rather than increasing the evidence for linkage at 9q22.2-31.2 as expected, including the TGFBR1 genotypes into the analysis decreased the significance (P= 0.0005 to P= 0.001) for linkage. Additionally, no significant impact on the linkage evidence was found when the number of *6A alleles was added as a covariate in the regression analysis. These data indicate that in our population the *6A allele is not the causative susceptibility allele, nor is it in significant linkage disequilibrium with a causative allele close by. There remains an unidentified susceptibility locus for colorectal neoplasia at 9q22.2-31.2.

Empirical observations on detection of linkage disequilibria by testing homozygosity of haplotypes. *X. Sheng, S. Guha, R. Chakraborty* Ctr Genome Information, Univ Cincinnati, Cincinnati, OH.

Excess homozygosity (EH) of haplotypes has been advocated as a measure of linkage disequilibrium (LD) in the literature. In particular, EH has been claimed to have advantages to detect LD for highly polymorphic multiallelic markers, since it avoids haplotype determination from multilocus genotype data and provides a single statistic to measure LD. In spite of the fact that under the assumption of Hardy-Weinberg equilibrium, expected EH is algebraically related with LD, and dependence of EH on recombination distance of loci has been worked out, empirical data on the ability of detect significant LD by using EH for loci of varying recombination distance is not yet available. In this study, we used genome wide data on 784 microsatellite loci from 36 worldwide populations to examine the power of detecting significant LD by using EH. Six geographic grouping (Africa, Asia, Europe, Middle-East, America, and Oceania) of populations resulted in the following observation: (i) EH exhibits significant LD more often than the level of significance of the test procedure only for America and Middle-East when the loci are less than 10cM recombination distance apart (power in the range of 0.09 to 0.16); (ii) the power of detecting LD by permutation tests of EH is in general low, particularly for populations of higher heterozygosity; and (iii) for small populations, albeit for lower heterozygosity, power appears to be somewhat larger. We conclude that these observations are caused by the discrete sampling distribution of EH, particular when the homozygosity in at least one locus is not large, and sample size is small. For example, when at least one locus has a homozygosity of 0.1, measuring EH from genotype data on 100 individuals can give rise to only 11 distinct values of EH, resulting in low power and a non-monotonic decrease in power with increasing recombination distance. These statistical properties of EH apparently outweigh the advantages of using EH for studying background LD levels for microsatellite loci. (Research supported by the NIH grant GM 41399 to RC).

DNA repair gene polymorphisms and G2 chromosomal radiosensitivity. *C.S. Wilding[1], G.B. Curwen[1], E.J. Tawn[1], X. Sheng[2], J.F. Winther[3], J.D. Boice Jr.[4,5], R. Chakraborty[2]* 1) Genetics Department, Westlakes Research Institute, Moor Row, Cumbria, United Kingdom; 2) Center for Genome Information, University of Cincinnati College of Medicine, Cincinnati, OH 45267, USA; 3) Institute of Cancer Epidemiology, Danish Cancer Society, DK-2100, Copenhagen, Denmark; 4) International Epidemiology Institute, Rockville, MD 20850, USA; 5) School of Medicine, Vanderbilt University, Nashville, TN 37232, USA.

Prior research suggests major gene susceptibility for G_2 chromosomal radiosensitivity and association of enhanced chromosomal radiosensitivity with cancer predisposition. Based on this, we have undertaken a preliminary investigation of the association of polymorphisms in DNA repair genes with G_2 chromosomal radiosensitivity. Sixteen candidate polymorphisms (13 SNPs and 3 microsatellites) were examined at nine genes of four DNA repair pathways in 83 subjects, comprising 23 survivors of childhood and adolescent cancer, their 23 partners and 37 offspring. Genotypes at the Asp148Glu SNP site on the APEX gene of the BER pathway appeared to be significantly associated with cancer in survivors ($p = 0.001$, significant even after multiple test adjustment), due to the enhanced frequency of the APEX Asp148 allele in survivors in comparison to that in their partners. Analysis of variance (ANOVA) of G_2 radiosensitivity in the pooled sample, as well as family-based association test (FBAT) of the family-wise data, showed sporadic suggestions of association of G_2 radiosensitivity with polymorphisms at two sites (the Thr241Met SNP site on the XRCC3 gene of the HRR pathway for ANOVA, and the Ser326Cys SNP site on the hOGG1 gene of the BER pathway for FBAT analysis), neither of which remained significant after multiple test adjustment. These, together with near non-significance of the haplotype level association analyses, suggest that polymorphisms at one or more DNA repair pathway genes may be associated with G_2 radiosensitivity governing cancer predisposition. (Research supported by US Public Health Service grants CA104666, GM41399 from the US National Institutes of Health and a grant from Westlakes Research Institute).

Evaluation of the Third Wave InPlex CFTR Microfluidics Card Assay: Limitations and Controls. *H. Rennert, A. Khazanova, J. Sipley* Department of Pathology and Laboratory Medicine, NewYork Presbyterian Hospital-Weill Medical College of Cornell University, New York, NY.

The InPlex CFTR Microfluidic Card Assay (Third Wave, Madison, WI) is a new method for the detection of 46 Cystic Fibrosis (CF) mutations and polymorphisms; including the 25 American College of Medical Genetics (ACMG) recommended mutations, using single-tube multiplex Invader reactions. This assay uses target amplification of template DNA, followed by multiplex Invader reactions in a microfluidics card. Samples are scored as either WT, heterozygous (HET), homozygous mutant (MUT) or equivocal (EQ). We have analyzed a total of 76 controls that included 44 Coriell, 12 College of American Pathologists proficiency testing samples and 20 DNA samples from a reference laboratory. In addition, we have evaluated the tests performance using commercial single-tube controls obtained from AcroMetrix (28 mutations) and Molecular Controls (44 mutations). Of the 76 DNA samples tested, 73 samples generated the expected results while 3 samples yielded equivocal results. Of the three, two were deltaI507 samples that were called equivocal (EQ) for deltaF508, and one was a G551D/R553X that was called homozygous (MUT) for both mutations. All negative controls were called as expected. Both controls were able to accurately call as MUT the majority, but not all, mutations contained within these controls, generating a complex genotype composed of MUT, heterozygous (HET), EQ and low signals. The HET or EQ calls were predominantly seen for mutations adjacent to one another, such as G551D, G542X, S549N and S549R, and are likely a result of hybridization interference of allele-specific probes. These results demonstrate that certain mutations and genotypes are more likely than others to yield imprecise calls, and therefore test results for these mutations should be interpreted with caution. The use of commercial controls for this assay requires further optimization.

Sample size estimation under fixed statistical power and FDR (false discovery rate) control in microarray experiments using a nonparametric test. *J.G. ZHANG[1], J.F. LIU[1], H.W. DENG[1,2,3]* 1) Basic Medical Science, University of Missouri/Kansas, Kansas City, MO; 2) Laboratory of Molecular and Statistical Genetics, College of Life Sciences, Hunan Normal University, Changsha, Hunan, P. R. China; 3) The Key Laboratory of Biomedical Information Engineering of Ministry of Education and Institute of Molecular Genetics, School of Life Science and Technology, Xi'an Jiaotong University, P.R.China.

Motivation: One main task for DNA microarray analyses is to detect differentially expressed genes between different tissues or cell samples, such as healthy vs diseased. Microarrys data usually do not follow normal distributions, even after some data preprocessing and transformation. Nonparametric tests (e.g. Wilcoxon rank sum test) were recommended for analyses of microarray data. However, statistical power estimation for non-parametric tests, which is significant for planning experiments, is not available. In this paper, we develop a sample size estimation algorithm for Wilcoxon rank sum test under fixed FDR (false discovery rate)-controlling levels and pre-determined detecting statistical power. We elucidate how the experimental factors affect sample size estimation, FDR control and detecting power. Results: We investigate the situations when expressions of various genes are independent or correlated with each other at the same levels. We assume expression of each gene follows a normal-uniform mixture distribution. The sample sizes needed for two-sample Wilcoxon rank sum tests are computed by our newly developed algorithm. The computed results agree very well with the Monte Carlo simulation results. Factors such as sample allocation proportion for comparing groups, effect size of differentially expressed genes and noise levels (as reflected by the proportion of the uniform distribution in the mixed distribution of gene expression), can influence the sample size calculation under fixed FDR levels and detecting statistical power. We observe that correlation structures among gene expressions may increase variance of computed statistical power and FDR levels compared with the independent gene expression model.

Segregation of mtDNA in the female germline. *T. Wai, E.A. Shoubridge* Human Genetics, Montreal Neurological Institute-McGill University, Montreal, Quebec, Canada.

Mitochondrial DNA (mtDNA) is a high copy, maternally inherited genome coding for polypeptides essential for oxidative phosphorylation (OXPHOS). Typically, a cell contains one mtDNA genotype, homoplasmy, but in some circumstances two genotypes can coexist, heteroplasmy. Studies in animals and human pathogenic mtDNA mutations have demonstrated that there is a genetic bottleneck during oogenesis that can affect the segregation of mtDNA sequence variants. This genetic bottleneck is believed to occur during the development of primordial germ cells (PGC) to primary oocytes because of the amplification of an estimated 10 copies of mtDNA found in PGCs to the 105 copies that are found in oocytes. We hypothesize that modulating mtDNA copy number and OXPHOS function during oogenesis can affect mtDNA segregation, and ultimately the size and shape of the genetic bottleneck. We are testing this hypothesis in a mouse model heteroplasmic for two mtDNA sequence variants (NZB and BALB) using the Cre-lox recombination system to knock out genes essential to maintain mtDNA copy number (TFAM) or OXPHOS function (COX10), at early or late stages of oogenesis. mtDNA heteroplasmy levels and copy number are assessed in single cells throughout oogenesis to investigate the impact of copy number and OXPHOS on this genetic bottleneck. Oocytes from Heteroplasmic TFAM+/- mice demonstrate increased an increased heteroplasmic variance when compared to oocytes from TFAM+/+ littermates, yet the distribution of heteroplasmy in COX10+/- oocytes does not differ significantly from wild type littermates. Since previous studies have demonstrated that mtDNA copy number correlates directly with TFAM levels, this suggests that a reduction in mtDNA copy number, but not diminished OXPHOS, can constrict the size of the bottleneck.

Confirmation of PREDICTED and generation of novel sequences for the calcitonin (CALCR) and vitamin D3 (VDR) receptors in *Bos taurus*. *M.J. Paolella, K.M. Bilides, M.L. Fitzmaurice, C.E. Horbal, S.M. Lombardi, K.E. Mannion, V.L. Martucci, R.A. Nunez, J.E. Pachesa, B.K. Reidy* Science, Sacred Heart Academy, Hamden, CT.

As the recipients of a grant to engage in a 3-year study of osteoporosis, and the interest in the implications of the calcitonin (CALCR) and the vitamin D3 (VDR) receptors, another group of students chose to continue to focus on these genes for the second year. After having confirmed a portion of the PREDICTED *Bos taurus* sequence for CALCR and COLIA1 last year (gb: DQ114140 and DQ114141), but without conclusive results for VDR, and the sustained research on CALCR as a major influence of bone mineral density, the genes offered an opportunity to further the students knowledge on the relationship of these two genes. GenBank sequences of these genes from related organisms, specifically *Homo sapiens* and *Rattus norvegicus*, were aligned and the areas of greatest similarity were BLASTed against unidentified genomic *Bos taurus* contigs. Results guided primer design to amplify the significant areas of homology using multiple bovine templates including L1 Dominette 01449 (courtesy of USDA). Successful PCR products were purified, quantitated and sequenced in the schools ABI Prism 310 Genetic Analyzer with the same primers used in PCR. Forward and Reverse sequencing results were aligned with each other, BLASTed, and compared to CALCR and VDR genes in related organisms. Student results confirmed a portion of the PREDICTED *Bos taurus* VDR sequence variants and revealed partial coding regions. Student results for the bovine CALCR sequence, reflective of the *Sus scrofa* and *Homo sapiens* CALCR genes, determined the 3UTR for *Bos taurus*. Sequencing results are posted in GenBank: DQ519355, DQ 519356, and in later June, 2006: DQ659276 and DQ659277.

New molecular findings in Turkish cystinosis patients. *B. Tinloy[1], R. Topaloglu[2], T. Coskun[2], M. Gunay-Aygun[1], Y. Anikster[3], A. Jeong[1], A. Bakkaloglu[2], N. Besbas[2], S. Kalkanoglu[2], W.A. Gahl[1], R. Kleta[1]* 1) SHBG, MGB, NHGRI, NIH, Bethesda, MD, USA; 2) Hacettepe University Faculty of Medicine, Ankara, Turkey; 3) Sheba Medical Center, Tel Hashomer, Israel.

CTNS, located on chromosome 17p13, codes for cystinosin, a protein that facilitates obligatory lysosomal cystine egress. Without proper functioning of cystinosin, patients manifest nephropathic cystinosis, developing renal Fanconi syndrome in infancy and glomerular kidney failure within the first 10 years of life. Many cystinosis patients of European descent have a common 57 kb deletion (allele frequency 50-60%). Consequently, molecular analyses involve an initial multiplex PCR, checking for the presence or absence of this founder deletion, and subsequent sequencing of the 9 coding exons of CTNS. Early and accurate diagnosis is critical because treatment with cysteamine can prevent many disease complications. Here we report our molecular findings in 12 Turkish cystinosis patients. Six of the patients reached end-stage renal failure at ages ranging from 6.5 to 15 years, necessitating renal replacement therapy. None of the 12 nephropathic cystinosis patients carried the 57 kb deletion. Instead, one patient had a new homozygous 10 kb deletion of exons 4 to 9 of CTNS. One patient was homozygous for a known 4 bp deletion in exon 3, i.e., c.357del GACT. Two patients were homozygous for new missense mutations in exons 7 and 9, i.e., c.451G>A (R151G) and c.518A>G (Y173C), respectively. The most common mutation in our Turkish patients was a new exonic splice site mutation in exon 9, i.e., c.681G>A (E227E). Of 24 alleles studied, 7 carried this mutation, which is expected to disrupt proper splicing. Two patients were compound heterozygous for one of the above-mentioned mutations and a known missense mutation in exon 12, i.e., c.1015G>A (G339R). In two patients, we could not find any disease-causing mutations and in two others, we could find only one disease-causing mutation. In summary, cystinosis patients of Turkish ancestry show CTNS mutations different from those of Western European patients. These findings are pertinent to molecular-based screening of cystinosis.

MCMC provides practical linkage analysis on general pedigrees with many STRs or dense SNPs. *E. Wijsman*[1,2], *J. Rothstein*[2], *E. Thompson*[3] 1) Dept Medical Genetics; 2) Dept. Biostatistics; 3) Dept. Statistics; Univ Washington, Seattle, WA.

Linkage analysis needs to adapt to full-chromosome multipoint linkage analysis with either SNPs or STRs. While exact computational tools are available for use with small pedigrees, equivalent exact computation for large pedigrees remains intractable. Markov chain Monte Carlo (MCMC) based methods currently provide the only computationally practical option. No systematic comparison of performance of MCMC-based programs is available, nor have these programs been systematically evaluated for use with dense SNPs. We used simulated data to evaluate performance of two MCMC-based linkage analysis programs: lm_markers (LMM) from the MORGAN package and SimWalk2 (SW). Pedigrees consisted of 14, 52, or 98 individuals in 3-6 generations with up to 4 generations of missing data in the largest pedigree. 100 replicates of markers and trait data were simulated on a 100-cM chromosome, with up to 10 STRs and up to 200 SNPs used simultaneously for computation of multipoint LOD scores. Exact computation was available for comparison in most situations, and comparison with a perfectly informative marker or inter-program comparison otherwise. Both programs were fast and accurate with STRs. For example, computation on the 52-member pedigree (PED 52) for 3 markers required 2-2.5 min. with LMM, SW, or Vitesse; the median discrepancy, d, relative to exact LOD scores was 10%;, and even for 10 markers, computation times for the MCMC programs increased to only 5.7 and 14 CPU min., respectively. In contrast, for large numbers of dense SNPs only LMM was able to provide accurate results in computationally practical time. For PED52 and an analysis of 67 dense SNPs, LMM required only ~11 CPU min/pedigree with a median d=19%, compared to ~11 CPU hrs/pedigree for SimWalk2 yielding a median d=62%. Similar results were obtained for the smallest pedigree, with LMM requiring 2 min to achieve a median d=10% of the truth, while SW required 66 min to achieve a median d=20% of the truth. Thus the MORGAN package provides a computationally practical option for accurate linkage analyses in genome scans with both large numbers of SNPs and large pedigrees. Supported by NIH GM46255.

A novel mutation in the RAB27A gene causing Griscelli Syndrome. *M. Tuchman[1], R. Kleta[1], M. Gunay-Aygun[1], M. Huizing[1], W. Westbroek[1], A. Helip-Wooley[1], J.M. Hertz[2], W.A. Gahl[1]* 1) SHBG, MGB, NHGRI, NIH, Bethesda, MD, USA; 2) Department of Clinical Genetics, Aarhus University Hospital, Denmark.

Griscelli syndrome (GS) is an autosomal recessive disorder characterized by a pigment abnormality, silvery gray hair (caused by uneven distribution of pigment in the hair shaft), and an immune regulation defect. Three genes (RAB27A, Myosin 5A, and melanophilin) are associated with different types of GS. These genes function in melanosome movement into the periphery of melanocytes. RAB27A, located on chromosome 15q21, encodes a small GTPase that also plays a crucial role in granule release by T cells of the immune system. Therefore, mutations in RAB27A make patients more susceptible to viruses and other infectious agents. GS patients exposed to viral infections can develop an acute syndrome characterized by fever, hepatosplenomegaly, leucopenia, and neurological problems. We describe a novel mutation in the RAB27A gene of a six-year old Afghani male who is a product of a consanguineous marriage. The mutation, in exon 2, codon 43, changes a glycine (GGC) to a serine (AGC). Glycine 43 is conserved among various species RAB27A genes and also among other Rab genes. It is located within the switch I domain that is thought to play a role in binding of regulators to the RAB27A peptide. This discovery confirms the diagnosis of the patient and confirms the functional importance of this specific region of the gene. Although GS is rare, identification of the genes associated with this disease has provided some understanding of fundamental aspects of cell function, such as vesicle trafficking and immune modulation. Additional investigations involving mutation detection of other GS patients can provide insights into the molecular regulation of intracellular vesicle trafficking and may allow the design of targeted therapies in the future.

Redefining a locus for autosomal dominant Fanconi syndrome with kidney failure. *H. Stanescu[1], R. Unwin[2], T.J. Heikkila[2], C. Willoughby[2], C.M. Laing[2], M. Kashgarian[3], K. O'Brien[1], N. Siegel[3], W.A. Gahl[1], S. Povey[2], R. Kleta[1]* 1) SHBG, MGB, NHGRI, NIH, Bethesda, MD, USA; 2) UCL, London, UK; 3) Yale Medical School, New Haven, CT, USA.

Renal Fanconi syndromes are defined by the presence of glucosuria, generalized amino aciduria, phosphaturia, small molecular weight proteinuria and proximal tubular acidosis. Clinical consequences can be severe, involving growth disorders, rickets, and electrolyte imbalances. Cystinosis is the most common identifiable reason for renal proximal tubular failure in childhood. In this lysosomal storage disease, enhanced apoptosis of renal tubular cells has been proposed as the mechanism. For other types of renal Fanconi syndrome, the responsible cellular mechanisms are currently unknown. Familial occurrences of renal Fanconi syndrome appear to be rare. However, we have studied three families in the UK and the US with an autosomal dominant pattern of renal Fanconi syndrome and subsequent glomerular failure, including the original family described by Dent and Harris in 1951. Whole genome mapping with 2000 highly polymorphic markers revealed that all three families are compatible with a linked locus on chromosome 15q with a total lod score above 4. This locus of approximately 17 Mb contains 169 genes (NCBI build 36.1) and was described earlier using a different US family. Our data confirm this locus. Cloning the gene for this form of autosomal dominant renal Fanconi syndrome with subsequent kidney failure will give invaluable insights into renal proximal tubular functions. We are attempting to recruit more patients and to establish active collaborations.

The use of genetic testing by pediatric otolaryngologists. *S. Prucka[1], R.D. Duncan[2], B.J. Wiatrak[2], R. Smith[4], N.H. Robin[1, 3]* 1) Genetics, University of Alabama at Birmingham (UAB), Birmingham, AL; 2) Surgery, UAB, Birmingham, AL; 3) Pediatrics, UAB, Birmingham, AL; 4) Internal Medicine, Otolaryngology, and Pediatrics, University of Iowa, Iowa City IA.

Clinical genetic testing for *GJB2* was introduced in the late 1990s and has become an important tool in evaluating children with sensorineural hearing impairment (SNHI). Prior to the introduction of genetic testing clinicians ordered an extensive battery of tests, which often had a low yield, to evaluate the underlying etiology of a childs SNHI.

To assess the use of genetic testing by pediatric otolaryngologists in evaluating a child with SNHI, a questionnaire was developed and made available to pediatric otolaryngologists through the American Society of Pediatric Otolaryngology (ASPO) website (www.aspo.us). In total 63 ASPO members completed the questionnaire, but not all respondents answered every question of this survey. We found that the majority (42/61; 69%) use genetic testing of *GJB2* as an initial test in their work-up. However, while 30/42 (71%) stated that they provide genetic counseling to their patients we also found that 17/38 (45%) answered questions regarding recurrence risks incorrectly, or stated that they did not know the correct response. In addition, the majority of individuals (36/61; 59%) indicated using other tests, such as ECG, inner-ear CT, MRI, audiometry on family members, congenital infection screening, and/or thyroid function testing, as part of the initial evaluation either before or in conjunction with genetic testing. **CONCLUSIONS**: This questionnaire has demonstrated that many otolaryngologists do not understand the full implications of genetic testing. In addition, while many pediatric otolaryngologists use DNA-based testing in their initial work-up of a child with SNHI over half still use other tests that are both expensive and unnecessary for the initial evaluation. Although genetic testing is playing a larger role in clinic practice, these results demonstrate that knowledge is still lacking in this physician population. *This work was supported by NIH NIDCD R03 DC006217-01 (to NHR).*

Synergistic effect of two connexin-26 mutations results in profound hearing loss and inner ear malformation. *B. Tinkle[1], A. Bedard[1], J. Greinwald[2]* 1) Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 2) Departments of Otolaryngology and Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Mutations in connexin-26 (GJB2) are the most common genetic cause of non-syndromic deafness. Most mutations in GJB2 are recessive but few are autosomal dominant. We report a compound heterozygote with both a recessive and a novel dominant mutation in GJB2 with profound sensorineural hearing loss and inner ear malformations. The proband was identified shortly after birth with profound hearing loss. His father, paternal uncle, and paternal grandfather had juvenile-onset hearing loss consistent with an autosomal dominant, non-syndromic pattern. The child underwent audiologic, radiographic, and molecular studies. Audiometry revealed profound sensorineural hearing loss (SNHL). Temporal bone CT scans showed abnormally partitioned cochlea and enlarged vestibular aqueducts (EVA). Molecular studies involved sequencing of GJB2 and SLC26A4 (Pendrin) as well as connexin-30 (GJB6) deletion analysis. Both SLC26A4 and GJB6 studies were normal. Sequencing of GJB2 demonstrated the common 35delG mutation as well as a novel 604T>C (C202R) missense mutation. Morle et al. (2000) reported a similar missense mutation (C202F) as a cause of autosomal dominant hearing loss of juvenile-onset similar to the paternal family history. Parental studies were attempted but only the mother was available. She carried the 35delG mutation suggesting that the father contributed the C202R mutation to the proband which was also consistent with the fathers phenotype and familial inheritance pattern. Although EVA is seen in association with idiopathic juvenile-onset hearing loss as well as Pendred and other syndromes, in a study of 500 individuals with hearing loss, Yaeger et al (2006) found no individual with EVA who also had a GJB2 mutation. However, in our patient, it is most likely that the synergistic effect of the 35delG (null) mutation and the dominant C202R in GJB2 were the cause of the inner ear malformation and SNHL.

Comparison of two admixed populations for MALD: 2,000 years versus 200 years. *S. Xu[1], W. Huang[2, 3], Y. Wang[2], H. Wang[2], J. Qian[1], L. Jin[1, 2, 4]* 1) Institute of Genetics, Fudan University, Shanghai, China; 2) Chinese Human Genome Center at Shanghai, Shanghai, China; 3) Rui Jin Hospital, Shanghai, China; 4) CAS-MPG Partner Institute of Computational Biology, Shanghai, China.

Mapping by admixture linkage disequilibrium (MALD) has been shown to be a potentially powerful approach to mapping genetic variants that are involved in human disease. The strategy of MALD has good prospect to performing in many admixed populations with similar histories to African-American. However, challenges are remained for older admixed populations. In this study, using more than 21,000 SNPs spanning over almost entire chromosome 21, we investigated the genetic structures of Uyghurs in China (UIG) and African-American (AfA). Our results showed both UIG and AfA are typical admixed populations, the overall admixture proportions were estimated as UIG with 52.1% European ancestry and 47.9% Asian ancestry, AfA with 19.8% European ancestry and 80.2% African ancestry. Under the assumption of a hybrid isolation (HI) model, the admixture event of UIG was estimated to have taken place about 92-98 generations or 1840-1960 years ago assuming 20 years per generation, while the admixture event of AfA was estimated to have taken place 6-8 generations ago, or 120-160 years ago. Comparing with AfA, we found it is not easy to screen ancestry informative markers (AIMs) with either enough number or enough information for inferring ancestral origins of chromosomal segments in Uyghurs. More powerful methods would be needed for admixture mapping in older admixed populations.

Genome -wide Association Studies Incorporating Searching Genetic Interaction Networks. *G. Pen*[1], *L. Jin*[1,3], *M. Xiong*[1,2] 1) Genetics, Fudan University, Shanghai, China; 2) Human Genetics Center, University of Texas Health Science Center at Houston; 3) CAS-MPG Partner Institute for Computational Biology.

Underlying genetic mechanism of complex diseases involves many genes that influence disease susceptibility primarily through genetic interaction networks and interactions with environments. Incorporating gene-gene interactions and gene interactions networks into genome-wide association studies are an essential and challenging problem in the genetic studies of complex diseases. An exhaustive evaluation of the all possible interactions between SNPs is impossible even if we use a large supercomputer. Therefore, it is urgent to develop efficient and powerful statistical methods and computational algorithms for searching combinations of SNPs, associated with disease and construction of genetic interaction networks, which will largely determine whether genome-wide association will success or not. To date, mathematical formulation of identification of subsets of SNPs or genes and construction of genetic interaction networks has not been well developed. In this report, we propose to formulate them as a combinatorial optimization or a feature selection problem, i.e., given a set of candidate SNPs or genes, selects the best sets in an optimization or classification task. In practice, we need to define: (1) an object function, (2) search algorithm and (3) validation procedure. We develop a novel statistic that can test association of networks with the diseases as the object function. Although genetic algorithms (Gas) that are randomized, evolutionary and population-based algorithms have been the most commonly used search algorithms. capturing the relationships among the variables of the problem (SNPs) is an essential issue for the success of Gas. To overcome the problems of GAs, we develop Estimation of Bayesian Network Algorithm (EBNA) that combines evolutionary computation and probabilistic graphical models as a search engine to discover high-order genetic interactions and genetic interaction networks associated with the disease. The proposed methods for Genome -wide association studies incorporating searching genetic interaction networks are applied to two real data sets. The results show that the proposed methods may open a new avenue for network based approach to genome-wide association studies.

Clinical features and molecular analysis of eight LHON Chinese families carrying the ND6 T14484C mutation. *X. Zhou[1], J. Qu[1,2], Y. Sun[3], F. Zhao[2], L. Yang[1], Y. Tong[1,4], Q. Wei[3], R. Li[5,6], Y. Qian[5,6], F. Lu[1], M.X. Guan[5,6]* 1) School of Ophth and Opto, Wenzhou Medical College, Wenzhou, Zhejiang, China; 2) Zhejiang Provincial Key Laboratory of Medical Genetics, School of Life Sciences, Wenzhou Medical College, Wenzhou, Zhejiang 325003, China; 3) Department of Ophthalmology, Dongfang Hospital, Beijing University of Chinese Medicine and Pharmacology, Beijing 100078, China; 4) The First Affiliated Hospital, Fujian Medical University, Fuzhou, Fujian 350005, China; 5) Division of Human Genetics, Cincinnati Childrens Hospital Medical Center, Cincinnati, Ohio 45229, USA; 6) Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45229, USA.

With the aim of investigating the molecular basis of development of Lebers hereditary optic neuropathy (LHON), a systematic and extended mutational screening of mtDNA has been initiated in the large clinical population of Ophthalmology Clinic at the Wenzhou Medical College, China. In the current study, we report the clinical, genetic and molecular characterization of eight Chinese families with LHON. Clinical and genetic evaluations revealed the variable severity and age-of-onset in visual impairment in these families. Penetrances of visual impairment in these Chinese families ranged from 20% to 55%. Furthermore, the average age-at-onset in those Chinese families ranged from 11 to 22 years old. In addition, the ratios between affected male and female matrilineal relatives in these Chinese families ranged from 1.5 to 5, respectively. Sequence analysis of the complete mitochondrial genomes in these pedigrees showed the distinct sets of mtDNA polymorphism, in addition to the identical T14484C mutation associated with LHON in these families. The fact that mtDNA of those pedigrees belonged to different haplogroups C, D, F, and M suggested that the T14484C mutation occurred sporadically and multiplied through evolution of the mtDNA in China. Furthermore, nuclear modifier gene(s) or environmental factor(s) or mitochondrial haplotype(s) seem to account for the penetrance and expressivity of LHON in these Chinese families carrying the T14484C mutation.

Phenylalanine blood levels and clinical outcomes in phenylketonuria: A systematic review and meta-analysis. *S.E. Waisbren[1], H. Leibowitz[1], K. Fahrbach[2], K. Noel[2], A. Dorenbaum[3], H. Levy[1]* 1) Children's Hospital, Boston, Boston, MA; 2) United BioSource Corporation Medford, MA; 3) BioMarin Pharmaceutical, Inc., Novato, CA, USA.

Blood phenylalanine (Phe) levels provide a practical and reliable method to diagnose and manage phenylketonuria (PKU) patients. To examine the question of whether blood Phe levels can be used as a predictive biomarker of clinical outcomes for the development of new PKU treatments, we conducted a systematic literature review and meta-analysis of all published trials from January 1980 through February 2004 that included Phe levels, neurological outcomes, and dietary compliance in PKU patients and we assessed the global relationship between blood Phe and IQ. Within-study correlations between blood Phe and IQ were extracted from 43 studies. Correlations derived from meta-analyses between IQ and blood Phe were significant during critical periods ranging from 0 to 12 years of age ($r = -.35$, $n = 459$); each 100 mol/L increase in blood Phe during the critical period predicted an average 1.3 to 3.1 point decrease in IQ over a range of blood Phe from 423 to 750 mol/L. Similar correlations were found between IQ and lifetime blood Phe levels ($r = -.34$, $n = 436$); each 100 mol/L increase in lifetime blood Phe predicted an average 1.8 to 3.8 point decrease in IQ over a range of blood Phe from 394 to 666 mol/L. Within-study correlations between IQ and concurrent blood Phe levels were also significant ($r = -.31$, $n = 666$). Each 100 mol/L increase in concurrent blood Phe predicted an average 0.5 to 1.6 point decrease in IQ, over a blood Phe range from 390 to 1863 mol/L. Compliance with the PKU diet was reported in 24 studies involving 928 PKU subjects. A high level of non-compliance was seen even in children ages 0-6 yrs. This percentage increased significantly as children got older. These results confirm previous studies reporting a significant correlation between concurrent and long-term blood Phe levels and IQ in PKU patients and highlight the need for development of new PKU therapies.

First report: Phenotypic and Molecular analysis in an extensive Colombian Pedigree with Fabry disease. *P. Paez[1], A. Lopez[1], S. Ospina[1], N. Gelvez[1], C. Duran[1], J.C. Prieto[1,2]* 1) Dept Cundinamarca, Inst de Genetica Humana, Bogota, Colombia; 2) Dept Genetica, Hospital la Victoria, Bogota, Colombia.

Fabry disease is an X-linked inborn error of glycosphingolipid metabolism resulting from mutations in the. alpha-galactosidase A (alpha-Gal A). Classically affectad males present acroparesthesias, hypohidrosis, angiokeratomas, corneal opacities, and abdominal pain, renal failure and cerebrovascular and cardiovascular complications. Fabry female carriers can be asymptomatic or clinically affected. We evaluated a Colombian family with classic criteria for Fabry disease. Phenotype analysis: 5 affected males and 2 obligate carriers from this family were analized, all affected and obligate carriers were studied with alpha Gal A levels, laboratory studies, echocardiogram, EKG and specialized valoration by neuropsicology and ophthalmology. Molecular analysis: Genomic DNA was isolated from affected males and obligate carriers females and the entire -Gal A coding region and flanking sequences were amplified by PCR and analysed by automated sequencing. STR loci (DXS101 and DXS7424) were analised determined in 50-X chromosome haplotypes from unrelated individuals and the frequencies of observed alleles. % heterozygosity and PIC were determined. One family with Fabry disease patients was referred by carrier detection for segregation analysis of highly polymorphic STR loci. The proband and other affected males were identified to be hemizygotes of GLA mutation. A C-to-T transition of g11002 in codon 342 of exon 7 (CGA to TGA), substituting a arginine codon by a termination signal (R342X), and deletion of 87 residues. Mothers of the affected males were heterozygotes carrying the same mutation as their sons. Haplotype analyses with polymorphic markers used for this purpose was informative for DXS7424 y DXS10; analyses indicate that all affected males and obligate carriers from this family have the allele that transmits the disease. The frequencies of all observed alleles, % heterozygosity and PIC were estimated and compared in several ethnic groups. Results derived from this study area useful for carrier detection and genetic counseling in Fabry disease.

Atypical Presentation of a Patient with Pityriasis Rubra Pilaris. *J.C. Prieto[1,3], P.M. Hurtado[1], A. Motta[2]* 1) Inst de Genetica Humana, Univ Javeriana, Bogota Cundinamar, Colombia; 2) Departamento Dermatologia, Hospital Simon Bolivar, Bogota, Colombia; 3) Departamento de Genetica, Hospital la Victoria, Bogota, Colombia.

Introduction. Pityriasis rubra pilaris is a very rare papulosquamosus disease which has chronic course and still unclear etiology. Already, we know that this dermatosis occurs as often in male as in female, can be present in each age and has genethics background. Diagnostic and especially therapy of those skin diseases is very difficult and can make a lot of problems not only dermatologist. Case report. A 12 year-old female was referred for genetic evaluation because of a history of reddish-orange patches on the skin, severe flaking, uncomfortable itching, and thickening of the skin on the feet and hands, since she was 2 year-old. This paper presents the case of a 12-year-old woman with diagnosed and histological confirmed Pityriasis rubra pilaris, and also hypotiroidism documented two months ago. This patient also presents short stature. Discusion. Pityriasis rubra pilaris is a papulosquamous disorder of unknown cause that is characterized by follicular hyperkeratosis, perifollicular erythema and that may progress to generalized erythroderma, and palmoplantar hyperkeratosis. Lesions are characteristically orange or yellow, with approximately 1-cm islands of sparing. Five types of pityriasis rubra pilaris have been described, which differ with respect to age, clinical features, course, and prognosis. The five types include the classic adult and juvenile types (I and III), the atypical adult and juvenile types (II and V), and the circumscribed juvenile type (IV). We didnt find in the literature, reports of Pytiriasis rubra pilaris in association with short stature and hypotiroidism.

Application of array-CGH method to the study on the genetic effects of atomic bomb radiation. -Copy number variants detected in the pilot study-. *N. Takahashi[1], K. Sasaki[1], M. Kodaira[1], Y. Satoh[1], Y. Kodama[1], H. Omine[1], Y. Shimoichi[1], K. Sugita[2], H. Katayama[2], N. Tsuyama[3]* 1) Dept Genetics, Radiation Effects Res Fdn, Hiroshima, Japan; 2) Dept Info Tech, Radiation Effects Res Fdn, Hiroshima, Japan; 3) Dept Anal Mol Med Dev, Hiroshima Univ Grad Sch Biomed Sci, Hiroshima, Japan.

[Purpose] We have studied the effects of atomic-bomb radiation on human germline cells at the genome level. To conduct this study at the genomewide level, we have introduced DNA micro-array based comparative genomic hybridization (array CGH) method. The pilot study using arrays having about 2,240 target Bac-clones revealed that, using the experimental conditions established by us, deletion-type and amplification-type variants with the size of 50 kb or more could be reliably detected. Before launching a large-scale study using the array-CGH, the feasibility of this technique was validated in a pilot study. We will summarize the results in the pilot study. [Experiment] We used an array with about 2,240 Bac-clones. These target clones were distributed in entire human autosomes at an interval of about 1.2 Mb. We examined genomic DNA obtained from 40 offspring of A-bomb survivors and 40 controls. [Results and Discussion] In the pilot study, a total of 253 copy numbers variants (CNVs) were detected. Among them, 14 were rare CNVs, each of which was detected in only one person in this population. Out of these 14, eight CNVs were amplifications and six were deletions. The unit sizes of rare CNVs were in the range of 50 kb to 500 kb (means were 73 kb in deletion type and 161 kb in amplification type). Furthermore, family studies showed that all of these CNVs were inherited from one of the parents. The remaining 239, which were common CNVs observed in two or more persons, were identified on 16 kinds of targets. Of those CNVs, 165 were amplifications and 74 were deletions. This mean number of CNVs (253/80 or 3/person) was considerably smaller than that reported previously by others (about 10/person) on individuals from different geographic backgrounds.

Inferring TGF-B pathway using time series data. *X. Yang[1], P. Yan[1], X. Sun[1], L. Jin[1, 3], M. Xiong[2], X. Zhou[2], D. Lu[1]*
1) State Key Laboratory of Genetic Engineering, School of Life Sciences, Fudan University, Shanghai, China; 2) University of Texas - Houston, TX, USA; 3) CAS-MPG Partner Institute for Computational Biology, Shanghai, China.

The TGF-b signal acts like a powerful molecular traffic light, which regulates growth and proliferation of cells, blocking the growth of many different cell types. Despite its importance in many biological processes such as inflammation, tissue repair and bone formation, its complete structure and computational models have not been well developed. Particularly, its dynamics to respond the external stimuli have not systematically investigated. To study dynamical behavior of TGF-b pathway, we performed three experiments to measure the expression levels of total 48 genes at 11 time points. We use state-space equations to represent dynamics of TGF-b pathway, extended kalman filter (EKF) to estimate both the states and parameters in the state space model (SSM) and EM algorithm to estimate system noise covariance Q and measurement noise covariance R of SSM. To improve the accuracy in estimation of state, we assume that the full set of observations is available, and use a full set of observation to predict the state at the current time. The concrete structure of TGF-b pathway is constructed and the accuracy of prediction is very high. The developed SSM of the TGF-b pathway can be used to study how TGF-b pathway responds the external stimuli and to aid the development of the treatment.

Haplotype inference error in association studies: A two-locus model. *Y. Wang*[1], *M. Xiong*[1, 2], *L. Jin*[1, 3] 1) State Key Laboratory of Genetic Engineering, School of Life Sciences, Fudan University, Shanghai, China; 2) University of Texas - Houston, TX, USA; 3) CAS-MPG Partner Institute for Computational Biology, Shanghai, China.

Haplotypes analyses have become increasingly common in association studies. Since experimental determination of haplotype is expensive, computational approaches were developed to extract haplotype information from genotyping data. However, haplotype inference error (HIE) is an intrinsic problem in all these computational approaches. It is worth systematically investigating how the error of haplotype frequency estimation affects the results of association studies by both theoretical analysis and intensive simulation studies over large parameter spaces. In this study, we derive the general formula to calculate the mean and variance of the HIE and the several statistics in association studies as well. We then use a two-locus model to demonstrate the magnitude of HIE and its effects on association study and the measure of linkage disequilibrium by both theoretical analysis and large-scale simulations. We show that the chi-square is usually about 10%-50% inflated depends on the frequency of four haplotypes. So an adjustment is suggested to be operated on the original chi-square statistic to avoid false positive.

Single nucleotide polymorphisms in *TNFSF15* confer susceptibility to Crohns disease. *K. Yamazaki[1], D. McGovern[2,3], J. Ragoussis[2], M. Paolucci[2], H. Butler[2], D. Jewell[2,3], L. Cardon[2], M. Takazoe[4], S. Saito[5], A. Iida[5], A. Takahashi[6], T. Tsunoda[6], M. Lathrop[7], Y. Nakamura[5], A. Hata[1]* 1) Laboratory for Gastrointestinal Diseases, SNP Research Center, RIKEN, Yokohama, Japan; 2) The Wellcome Trust Centre for Human Genetics,Oxford, UK; 3) Gastroenterology Unit, University of Oxford, Oxford, UK; 4) Department of Medicine, Division of Gastroenterology, Social Insurance Chuo General Hospital, Tokyo, Japan; 5) Laboratory for Genotyping, SNP Research Center, RIKEN, Yokohama, Japan; 6) Laboratory for Medical Informatics, SNP Research Center, RIKEN, Yokohama, Japan; 7) Centre National de Genotypage, Evry Cedex, France.

The inflammatory bowel diseases (IBD), Crohns disease (CD) and ulcerative colitis, are chronic inflammatory disorders of the digestive tract. The pathogenesis of IBD is complicated, and it is widely accepted that immunologic, environmental and genetic components contribute to its etiology. Our previous studies indicated that there may be ethnic differences in genetic susceptibility to CD that are not explained by the minor demographic and phenotypic differences between the populations. In order to identify genetic susceptibility factors in CD, we performed a genome-wide association study in Japanese patients and controls using nearly 80,000 gene-based single nucleotide polymorphism (SNP) markers, and investigated the haplotype structure of the candidate locus in Japanese and European patients. We identified highly significant associations ($p = 1.71 \times 10^{-14}$ with odds ratio of 2.17) of SNPs and haplotypes within the *TNFSF15* (the gene encoding tumor necrosis factor superfamily, member 15) genes in Japanese CD patients. The association was confirmed in the study of two European IBD cohorts. Interestingly, a core *TNFSF15* haplotype showing association with increased risk to the disease was common in the two ethnic groups. Our results suggest that the genetic variations in the *TNFSF15* gene contribute to the susceptibility to IBD in the Japanese and European populations.

Molecular diagnosis for fragile X syndrome in Korea. *S.J. Park, J.A. Yang, S.Y. Jeong, H.J. Kim* Dept Medical Genetics, Ajou Univ School of Medicine, Suwon, Korea.

Fragile X syndrome (FXS) is the commonest cause of inherited mental retardation in males. Several population-based studies in Caucasians of mostly northern European descent have established that the prevalence is probably between one in 6,000 to one in 4,000 males in the general population and one in 8000 females. The FXS is the prototype of triplet repeat disorders, caused by the expansion of a CGG repeat in the 5'UTR of the *FMR1* gene which is located in the Xq27.3 region of the long arm of the X chromosome, eventually causing promoter methylation and transcriptional silencing of the gene. A full mutation is found in patients with mental retardation and corresponds to large expansions of the repeat (>200 repeats). Premutations are moderate expansions (5-200 repeats) and are found in normal transmitting males and in the majority of clinically normal carrier females. About 15% of patients show a mosaic pattern consisting of both full mutations and premutations. We screened for FXS in 96 unrelated individuals (male 80 and female 16) with mental retardation of unknown cause. We have used PCR and Southern blotting (nonradioactive) for determination of a CGG repeat size and methylation-specific PCR (MS-PCR) for detection of the methylated allele in male. We found five male patients (6.3%, 5 in 80 male examined) with full mutation and the CGG repeat size range was 300-500. Among them, three patients revealed to have a mosaic full mutation and normal size alleles. We also found one premutation carrier male with the approximately 100 CGG repeats. These results indicate that a high incidence of FXS is found in Korean males with inherited mental retardation, compare to other ethnic groups, providing an insight into the prevention of FXS by genetic counseling and prenatal diagnosis.

S100A8, as a candidate of NF-kappa B pathway, participates in the genesis of laryngeal squamous cell carcinoma. *K.L. Sun[1], W.N. Fu[1], D.F. Huang[1], Y. Guo[1], C. Shang[1], Z.M. Xu[2], X.H. Sun[2]* 1) Medical Genetics, China Medical University, Shenyang, Liaoning, China; 2) The 463 Hospital of PLA, Shenyang, China.

S100A8, as a candidate of NF-kappa B pathway, participates in the genesis of laryngeal squamous cell carcinoma Kailai Sun1, Dai-Fa Huang1, Wei-Neng Fu1, Yan Guo1, Chao Shang1, Zhen-Ming XU2, Xing-He Sun2 1. Department of Medical Genetics, China Medical University, Shenyang, 110001, P.R. China; 2. The 463 Hospital of PLA, Shenyang, 110042, P.R. China To explore the function and molecular mechanism of S100A8 in laryngeal squamous cell carcinoma. Semi-quantitative RT-PCR, immuno- histochemistry analysis and Western blotting were used to investigate the association of S100A8 with LSCC. RNA interference was applied to evaluate the function of S100A8 in NF-B pathway. The co-immunoprecipitation combined with peptide mass fingerprinting based on matrix-assisted laser adsorption/ionization time-of-flight mass spectrometry were used to reveal the interacted proteins to S100A8. Both mRNA and protein levels of S100A8 increasing in LSCC demonstrated that S100A8 was involved in the genesis of LSCC. Increased apoptotic cells and decreased trans-membrane cells after RNAi S100A8 in Hep-2 cells were observed. No changes of P65, RELB, IKK and IKKmRNA levels were found in Hep-2 cells after RNAi S100A8, but S100A8 mRNA was down-regulated in Hep-2 cells after RNAi P65. Some proteins interacted with S100A8 were found. They were MHC class I HLA-B,similar to T-box 1 isform C, sarcolemmal associated protein 1 and hypothertical protein L0C80154. The abnormalities of S100A8 are common events in LSCC. S100A8 has the abilities of inhibiting apoptosis and promoting metastasis. S100A8 might be a new member of NF-B pathway and probably lie in the downstream of P65. Key words: laryngeal squamous cell carcinoma (LSCC); RNA interference (RNAi); S100A8; NF-B pathway.

Systemic assessment of MMP2 gene and the risk for endometriosis and adenomyosis. *R.S. Wu[1], H.Y. Hsieh[2], M.T. Wu[3], M.C. Huang[4], E.M. Tsai[5], S.H. Juo[1]* 1) Graduate Institute of Medical Genetics,Kaohsiung Medical University,Taiwan; 2) Graduate Institute of Behavior Sciences,Kaohsiung Medical University,Taiwan; 3) Departments of Occupational Medicine and Family Medicine, China Medical University & Hospital,Taiwan; 4) Department of Nutrition,Kaohsiung Medical University Hospital,Taiwan; 5) Department of Obstetrics and Gynecology, Kaohsiung Medical University Hospital,Taiwan.

Background: To test if the MMP2 gene are associated with the risk of endometriosis and adenomyosis. **Methods**: We performed case-control studies, where the cases had at least one of the two diseases and controls were disease free women. We selected a functional promoter single nucleotide polymorphism (SNP1) along with 16 tagging SNPs (SNPs2-17). 107 patients had pure endometriosis, 102 had pure adenomyosis, 20 had both diseases. Two types of controls were used: 203 hospital-based and 183 community-based controls. Logistic regression model was used to assess the genotypic effect. **Results**: Genotype distributions were in Hardy-Weinberg equilibrium for both cases and controls. We found significant association at SNPs16 and 17 for both endometriosis and adenomyosis.The risk genotype CC at SNP16 had an OR of 2.41(P=0.014) and AC at SNP17 had an OR of 2.05(P=0.0027) when comparing endometriosis with hospital-based control. The results by comparing endometriosis with community controls were similar to those in the hospital controls, but somewhat less significant. Using the hospital controls, the OR slightly increased for severe endometriosis at SNP16 (OR=2.58;P=0.012)and SNP17 (OR=2.12;P=0.003). SNPs16 and 17 were also associated with adenomyosis, although the results were not as significant as endometriosis. The risk genotype CC at SNP16 had an OR of 2.23(P=0.037) and AC at SNP17 had an OR of 1.91(P=0.011). Similarly, the results were more significant when hospital-based subjects were used as controls than community controls. **Conclusions**: The present study found significant association between endometriosis and adenomyosis. The association was more significant endometriosis than adenomyosis. The association slightly increased between the SNPs and severe endometriosis.

The R192 allele of the PON1 gene is associated with reduced serum arylesterase activity in autistic patients and in their first-degree relatives. *A.M. Persico[1,2], L. Gaita[1,2]* 1) Lab. of Molecular Psychiatry & Neurogenetics , University "Campus Bio-Medico", Rome, Italy; 2) I.R.C.C.S. Fondazione S. Lucia, Rome, Italy.

The PON1 gene is located in ch 7q21.3 and encodes paraoxonase, the serum enzyme responsible for organophosphate inactivation. This enzyme also exerts an arylesterase activity, which is strictly correlated with PON1 gene expression and directly reflects the amounts of enzyme present in the serum. We have recently described a significant case-control and family-based association between the R192 allele of the PON1 gene and autism in Caucasian-American, but not in the Italian families. In conjunction with our prior results, this finding points towards possible contributions of prenatal organophosphate exposure to autism pathogenesis in the U.S.A., and not in Italy. To explore whether the R192 allele associated with autism also affects PON1 gene expression in families with autistic probands, we have measured serum arylesterase activity and genotyped at the Q192R polymorphism in 183 autistic patients and first-degree relatives belonging to 39 simplex and multiplex Caucasian-American families recruited by the AGRE consortium. Overall, the presence of one or two copies of the R192 allele is strongly associated with significantly lower serum arylesterase activity both in autistics and in their first-degree relatives (two-way ANOVA $F=7.7$, 2 df, $p<0.001$). Furthermore, autistic patients display a trend towards reduced arylesterase activity compared to first-degree relatives, regardless of Q192R genotype ($F=2.47$, 3 df, $p=0.06$). We are now in the process of replicating previous results showing no effect of the Q192R genotype on arylesterase activity among normal controls. These findings indicate that in autistic patients, and to a lesser extent in their first-degree relatives, the R192 allele is associated with decreased PON1 gene expression, which may enhance the risk to undergo altered neuronal migration in the event of prenatal exposure to organophosphate insecticides. Supported by Cure Autism Now, Telethon-Italy (GPP02019), the Fondation Jerome Lejeune and the National Alliance for Autism Research.

Mitochondrial DNA depletion is a major cause of multiple respiratory chain defects. *E. Sarzi[1], A. Bourdon[1], D. Chretien[1], M. Zahrate[1], J. Corcos[1], A. Slama[2], V. Cormier-Daire[1], P. de Lonlay[1], A. Munnich[1], A. Rotig[1]* 1) INSERM U781, Necker Hosp, Paris, France; 2) Laboratoire de Biochimie 1, Hôpital Bicêtre, Le Kremlin-Bicêtre.

Mitochondrial DNA depletion syndrome (MDS) is a clinically and genetically heterogeneous condition characterized by reduction in mtDNA copy number responsible for multiple oxidative phosphorylation (OXPHOS) enzyme deficiency. In order to determine the actual incidence of mtDNA depletion in multiple respiratory chain deficiency, we have carried out the real-time PCR quantification of mtDNA in liver or muscle tissue of 100 of 270 children with unexplained multiple OXPHOS deficiency. Half of the patients presented a reduction in mtDNA copy number below 50% of control values in liver and/or muscle (50/100). Most patients (16%) presented a neonatal form with severe liver involvement, a second group was represented by Alpers syndrome (4%) and 20% of the patients presented encephalomyopathy. DGUOK or POLG mutations could be identified in 22% of patients with liver disease, POLG mutations were consistently found in all but one patient with Alpers syndrome. Two patients carried a homozygous TK2 or MPV17 mutation respectively. Our findings show that mtDNA depletion is a very frequent cause of multiple respiratory chain deficiency with a incidence of at least 18% of children with unexplained OXPHOS deficiency.

CLINICAL AND MORPHOLOGICAL CHARACTERIZATION OF THE MACROCEPHALIC ENDOPHENOTYPE IN AUTISM. *R. Sacco[1,2], R. Militerni[3], C. Bravaccio[3], A. Frolli[3], M. Elia[4], S. Trillo[5], P. Curatolo[6], B. Manzi[6], C. Lenti[7], M. Saccani[7], A.M. Persico[1,2]* 1) Lab. of Mol. Psychiatry & Neurogenetics, Univ. Campus Bio-Medico, Rome, Italy; 2) IRCCS "Fondazione S. Lucia", Rome, Italy; 3) Child Neuropsychiatry, II Univ. of Naples, Italy; 4) Neurology Serv., IRCCS "Oasi Maria S.S.", Troina (EN), Italy; 5) Assoc. "Anni Verdi", Rome, Italy; 6) Child Neuropsychiatry, II Univ. of Rome, Italy; 7) Child Neuropsychiatry, Univ. of Milan, Italy.

Macrocephaly (i.e., cranial circumference > 97th percentile) represents one of the most consistent endophenotypes described in autism research, present in approximately 20% of patients. Since this subgroup may share at least some pathogenetic mechanisms, we defined its clinical and physical characteristics in a sample of 200 autistic patients. Macrocephaly, present in 56 (28.0%) of our patients, is not an isolated morphological feature, but is instead part of a broader macrosomy phenotype, characterized by strong correlations between cranial circumference (CC), weight (W) and height (H) (Spearman rho: CC-W=0.45, CC-H=0.49, H-W=0.62, all $p<0.001$). Clinically, macrocephalic patients appear more severely impaired (for example, VABS Scores: Communication, $p<0.001$; Daily Living Skills $p<0.01$; Socialization $p<0.01$; Motor Skills $p<0.05$), with levels of impairment matched only by patients with cranial circumferences < 25th percentile. Interestingly, they also display enhanced frequencies of hyperkinesia ($p<0.05$), motor stereotypies ($p<0.05$), history of food allergies in first-degree relatives ($p<0.05$) and possibly of allergies in the patient ($p=0.065$). Instead, the development of verbal language, when present, occurs significantly earlier ($p<0.05$) in macrocephalic children compared to other patients. Our study conclusively proves that macrocephalic autistic children are in reality macrosomic, it outlines their spectrum of severe clinical symptoms, and points towards pathogenetic links with immune dysfunction and altered control of cell cycle progression as possibly playing a more prominent role in this subgroup of patients. Supported by Telethon-Italy (GPP02019), the National Alliance for Autism Research, Cure Autism Now, and the Fondation Jerome Lejeune.

Association between an endoglin gene polymorphism and systemic sclerosis related pulmonary arterial hypertension. *J. Wipff[1,2], A. Kahan[2], E. Hachulla[3], J. Cabane[4], O. Meyer[5], L. Mouthon[6], L. Guillevin[6], C. Boileau[1], Y. Allanore[1,2]* 1) INSERM U 781, Necker Hospital, Paris, France; 2) Rheumatology A, Cochin hospital, Paris, France; 3) Internal Medecine department, Claude Huriez Hospital, Lille, France; 4) Internal Medicine Department, Saint-Antoine hospital, Paris, France; 5) Bichat Hospital, Rheumatology department, Paris, France; 6) Internal medecine Department, Cochin hospital, Paris, France.

Background Systemic sclerosis (SSc) is a connective tissue disorder characterized by early generalised microangiopathy with endothelial activation and apoptosis. Endoglin gene (ENG) encodes an accessory receptor for the TGFbeta superfamilly crucial for maintaining vascular integrity. ENG mutations are responsible of one of the two major types of Hereditary Haemorrhagic Telangiectasia. An intron 7 insertion polymorphism of ENG has been reported to be associated with intracranial saccular aneurysms. Objective. To investigate the association between the polymorphism in ENG intron 7 and SSc in a multicentric French Caucasian population. Methods. The insertion polymorphism was investigated in 280 SSc patients: mean age was 5713 years, 29/280 had associated pulmonary arterial hypertension (PAH) diagnosed by catheterism. The control group consisted in 140 patients with osteoarthritis. Allelic and genotypic frequencies were compared between cases and controls using a ² test. Results. The polymorphism was in Hardy Weinberg equilibrium. The frequency of the polymorphism did not differ between SSc patients and controls (19.3% versus 23.9%, NS). However, there was a significant lower frequency of 6bINS allele in SSc patients with associated PAH compared to controls (10.3% versus 23.9%, p=0.01) and a trend in comparison to SSc patients without PAH (10.3% versus 20.3%, p=0.05). Genotype carrying 6bINS allele was also less frequent in SSc patients with PAH than in controls (20.7% vs 42.9%, P = 0.02). Conclusion. The frequency of ENG intron 7 insertion polymorphism differs between SSc patients with or without PAH which suggests the implication of ENG in this devastating vascular complication of SSc. The sequencing of ENG among SSc patients is ongoing.

Mitochondrial DNA Lineages in the Yukaghir, Chukchi and Siberian Eskimos, and Resettlement of Arctic Siberia after the Last Glacial Maximum (LGM). *N.V. Volodko[1], R.I. Sukernik[1], E.B. Starikovskaya[1], M.A. Lvova[1], D.C. Wallace[2]* 1) Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences, 10, Lavrent'ev Ave, Novosibirsk, Russian Federation, 630090; 2) Center for Molecular and Mitochondrial Medicine and Genetics, University of California, Irvine, 2014, Hewitt Hall, Irvine, CA 92697-3940.

In this study, we filled important gaps in the previously unidentified internal sequence variation within haplogroups A, C and D prevailing in the former western Beringia through extended survey of mtDNA diversity in the Yukaghir, Chukchi, and Siberian Eskimos. By integrating 71 entire A, C, and D mtDNAs selected from ~350 individuals with those previously revealed in other Siberian populations, we were able to identify novel A, C, and D lineages. Unlike the Chukchi and Siberian Eskimos of Chukotka, who harbored the Beringian specific A2a, A2b and D2, distinguished by the mutations 3330 and 16292, 11365 and 16265, and 11215, respectively, the core of the genetic makeup of adjoining Yukaghir tribes in the west consisted of a unique combination of C and D mtDNA lineages that apparently were continental Siberian. A phylogeny of the entire D revealed a wide range of distinct lineages (D1-D13) identifiable by characteristic mutations. Of these, the Yukaghir D3-D9 were a subset of the Siberian/Asian haplogroup D variation, and their coalescence ages ranged from 8,000 to 43,000, in consistence with the range of their distribution in Siberia/Asia. The ages of Paleosiberian C2 and C3 (~18,000 years), when compared to that of Native American C1 (~23,000) and entire C (~28,000), have major implications for the origin of the Yukaghir. On the other hand, the estimated ages of the Chukchi-Eskimo subset of the Beringian A2a and A2b (~5,000), and D2 (~10,000) define the time frame for the fission-fusion events in the Chukotka and adjacent Alaska. Overall, our data suggest that the direct ancestors of the Yukaghir were primarily drawn from the southern belt of Siberia after ~13,000 years ago when environment conditions changed permitting re-colonization of the Siberian arctic and subarctic after the LGM.

Prognostic evaluation of trisomy 13 syndrome: Review of 23 cases. *K. Sameshima[1], H. Yoshihashi[1], Y. Igarashi[1], Y. Itani[2], M. Yamanaka[3], K. Kurosawa[1]* 1) Division of medical genetics, Kanagawa Children's Medical Center; 2) Division of Neonatology, Kanagawa Childrens Medical Center; 3) Division of Obstetrics and Gynecology, Kanagawa Childrens Medical Center, Yokohama, Japan.

Trisomy 13, known as Patau syndrome, is the third most common autosomal trisomy, and occurs at a frequency of 1 of 5,000 births. Triosmy 13 is associated with multiple severe structural abnormalities, and represents high infant mortality rate. From January 1995 to December 2005, all 23 cases of trisomy 13 diagnosed at our hospital were reviewed, including 15 cases prenatally diagnosed. Data are presented on pregnancy, delivery, medical complications, growth, cause of death, cytogenetics, and recurrence risk. We also examined median survival time or median age at death and factors associated with longer survival. Median survival time was 18 days. 96% of infants died within the first year. Major anomalies detected by means of ultrasound(US) included holoprosencephaly(9[39%]) or other central nervous system anomalies(16[69%]), cleft lips and palates(15[65%]) and cardiac (18[78%]) defects. The presence of holoprosencephaly is associated with significant shorter lifespan, but the presence of a congenital heart defect is not. Although the ascertainment of cases is based toward survivors, the data provide information useful in counseling families of newborn infants, and valuable insights into the natural history of trisomy 13. When prenatal or postnatal decisions need to be made regarding the care of fetus or child, it is important that the full spectrum of the natural history of these conditions be presented to the parents to enable them to make the best possible decisions for their family.

Could the expression of Jagged1 gene mutations play a role in modulating a CADASIL-like phenotype? *C. Ungaro, T. Sprovieri, FL. Conforti, A. Patitucci, A. Magariello, AL. Gabriele, M. Muglia, R. Mazzei* ISN-CNR, Mangone , Cosenza, Italy.

The aim of this study was to investigate the possible role of Jagged1 gene mutations in modulating clinical features in patients with CADASIL-like phenotype resulted negative for Notch3 gene mutations. Sixty-five CADASIL-like patients without Notch3 gene mutations were investigated for 5 out 26 exons of the Jagged1 gene. Amplicons were analyzed by DHPLC and direct sequencing. The sequence of exons 3,4 and 23 revealed the presence of four already described polymorphisms in Jagged1. 1001C/T in exon 4 was found in 3 patients, IVS23+18del(T) in 24 patients, IVS4-15T/C in 11 patients, IVS3-43C/T in one patient; in four patients we found both the polymorphism 1001C/T and IVS4-15T/C. CADASIL is an autosomal dominant disorder caused by mutations in Notch3 gene, encoding a transmembrane protein involved in cellular signalling and cell differentiation. Notch receptor signalling is a conserved fundamental mechanism modulating several processes through interaction with ligands of the Delta/Serrate family, like that Jagged1. Mutations in the human Jagged1 gene cause Alagille syndrome (AGS). In some patients (16%), the literature documents multiple case reports of intracranial vessel abnormalities and other vascular anomalies in AGS (1). Intracranial bleeding, which ranges from massive fatal events to asymptomatic cerebral infarcts, is a recognized complication and cause of mortality in AGS. Further evidence of the role of Jagged1 and Notch in vascular development is provided by the phenotype of targeted Notch pathway mutants. In fact, mice homozygous for mutation in Jagged1 die of haemorrhage during early embryogenesis because of defects in angiogenic vascular remodelling in the yolk sac and embryo (1). As mutations in the gene for Notch3 receptor cause adult onset CADASIL which is associated with intracranial bleeding, the mechanism of the vasculopathy in CADASIL may be similar to that seen in AGS (2). Conclusion: The present study demonstrates that the Jagged1 gene does not play a role in patients with a CADASIL-like phenotype.

Gossypol-induced abnormal chromosome pairing during meiotic prophase of male mice (Mus musculus).

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Gossypol, a reported male antifertility compound isolated from the seed, roots, and stem of the cotton plant (Gossypium sp.), was assayed for its effects on synaptonemal complexes(SCs) damage in male mice. The mice were taken orally daily gossypol acetic acid (XIAN NORTH PHARMACEUTICAL COMPANY LTD) (suspension in 1 % methylcellulose with an oral administration dose of 30 mg /kg body weight) for 4 and 10 weeks. Other treated groups were administered daily dose of 60 mg /kg body weight for 4 weeks. Whole-mount SC spreads were prepared by surface spreading-silvers staining transmission electron microscope (TEM) technique. The structure and pairing of chromosomes and SCs from control male mice (untreated by gossypol) and experiment mice (treated by gossypol) were observed by light microscope (LM) and TEM. SC aberrations in spermatocyte of treated male mice could be induced by gossypol. SC abnormalities were classified as various forms of breakage and synapsis errors. It included various forms as follows: breakage of the SC; lateral element break; asynapsis; non-homologous synapsis; XY-mispairing; XY separation; synapsis irregularity. The chromosomes abnormalities in MI stage were showed as follows: univalents of autosomes; isochromatid break of autosomes; multivalent; univalent of XY. More serious damage could be observed as the increased dosage or prolonged treated time of gossypol, but no special aberration forms were produced in high dosage gossypol. It is concluded that we can evaluate the genetic risk induced by gossypol in males by SC analysis. The relationship between the behavior of SC and the mechanism of infertility was discussed.

Mutation screening of the *ATRX* gene in Japanese familial mental retardation. *T. Wada[1], S. Saitoh[2], Y. Fukushima[1]*
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Mental retardation (MR) is relatively common and about 2-3 % of population has MR (IQ <70). It is important to know the pathogenesis of MR to develop well-grounded medical treatment. Up to now, more than 60 genes, including more than 20 genes for non-syndromic X-linked MR (XLMR), have been identified to be involved in MR, and the European XLMR Consortium has made great contribution to this accomplishment. The Japanese Mental Retardation Consortium was started in 2003, and more than 50 MR families have been registered. We have participated in the Consortium and conducted our study on the MR families. X-linked -thalassemia/mental retardation syndrome (ATR-X, OMIM #301040) is a syndromic form of XLMR, and it is caused by a mutation in the *ATRX* gene. An *ATRX* gene mutation is involved not only in other syndromic XLMRs but also in non-specific XLMR in males. Recently we reported that the *ATRX* gene mutation caused MR in females by escaping a skewed X chromosome inactivation (Wada T et al. Am J Med Genet, 2005). In this study, we performed mutational analysis of the *ATRX* gene in 33 mentally retarded probands of the MR families by a new method we have established, using mismatch-specific endonuclease (Surveyor^{TM}) (Wada T et al. Am J Med Genet, 2006, in press), in order to evaluate the frequency of *ATRX* mutations in familial MR cases in Japan.We have not found any mutations in these families so far. Although it is expected that an *ATRX* mutation may not be such a frequent cause of MR as *FMR1*, *MECP2* or *ARX* mutations, our data should provide further information of the molecular genetic etiology of familial MR in Japan. Our study would also be important to establish the genotype-phenotype correlations of the *ATRX* gene mutations and to understand the function of ATRX protein.

The novel L1649Q mutation in the *SCN1A* epilepsy gene is associated with familial hemiplegic migraine: genetic and functional studies. *K.R.J. Vanmolkot[1], M. Pusch[2], B. de Vries[1], A.H. Stam[3], E. Babini[2], T. Freilinger[4], M.A. Welch[5], N. Ramadan[5], R.R. Frants[1], M.D. Ferrari[3], M. Dichgans[4], A.M.J.M. van den Maagdenberg[1, 3]* 1) 1Department of Human Genetics, Leiden University Medical Centre, Leiden, The Netherlands; 2) Istituto di Biofisica, Genova, Italy; 3) Department of Neurology, Leiden University Medical Centre, Leiden, The Netherlands; 4) Department of Neurology, Klinikum Grohadern, Ludwig-Maximilians-Universität, München, Germany; 5) Department of Neurology, Rosalind Franklin University of Medicine and Science, The Chicago Medical School, Chicago, IL.

Familial hemiplegic migraine (FHM) is an autosomal dominant severe subtype of migraine with aura characterized by hemiparesis during the attacks. Mutations in the calcium channel gene *CACNA1A* (FHM1) and the Na,K-ATPase gene *ATP1A2* (FHM2), have led to the conclusion that abnormalities in ion fluxes play an important role in the pathophysiology of FHM. This was further strengthened by the recent discovery of the Q1489K mutation in the sodium channel gene *SCN1A* (FHM3) located at chromosome 2q24. Interestingly, mutations in *SCN1A* have also been associated with severe myoclonic epilepsy of infancy (SMEI) or generalized epilepsy with febrile seizures (GEFS+). Here we performed mutation scanning in 10 FHM families without mutations in the *CACNA1A* and *ATP1A2* genes. We identified a novel missense mutation, L1649Q, in a North American family with pure FHM without associated ataxia or epilepsy. Investigation of the biophysical consequences of the mutation by patch-clamp experiments in transiently transfected human tsA201 cells, using the closely related SCN5A cDNA, revealed a slower inactivation and a two-fold faster recovery from fast inactivation. Our findings confirm the role of neuronal Nav1.1 sodium channels in FHM, strengthen a common channelopathy mechanism of FHM and further support a possible link between epilepsy and migraine.

Synergistic association of neuronal UCP genes with schizophrenia. *K. Yasuno[1], S. Ando[2], S. Misumi[1], S. Makino[2], T. Muratake[3], N. Kaneko[3], H. Amagane[3], T. Someya[3], H. Inoko[1], H. Suga[4], K. Kanemoto[4], I. Inoue[1], G. Tamiya[2]* 1) Dept Mol Life Sci, Tokai Univ Sch Med, Kanagawa, Japan; 2) Div Hum Mol Genet, Dept Neurol, Tokushima Univ Sch Med, Tokushima, Japan; 3) Dept Psych, Niigata Univ Grad Sch Med and Dent Sci, Niigata, Japan; 4) Dept Neuropsych, Aichi Med Univ, Aichi, Japan.

Accumulating evidence suggest an involvement of mitochondrial dysfunction in schizophrenia (SZ). Three mitochondrial uncoupling proteins (UCPs), UCP2, UCP4 and UCP5 are present in selected neurons and their activities allow controlled proton leak back into the mitochondrial matrix, thereby reducing the membrane potential. These neuronal UCPs may regulate calcium homeostasis and productions of ATP and reactive oxygen species. Since dysregulations of these mechanisms have been implied in SZ, functional mutatinos in neuronal UCP genes may affect the risk of SZ. To explore this possibility, we conducted a case-control association study using a Japanese sample of 335 cases and 501 controls. We determined 7 tag SNPs (3 in UCP2, 3 in UCP4 and 1 in UCP5) from genotyped 16 SNPs following Carlson et al (Am J Hum Genet 74:106-120, 2004) and tested them for association with SZ. Four tag SNPs were significantly associated with SZ even after correcting for multiple comparisons: A55V (P=0.004) and rs649446 (P=0.007) in UCP2, and rs10807344 (P=0.003) and rs2270450 (P=0.004) in UCP4. We further explored the possibility of gene-gene interactions using the multifactor dimensionality reduction and the logistic regression. We observed a significant (P<0.05) synergistic interaction between UCP2 (A55V) and UCP4 (rs10807344). The highest OR was obtained for homozygotes of risk alleles at both loci while the lowest for individuals homozygous of risk alleles at UCP2 and having 1 or 2 non-risk alleles at UCP4. Individuals in the former class have 7.6-fold risk compared with the latter. These results suggest that dysfunctions of neuronal UCPs are likely to be involved in the genetic etiology of SZ accompanied with the interaction between them. Our findings thus offer new avenues for a better understanding of the roles of mitochondria in SZ.

Analysis of epigenetic abnormalities in an autistic population. *M. Rosales, I. Cuscó, B. Gener, M. Del Campo, L.A. Pérez Jurado* Genetics Unit, Experimental & Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain.

The Austistic Spectrum Disorders (ASD) comprise a group of neurodevelopmental diseases characterized by impairments in social interaction, communication problems and restricted range of interests. There is strong evidence for a neurobiological and genetic etiology of ASDs, but no major genes have been found, concordance in MZ twins is less than 100% and the phenotypic expression of the disorder varies widely. Therefore, a mixed genetic/epigenetic component to the disease has been proposed, supported in part by its association with several imprinted regions. *Methods*: To search for epigenetic alterations in our autistic population consisting of 88 children and 97 adults (all meeting DSM-IV criteria), we have first screened for chromosomal rearrangements by karyotyping, Q-PCR and a-CGH; and fragile-X, and then performed a battery of assays: 1) assessment of methylation patterns at SNRNP imprinted domain by QAMA; 2) genotyping of MTHFR alleles; 3) X-inactivation patterns in female patients and patients' mothers; 4) assessment of mono-biallelic expression at 44 putatively imprinted genes by RTPCR from lymphocyte RNA (69 cases / 7 controls) using 61 SNPs in 3 multiplex Sequenom assays. *Results*: QAMA confirmed the maternal 15q11-q13 duplication found in 4 patients and identified a patient with an epigenetic mutation at the same region in a mosaic pattern (verified by Southern Blot). MTHFR allele distribution and X-inactivation patterns did not differ significantly between patients and controls. Out of the 51 informative SNPs analysed in the 38 genes detectable by RTPCR, average heterozygosity was 30%. Eighteen genes showed consistent biallelic expression in all cases while 7 genes were always monoallelic, reflecting their imprinted status. Variable expression, either mono or biallelic, was found in several patients but not in controls for 13 genes, which are therefore candidates for epigenetic defects in our ASD sample. *In conclusion*, our preliminary data suggest that epigenetic abnormalities and alterations in the patterns of allelic expression of imprinted genes might be pathogenically related with ASD in a proportion of cases.

Parkin mutations in patients with early onset parkinsonism. *F.E. Rocca, F. Annesi, P. Tarantino, I.C. Cirò Candiano, S. Carrideo, D. Civitelli, G. Provenzano, E.V. De Marco, G. Annesi* Inst. of Neurological Sciences, National Research Council, Mangone, Cosenza, Italy.

Parkin gene mutations are reported to be a major cause of early-onset parkinsonism (age-at-onset 45 years) in families with autosomal recessive inheritance and in isolated early-onset Parkinsons disease (IEOPD). The mutations in the parkin gene are extremely varied and include many different point mutations and exonic rearrangements. The Parkin protein is an ubiquitin E3 ligase that functions to prepare substrates for protein degradation mediated through the ubiquitin-proteasome system. To evaluate the frequency of parkin mutations in patients with IEOPD, we studied 57 patients from Southern Italy. Our patients were selected according to the following criteria: parkinsonism; no family history of PD and age at onset 45 years. All patients were screened for mutations in the parkin gene by a combination of gene dosage and sequencing of entire coding region Among the 57 patients, 10 (17,5%) had mutations in the parkin gene. Five carried single heterozygous mutations (Thr55Ile, Asp18Asn, Lys32Thr, Leu261Leu, Arg42Pro); one was a compound heterozygous (Leu174Leu and Arg402Cys); four carried homozygous mutations (202-203delAG, ex2-3del, ex3 del). Six of these were previously described and four were novel nucleotide changes. Genomic rearrangements were excluded by absolute quantification in real time PCR. The frequency of IEOPD patients carrying a single heterozygous parkin mutation in this study is similar to that reported by other authors. Most of the variants found in our patients are localized in two important parkin regions: the N-terminal ubiquitin-like that might mediate protein-protein interactions, which have an impact on the stability of parkin; the C-terminal cassette of two Ring finger domains (R1-R2) that are responsible for ubiquitination of target protein. Our patients with IEOPD may suffer from haploinsufficiency, due to a loss of one parkin allele wich reduces normal expression and subsequent enzymatic activity, thus representing a risk factor for the disease. Functional studies are required to clarify the role of single heterozygous parkin mutations in IEOPD.

HNPP due to a novel frameshift mutation of the PMP22 gene. *A. Patitucci[1], M. Muglia[1], R. Rizzi[2], C. Ungaro[1], FL. Conforti[1], AL. Gabriele[1], A. Magariello[1], R. Mazzei[1], L. Motti[2], R. Saladini[2], T. Sprovieri[1], N. Marcello[2], A. Quattrone[1,3]* 1) ISN-CNR, Mangone , Cosenza, Italy; 2) Department of Neurology, Arcispedale S. Maria Nuova, Reggio Emilia, Italy; 3) Institute of Neurology, University "Magna Graecia", Catanzaro, Italy.

Hereditary neuropathy with liability to pressure palsies (HNPP) is an autosomal dominant inherited disorder characterized by recurrent sensory or motor dysfunction, often precipitated by minor trauma. Electrophysiological studies demonstrate an underlying generalized neuropathy with moderate slowing of conduction velocities, predominant over entrapment sites and prolonged distal latencies. Characteristic pathological changes are sausages-like myelin thickening, commonly referred to as tomacula. In 85% of HNPP cases the genetic defect is a 1.5 Mb deletion on chromosome 17p11.2, encompassing the gene for the peripheral myelin protein (PMP22). Point mutations in the PMP22 gene are responsible for rare HNPP phenotype. Up to now, 11 point mutations of PMP22 have been associated with HNPP. We investigated a 17 years old girl who was referred at our clinic because she noted a four months history of severe hypesthesia at the 4th and 5th fingers of the left hand, that persisted for about two months, and then spontaneously regressed. Electrodiagnostic test revealed a sensory motor demyelinating neuropathy showing a conduction block in the left ulnar nerve at the elbow. Screening of the entire coding sequence of the PMP22 gene by DHPLC analysis showed a modified pattern for exon 2. Direct sequencing of exon 2 revealed a heterozygous deletion of one tymidine at position 11. The deletion was also confirmed by cloning of fragment containing exon 2. The mutation was also detected in the fathers patient, but it was not found in 100 normal chromosomes. The novel mutation here reported c.11delT creates a shift on the reading frame starting at codon 4 and lead to the introduction of a premature stop at codon 6. The c.11delT would lead to a truncated protein that is rapidly degraded resulting in reduced dosage of PMP22, comparable to the 1.5Mb deletion.

Analysis of Genes Affecting Susceptibility to Systemic Lupus Erythematosus. *T. Tahira[1], T. Horiuchi[2], D. Sakaguchi[1], M. Yamai[1], H. Miyagawa[2], H. Tsukamoto[2], K. Hayashi[1]* 1) Res Ctr Genetic Info, Med Inst Bioreg, Kyushu Univ. Fukuoka, Japan; 2) Med. and Biosys. Science, Kyushu Univ. Grad. School of Med. Sciences, Fukuoka, Japan.

A genetic predisposition has been implicated in the occurrence of systemic lupus erythematosus (SLE). To identify SLE-susceptibility genes in Japanese population, we carried out case-control association studies for candidate genes using PLACE-SSCP analysis of pooled DNA. In this method, allele peaks are separated by capillary electrophoresis in non-denaturing conditions and precisely quantified. Candidate genes selected were genes involved in the lymphoid activation and apoptosis, those responsible for SLE-like symptoms in mice and those previously reported to be associated with SLE in the case-control studies. A total of 700 SNPs in 130 genes were subjected to allele frequency quantification by PLACE-SSCP analysis. The SNP that showed largest estimated allele frequency differences between cases and controls was rs752637 in interferon regulatory factor 5 (IRF5) gene. The association of this SNP with SLE was confirmed by sequencing 692 control and 506 SLE patients (p=0.00018). We also analyzed rs2004640 in IRF5, because T allele of this SNP that create exon 1B donor site was reported as a risk allele for SLE in European (Sigurdsson et al. 2005; Graham, et al. 2006). Although allele frequency of this SNP in Japanese controls (28%) differed from European controls (44-56%), rs2004640 T allele showed strong association with SLE in Japanese descent (p=0.000018). During this sequence analysis, we found a new SNP 6 bp downstream of rs2004640 that was rare (1.1%) in Caucasian but common (19%)in Japanese. The SNP showed weak association with SLE (P=0.068) in Japanese. Another SNP in IRF5 gene (rs2280714), which was reported to associate with increased mRNA expression, showed weak association with SLE (P=0.027), consistent with the finding that this SNP do not confer risk by itself (Graham et al. 2006). In this study, we show for the first time that a haplotype of IRF5 is associated with increased risk of SLE in Asian samples. These results support the involvement of IRF5 in the pathogenesis of SLE.

LD Tag SNPs Selection for candidate gene association studies using HapMap and gene resequencing data. *Z. Xu*[1], *N.L. Kaplan*[2], *J.A. Taylor*[1,3] 1) Epidemiology Branch, NIEHS, RPT, NC; 2) Biostatistics Branch, NIEHS, RPT, NC; 3) Laboratory of Molecular Carcinogenesis, NIEHS, RPT, NC.

The recently completed HapMap provides linkage disequilibrium (LD) information on a sample of 3.7 million SNPs that can be used for whole genome association studies. HapMap data can also be used for LD tag SNP selection in candidate genes, although its performance has yet to evaluated against gene resequencing data where there is near-complete SNP ascertainment. The Environmental Genome Project (EGP) is the largest gene resequencing effort to date with over 500 genes resequenced in 90 or more individuals, although individual ethnic information is only available for the 127 genes included in EGP Panel 2. For EGP Panel 2 genes we used HapMap data to select LD tag SNPs and evaluated gene tagging proportions against EGP resequencing data. Median gene tagging proportions (the proportion of common SNPs correlated with at least one tag SNP) were 48%, 78% and 72% for African, Asian, and European groups respectively. These low gene tagging proportions may be problematic for some candidate gene studies. We show that median gene tagging proportions can be improved to 80%, 90%, and 87% by adding a parsimonious set of tag SNPs selected from EGP ethnic-specific multi-SNP LD bins. This strategy cannot be implemented for the genes in EGP Panel 1, because individual ethnic information is missing. We demonstrate that ethnic-mixed data can still be used to improve HapMap gene tagging proportions, although not as efficiently as with ethnic-specific data. Despite that HapMap targeted non-synonymous SNPs, we estimate, using EGP data, that HapMap SNPs only tag ~26% of total non-synonymous SNPs. Finally, we investigated the possibility of developing a single panel of tag SNP that can be applied across multiple populations rather than using separate ethnic-specific tag SNP panels. We generalized the greedy algorithm proposed by Carlson et al. (2004) to pick multi-population tag SNPs from HapMap data. We also evaluate the utility of supplementing these SNPs with multi-population tag SNPs selected from ethnic-specific and ethnic-mixed EGP data.

Interactions between BBS proteins provide functional support for oligogenic inheritance in Bardet-Biedl syndrome. *A.J. Ross, J. Hill, P.L. Beales* Molecular Medicine Unit, Inst Child Health, London, United Kingdom.

Bardet-Biedl syndrome (BBS) is a pleiotropic disorder primarily characterized by retinal degeneration, obesity, polydactyly, kidney dysfunction, genitourinary tract malformations and cognitive impairment. Eleven causative genes (*BBS1-11*) have been identified to date and the underlying cellular pathogenesis is associated with basal body/ciliary dysfunction. Although it is usually transmitted as a recessive trait, some families display a complex inheritance pattern whereby mutations at different loci interact genetically to cause and/or modify the phenotype. BBS2 is one of the least well-studied of the BBS proteins. Its amino acid sequence provides few clues to function, with the exception of a region of shared homology with BBS1 and BBS7, and a predicted coiled-coil domain suggestive of protein-protein interactions. To identify binding partners for BBS2, we performed a yeast two-hybrid screen of an adult kidney cDNA library. We identified several candidate interactors including two other known BBS proteins, which were verified by co-immunoprecipitation studies. Furthermore, we show that some missense mutations within BBS2 ameliorate these BBS-BBS interactions. These data provide the first evidence of interactions between BBS proteins and provide a possible functional explanation underlying oligogenic inheritance.

Autosomal Dominant Early Onset Pagets Disease of the Bone and Progressive Proximal Myopathy Maps to Chromosome 16q22.3-q24.1 in a family. *G. Watts[1], E.C. Fulchiero[1], S. Ramdeen[1], S.G. Mehta[1], C. Zhao[2], V.E. Kimonis[1]* 1) Division of Genetics and Metabolism, Childrens Hospital, Harvard Medical School, 300 Longwood Avenue, Fegan 10, Boston, MA 02115; 2) Center for Medical Genetics, Molecular Genetics Laboratory, 1000 North Oak Avenue, Marshfield, WI, USA.

We previously reported this family with autosomal dominant Pagets disease of the bone and limb girdle muscular dystrophy (LGMD) and excluded the IBMPFD (inclusion body myopathy, PDB and frontotemporal dementia) locus on 9p and the loci for PDB and LGMD (Waggoner *et al*. 2001). The six affected individuals have progressive proximal muscle weakness at a mean age of 41 years. EMGs and muscle biopsies in several individuals showed non-specific myopathic changes that were negative for amyloid staining. Within affected muscle fibers numerous ringbinden, marked central nucleation and rare rimless vacuoles were observed. The myopathy either preceded or followed Pagets disease of the bone, which presented mainly in the long bones and eventually progressed to the spine, scapulae and other bones with a mean age of onset being 42 years. Radiographs reveal coarse trabeculation and patchy sclerosis. We identified linkage to chromosome 16q22.3-q24.1 utilizing a genome-wide scan, with a maximal LOD score of 2.25 for markers D16S515 and D16S3091. Haplotype analysis narrowed the disease locus to a 14.8Mb region flanked by markers GATA81D12M and D16S520. This new region is known to contain a quantitative trait locus (QTL) for bone mineral density (BMD), a highly heritable trait and a key osteoporotic fracture risk factor. Ralston *et al*. (2005) identified 8 QTL for regulation of BMD by a genome wide scan involving 3691 individuals from 715 families. Families had reduced BMD values at the lumbar spine or femoral neck in probands. One QTL with a LOD score of greater than 2.2 mapped to 16q23-q24 and was flanked by markers D16S3091 and D16S520. Identification of the gene for this unique disorder will facilitate our understanding of the complex interaction between bone and muscle pathogenesis and help develop treatment strategies for this and other related debilitating disorders.

Variations in adiponectin receptor genes and susceptibility to type 2 diabetes in US women: A tagging-SNP haplotype analysis. *L. Qi*[1], *A. Doria*[2], *D. Hunter*[1], *F. Hu*[1] 1) Department of Nutrition, Harvard School of Public Health, Boston, MA; 2) Joslin Diabetes Center, Department of Medicine, Harvard Medical School, Boston, MA.

Adiponectin has been associated with improved insulin sensitivity and a lower risk of type 2 diabetes. Recent studies indicate that the metabolic effects of adiponectin are mediated by adiponectin receptor 1 (gene symbol ADIPOR1) and adiponectin receptor 2 (gene symbol ADIPOR2). We conducted a prospective, nested case-control study of 714 incident cases of type 2 diabetes and 1,120 matching control subjects from the Nurses' Health Study. Six polymorphisms in ADIPOR1 and sixteen polymorphisms in ADIPOR2 genes were determined. A 3 UTR polymorphism, rs1139646 (C>G), showed significant association with greater diabetes risk (OR=1.26, 95%CI 1.03-1.53). Adjustment for age, BMI, and other diabetes risk factors improved the associations (OR=1.36, 95%CI 1.10-1.70). When haplotypes possessing the six ADIPOR1 polymorphisms were analyzed, we found that haplotype 112222 (1 codes the common allele and 2 codes the minor allele) was associated with 39% increased risk of type 2 diabetes compared with the most common haplotype 221111 (OR=1.39, and 95%CI 1.03-1.87; P=0.027). Polymorphism rs1139646 was associated with higher plasma levels of interleukin 6 (adjusted means: CC, 2.09; CG, 2.54; and GG, 2.37 ng/mL). There were no significant associations between ADIPOR2 polymorphisms, individually or in haplotypes, and the risk of type 2 diabetes. In conclusion, our data indicate a significant association between ADIPOR1 rs1139646 and diabetes risk but do not support an effect of ADIPOR2 variability on the disease.

An Apoptosis Gene Differentiates Childhood Absence Epilepsy from Juvenile Absence Epilepsy. *L.J. Strug[1], M. Durner[1], E. Cayanis[3], F. Zhang[1], D. Politis[1], I. Klotz[1], E. Dicker[1], D.A. Greenberg[1,2,3], Our collaborators, critical to this study. Space limitations make it impossible to name them here.* 1) Dept Biostatistics, Columbia University, New York, NY; 2) Psychiatry, Columbia University, New York, NY; 3) Genome Center, Columbia University, New York, NY.

Absence seizures in Juvenile Absence Epilepsy (JAE) and Childhood Absence Epilepsy (CAE) appear similar. Differentiating these two syndromes are the age of onset, and the frequency of the absence seizures. In linkage genome scans for the juvenile absence phenotype, we found evidence for linkage on chromosome 5p. There are at least two candidate genes in this region: Succinate dehydrogenase complex subunit A (SDHA) and Programmed cell death 6 (PDCD6). We examined this region for association with JAE and CAE, genotyping single nucleotide polymorphisms in and around SDHA and PDCD6. We used case-control and family-based association methods to determine the degree of association. We found that CAE is strongly associated with all SNPs tested in the PDCD6 gene (maximum chi-squared = 21.22, $p<0.0001$ at rs4957014). JAE, in contrast, was not significantly associated with PDCD6 or any SNPs in the region studied. These results were confirmed in the family-based association analysis. The odds of having CAE if one carries at least one copy of the associated allele at the rs4957014 locus is 3.2 times the odds for an individual without a copy of the allele at this locus (95%CI: 1.91 - 5.37). Our results suggest that not only does PDCD6 play a role in CAE susceptibility, but that different genes contribute to susceptibility in CAE and JAE; no association was seen in the JAE group. This implies that CAE and JAE cannot necessarily be treated as having the same genetic basis. Although there may be susceptibility genes common to the two (or more) syndromes, treating them as one genetic entity could mask the existence of genes for only one of the syndromes. It is especially interesting that this is at least the second apoptosis gene that appears to be related to a form of IGE.

Interstitial chromosome 3q deletion in a newborn with hydrocephalus and hypoplastic kidney. *S. Ramanathan[1], R. Woldenberg[2], S. Gupta[3], P. Koduru[3], L. Mehta[1]* 1) Div. of Medical Genetics, Schneider Children's Hospital at North Shore, Manhasset, NY; 2) Dept. of Radiology; 3) Dept. of Laboratories, North Shore University Hospital, Manhasset, NY.

We report on a female infant with facial dysmorphisms, hydrocephalus and a hypoplastic right kidney. She was delivered to a 35 year-old G4P1021. Prenatal history was significant for high MSAFP. Prenatal ultrasound showed polyhydramnios, suspicion of agenesis of corpus callosum, and small right kidney in the fetus. Fetal MRI showed mild to moderate hydrocephalus but was inconclusive for other abnormalities. Birth weight was 3030 gm. Head circumference was 34.6 cm (~75%). Facial features were mildly dysmorphic with frontal bossing, sharply depressed nasal bridge, prominent nasal tip, short philtrum and downturned mouth. Right ear lobule was large. There were bilateral single to bridged palmar creases. The 2nd and 4th toes were deviated with mild 2-3 digit syndactyly. There were no heart defects. Postnatal MRI showed large 4th ventricle communicating with a prominent retrocerebellar CSF space, suggestive of a Dandy Walker malformation (DWM) variant with compensated hydrocephalus. On ultrasound, kidneys measured 2.3 cm (right), and 4.7 cm (left). Peripheral blood chromosome analysis showed 46,XX,del(3)(q24q25). Deletions of this region on chromosome 3q have been previously reported in the literature to be associated with facial dysmorphisms, central nervous system malformations, congenital heart defects and mental retardation. Our patient shows several of the described findings. To our knowledge, this is the first case of deletion 3q24-q25 presenting with renal hypoplasia. Grinberg et al. have recently identified deletions of two linked Zinc finger genes, *ZIC1* and *ZIC4* in 7 individuals with DWM and interstitial deletions of the 3q24-q25 region. The finding of DWM variant in our patient with a similar deletion further supports the conclusion that heterozygous deletion of the *ZIC1* and *ZIC4* genes causes DWM. DWM is a heterogeneous disorder with variable expression. Patients such as ours can contribute to identifying the proportion of DWM associated with mutations/ deletions of *ZIC1* and *ZIC4*.

Genetic Testing for Hearing Impairment in African Americans. *D.M. Pekarek[1], S.K. Prucka[1], R.J.H. Smith[3], N.H. Robin[1, 2]* 1) Dept Genetics; 2) and Pediatrics, UAB, Birmingham, AL; 3) Depts Internal Medicine, Otolaryngology, and Pediatrics, University of Iowa, IA.

BACKGROUND: Mutations in GJB2 and GJB6 at the DFNB1 locus are the most common cause of genetic hearing impairment (HI) in persons of Northern European extraction and account for approximately 30% of simplex and 50% of mulitplex cases of congenital severe-to-profound non-syndromic deafness. However, DFNB1-related HI is infrequent in the African American (AA) population. Previous studies have demonstrated that AA are cautious about participating in medical research in general, further complicating efforts to determine the overall prevalence of DFNB1 mutations in AA. STUDY: We asked parents of deaf/hard of hearing (D/HOH) AA children to complete a questionnaire regarding their interest in genetic research and testing for HI and offered free testing. RESULTS: 34 families completed questionnaires, including 3 multiplex sibships, although not all questions were answered. Of the 34 families, 7 declined testing. Including siblings, a total of 31 persons had genetic testing. Of the group that underwent testing (T), the majority (15/27) were married; in the questionnaire only group (QO), the majority were single (4/7). In both groups, the majority had some post high school education and the majority had an income less than $15,000/yr. The majority in both groups strongly agreed or agreed that genetic research for deafness would benefit their childrens healthcare (7/7 in QO and 16/21 in T). Both groups strongly disagreed or disagreed that genetic research was discriminatory (5/6 in QO and 18/22 in T). Both groups strongly disagreed or disagreed that they mistrusted the researchers (6/7 in QO and 20/25 in T). Of those tested, one individual was found to have a GJB2 mutation, one subject had one abnormal allele, and one subject had a -6T->A, which is of unknown significance. CONCLUSIONS: Our results show that the prevailing attitude towards genetic testing for deafness among African-Americans is positive and most believe it to be non-discriminatory. Furthermore, as in previous studies, we found that mutations at DFNB1 are not a common cause of HI among AA.

An unusual structural rearrangement involving chromosome 2 in a patient with dysmorphic features, developmental delay and Pierre-Robin sequence. *V. Tonk[1], M. Sehrawat[2], M. Drummond-Borg[3], M. Ito[4], L. Lockhart[5], G. Velagaleti[5]* 1) Dept Pediatrics, Texas Tech Univ, Lubbock, TX; 2) PGIMS, Rohtak, India; 3) Texas Dept Health, Austin, TX; 4) Ctr Hum Genet, Boston Univ, Boston, MA; 5) Dept Pediatrics, Univ of Texas Med Branch, Galveston, TX.

The Pierre-Robin sequence (PRS) is a clinical entity characterized by cleft palate and micrognathia that results in glossoptosis. The overall incidence of PRS is 1/8500-14,000 births. Genetics appear to play a major role in the etiology of PRS. Studies have shown several chromosomal regions causally implicated in PRS including chromosome 2q24.1-q33.3. No candidate genes have been identified in this region, although GAD67 at chromosome 2q31 was considered as a potential candidate gene. We report here a case of a de novo unusual structural rearrangement involving the chromosome 2q22-q34 region. A 3-year old, Hispanic male was referred because of PRS, developmental delay and additional dysmorphic features. His prenatal and birth history was unremarkable. He was noted to have acrocyanosis and PRS. On physical examination, his height, weight and OFC were at 5th, 50th and 97th percentile, respectively. He had a large head with broad forehead and frontal bossing. Palpebral fissures were large and eyes were deep set. Nose was broad and up-turned. He had significant developmental delay. Chromosome analysis showed a structurally rearranged chromosome. Based on the G-banding analysis, the rearrangement appeared to be complex, similar to a recombinant chromosome. The karyotype was interpreted as 46,XY,der(2)del(2)(q31q32.2)dup(2)(q33.1q34)inv(2)(q22q24.3). Parental karyotypes were normal. To better characterize the rearrangement, FISH studies with BAC clones localized to q31.2, q31.3, q33.1 and q33.3 regions were carried out. BAC-FISH results confirmed the presence of inversion. Because of the ambiguity with banding analysis, additional studies with Affymetrix 250k SNP chip are being carried out. Results from the array analysis might help us narrow the region and identify potential candidate genes involved in PRS.

A new locus for autosomal recessive spastic paraplegia, mental retardation and brainstem dysraphia (SPG32) on chromosome 14q12-q21. *G. Stevanin[1,2], C. Paternotte[3], P. Coutinho[4,5], S. Klebe[1], J.L. Loureiro[4,5], V.T. Cruz[4], A. Durr[1,2], J.F. Prudhomme[6], J. Weissenbach[3], A. Brice[1,2], J. Hazan[3,7]* 1) IFR Neurosciences, INSERM U679-NEB, Paris, France; 2) APHP, Salpetriere Hospital, Dept of Genetics, Paris, France; 3) Genoscope, CNS, Evry, France; 4) Dept of Neurology, Sao Sebastiao Hospital, Santa Maria da Feira, Portugal; 5) UNIGENE, University of Porto, Portugal; 6) Genethon, Evry, France; 7) MRC centre for Developmental Neurobiology, King's College London, Guy's Hospital Campus, London, UK.

Hereditary spastic paraplegias (HSP) are a genetically heterogeneous group of neurodegenerative disorders characterized by progressive spasticity of the lower limbs. In the present study, we carried out a genome-wide linkage analysis on a consanguineous Portuguese family affected by an autosomal recessive complex form of HSP combining mild intellectual impairment, brainstem dysraphia and a clinically asymptomatic cerebellar atrophy. The disease has an early onset but a very benign course. We have mapped the disease locus, SPG32, to chromosome 14q12-q21 within a 30-cM interval, flanked by markers D14S264 and D14S58, which partially overlaps the initial SPG3A locus but excludes the atlastin gene.

Interacting proteins of Autoimmune Regulator (AIRE). *M.C. Rosatelli[1], D. Corda[1], E. Fiorillo[1], A. Cao[2], A. Meloni[2]* 1) Dipartimento di Scienze Biomediche e Biotecnologie, University of Cagliari, Cagliari, Italy; 2) Istituto di Neurogenetica e Neurofarmacologia, Consiglio Nazionale delle Ricerche, Cagliari.

The Autoimmune Polyendocrine Syndrome Type 1 (APECED; OMIM 240300) is a rare autosomal recessive disease that is more frequent in some isolated populations. It is characterised by three major clinical symptoms: hypoparathyroidism, Addisons disease, and chronic mucocutaneous candidasis. The disease is caused by mutations of the Autoimmune Regulator gene (AIRE). The AIRE protein has several functional domains such as, two PHD finger, 4 LXXLL motifs, a SAND domain and a HSR domain which are typical of transcription regulators. AIRE is able to activate in vitro transcription and binds with CREB binding protein. The AIRE expression is predominant in the thymic medullary epithelial cells and the monocyte-dendritic cell lines involved in the deletion of autoreactive T cells by negative selection. In this study we carried out functional studies by the two-hybrid technique in yeast cells in order to identify interacting proteins. The AIRE protein fused to a DNA-Binding Domain was used as bait to screen interacting proteins within a cDNA human thymus library. This approach allowed us to establish that 84% of isolated clones contained the cDNA which codified for the AIRE protein, confirming previous data on the homodimerization properties of the AIRE protein. Of the isolated clones, 12% contained the cDNA codifying for the UBC9 protein which represent the E2 enzyme of Sumoylation. Sumoylation is a posttranslational modification which mediates various processes such as nuclear localization, protein-protein interactions and the transcriptional repression and activation. By deletion study, we were able to map the domains of interaction in the N-term portions of both AIRE and UBC9. With the aim to confirm the interaction between AIRE and UBC9 we tested their binding by two-hybrid technique in mammalian cells and co-immunoprecipitation. In order to establish the role of AIRE in the Sumoylation process, we carried out the Sumoylation test in vitro. These experiments gave negative results and allowed us to exclude the involvement of AIRE in this posttranslational modification process.

Intra- and inter-population genotype reconstruction from tagging SNPs. *P. Paschou[1], M.W. Mahoney[2], A. Javed[3], J.R. Kidd[1], A.J. Pakstis[1], S. Gu[1], K.K. Kidd[1], P. Drineas[3]* 1) Department of Genetics, Yale University, New Haven, CT; 2) Department of Mathematics, Yale University, New Haven, CT; 3) Department of Computer Science, RPI, Troy, NY.

The optimal method for tSNP selection, the applicability of a reference LD map to unassayed populations, and the scalability of these methods to genome-wide analysis, remain important research questions. Taking advantage of recent results in computer science, we design novel matrix algorithms that address these issues and evaluate them on data from four genomic regions (248 SNPs typed over a total of 2.7Mb for approximately 2000 individuals from 38 worldwide populations). A separate dataset for the same genomic regions, available from the HapMap database (1336 SNPs for four populations), is also analyzed. We define the tSNPs selection problem as a reconstruction problem and attempt the accurate prediction of untyped SNPs based on a few carefully selected tSNPs. To justify the linear algebraic algorithms that we develop, we quantify the linear structure of the data using the Singular Value Decomposition (SVD) of matrices. We select tSNPs using a heuristic variant of a Monte-Carlo algorithm for approximating the SVD of large matrices. Next, we predict untyped SNPs using the CUR matrix decomposition. Our results show that keeping a small number of tSNPs suffices for 85-95% prediction accuracy for most studied populations. African populations prove to be the most resistant to extrapolation. Testing the portability of tSNPs across populations, we select tSNPs in each of our worldwide samples and measure the reconstruction error of unknown genotypes in the remaining populations. A considerable amount of tSNP transferability is revealed especially within geographic regions but also across them. The algorithms we employ for tSNP selection and unassayed SNP reconstruction do not require haplotype inference, are block-free multimarker tests, and they are, in principle, scalable even to genome-wide analysis. Algorithms from the computer science literature, proven already successful in mining large datasets, can become a powerful tool for mining information in the large volume of available genomic data.

Gene mutation analysis of genetic leukoencephalopathies in China. *X. Wu, Y. Jiang, J. Wang, Y. Wu, H. Wei, J. Xiao, J. Qin, Y. Yang, Y. Zhang* Pediatrics, Peking University First Hospital, No.1, Xi'an Men Street, West district, Beijing 100034, China.

Genetic leukoencephalopathies are a heterogeneous group of diseases with mainly white matter destruction or failed development caused by genetically determined molecular abnormality. Genetic analysis is always an important tool to diagnose these diseases. Therefore, we reported here our preliminary data of gene mutation analysis of genetic leukoencephalopathies, such as arylsulfatase A (ARSA) gene in Metachromatic leukodystrophy (MLD), glial fibrillary acidic protein (GFAP) gene in Alexander disease, MLC1 gene in Megalencephalic leukoencephalopathy with subcortical cysts (MLC), and Eukaryotic translation initiation factor 2B subunit (eIF2B5) in childhood ataxia with central nervous system hypomyelination/ vanishing white matter (CACH/VWM). From four Chinese patients with MLD, five DNA variations of ARSA gene were identified. Two frame shift mutations (177-180insCA and 1337-1338ins C) were novel and the other three were known missense mutation (p.R84W, p.G99V, and p. R288C). All 4 patients had heterozygous compound mutations. From 3 patients with Alexander disease, two different missense GFAP mutations were found in exon 1, which are all heterozygous, de novo mutations. Two patients carried the same arginine mutations (p. R88C), and one had tyrosine mutation (p. Y83H) which has not been reported yet. These results suggested that Exon1 of GFAP may be the hot spot mutated exon for Chinese infantile Alexander disease. That is similar with the results from USA. From 2 patients of MLC, we found 3 mutations, c.218G>A (p.Gly73Glu), c.823G>A (p.Ala275Thr), c.772-1G>C (splice-site mutation). Both two patients had heterozygous compound mutations. All these three mutations have not yet been reported. From 9 CACH/VWM patients (diagnosed on the basis of distinct clinical features and MRI characteristics), we had only finish part work for one subunit gene (eIF2B5) analysis, and found one homozygous missense mutation, c. 1759 A>G in a patient. Other analysis work is undergoing now. All these gene mutations were first reported in Chinese.

Large-scale In Vivo Enhancer Analysis of Extremely Conserved Human Noncoding Sequence. *L.A. Pennacchio[1,2], A. Visel[1], N. Ahituv[1,2], S. Prabhakar[1,2], I. Dubchak[1,2], E.M. Rubin[1,2]* 1) Genomics Division, Lawrence Berkeley Natl Lab, Berkeley, CA; 2) DOE Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, CA.

A significant challenge exists in identifying and characterizing the complete set of distant acting transcriptional enhancers present in the human genome. In this study, we leveraged extreme evolutionary sequence conservation as a filter to identify putative gene regulatory elements and characterized the in vivo enhancer activity of human-fish conserved and ultraconserved noncoding elements on human chromosome 16. We initially tested 165 of these extremely conserved sequences in a transgenic mouse enhancer assay and observed that 48 percent functioned reproducibly as tissue-specific enhancers of gene expression at embryonic day 11.5. While driving expression in a broad range of anatomical structures in the embryo, the majority of the 79 enhancers drove expression in various regions of the developing nervous system. Studying the set of DNA elements that specifically drove forebrain expression, we identified DNA signatures specifically enriched in these elements and used these parameters to rank all ~3,400 human-fugu conserved noncoding elements in the human genome. The testing of the top predictions in transgenic mice resulted in an enrichment for sequences with forebrain enhancer activity. These data dramatically expand the catalogue of in vivo-characterized human gene enhancers and illustrate the utility of such training sets for a variety of biological applications including decoding the regulatory vocabulary of the human genome.

As a community resource, we have established a database to visualize and query the activity of these enhancer sequences at http://enhancer.lbl.gov.

A Genome-wide Scan for Quantitative Trait Loci Underlying Obesity -Related Variation in 434 Caucasian Families. *L. Zhao[1], Y.J. Liu[2], P. Xiao[1], H. Shen[2], D.H. Xiong[1], Y.Z. Liu[1], R. Recker[1], H.W. Deng[1,2,3,4]* 1) Creighton Univ, Omaha, NE; 2) University of Missouri-Kansas City, Kansas City, MO; 3) Xi'an Jiaotong University, Xi'an, Shan'Xi, China; 4) Hunan Normal University, Changsha, Hunan, China.

Introduction: Obesity is a common complex disease that has strong genetic determination. Materials and Methods: To identify QTLs for regulation of obesity, we performed a large-scale whole genome linkage scan (WGS) involving 4,102 individuals from 434 families. All the subjects were genotyped with 410 microsatellite markers from the Marshfield screening set 14 spaced ~8.9 cM apart across the human genome. Using the variance component method implemented in SOLAR, we performed a linkage analyses in the total sample and in a specific gender. Results: Linkage evidence was found in the regions of 20p11-12 for fat mass (LOD = 3.31) and percentage fat mass (PFM) (LOD = 2.92). We also identified several suggestive linkage signals (threshold LOD=1.9) showing linkage to obesity, such as 5q35, 8q13, 10p12 and 17q11. Of them, 20p11-12 and 5q35 were particular interest because they were linked to obesity in multiple previous linkage studies and associated with multiple obesity candidate genes. Subgroup analysis revealed that the effect of 20p12 and 5q35 on obesity were present in whole sample, and males, but not in females. Conclusions: Together with the findings from other studies, the current study has further delineated the genetic basis of obesity and highlighted the importance of increasing sample size to confirm linkage findings and to identify new linkage regions. Our findings in this study laid a foundation for further replication and fine-mapping studies as well as for positional and functional candidate gene studies on obesity.

Life without sulfatases: a mouse model lacking all sulfatase activities. *C. Settembre[1], I. Annunziata[1], C. Spampanato[1], G. Cobellis[1], E. Nusco[1], M. Sardiello[1], E. Zito[1], M.P. Cosma[1], A. Ballabio[1,2]* 1) Telethon Institute of Genetics and Medicine (TIGEM), Naples, Italy; 2) Medical Genetics, Dpt. of Pediatrics, Federico II Univ., Naples, Italy.

Sulfatases are involved in several biological functions as diverse as degradation of complex molecules, production of steroid hormones and cell signaling. Humans have 17 different sulfatases and 8 diseases due to individual sulfatase deficiencies are known. In patients with Multiple Sulfatase Deficiency (MSD), the activities of all sulfatases are substantially reduced due to a defect in their post-translational modification. Recently, we and others identified the Sulfatase Modifying Factor 1 (SUMF1) gene, which is involved in the post-translational modification of sulfatases and is mutated in patients with MSD (Cosma et al and Dierks et al, Cell 2003). We have now generated a Sumf1 KO mouse model. In Sumf1-/- mice the activities of all sulfatases tested were found completely absent, indicating that sulfatase post-translational modification in mammals absolutely requires SUMF1. The residual sulfatase activities observed in MSD patients are likely due to hypomorphic SUMF1 mutations, as demonstrated by expression of SUMF1 mutant cDNAs in murine Sumf1-/- embryonic fibroblasts. Sumf1-/- mice display congenital growth retardation and frequent mortality within the first 2 months of age, with correlation between growth rate and lifespan. They also show striking skeletal abnormalities resembling those observed in human patients, including a flat facial profile, and a severe kyphosis. Histologically, we observed storage of glycosaminoglycans (GAGs) leading to progressive cell vacuolization and a massive inflammatory response (i.e. macrophage activation) in all examined tissues, particularly in the brain, suggesting an involvement of inflammation in the pathogenesis of disease manifestations. This exceptional mouse model, in which the function of an entire protein family has been completely abolished, offers an unprecedented opportunity to perform functional studies, to dissect disease mechanisms and to test new therapeutic strategies for diseases due to both single and multiple sulfatase deficiency.

High-throughput Screening Genomic Deletions and Duplications in the Chinese DMD Gene by Using Array-based MLPA Approach. *F. Zeng[1], Z.R. Ren[1], S.Z. Huang[2], C.L. Jin[3], M. Mommersteeg[4], M. Smit[4], S.Z. Huang[1], R. Beuningen[4], Y.T. Zeng[1], Y. Wu[4]* 1) Institute of Medical Genetics, Shanghai Jiao Tong University, Shanghai, P.R.China; 2) Institute of Basic Medical Science, the Chinese Academy of Medical Science, Beijing, P.R.China; 3) China Medical University, Shenyang, P.R.China; 4) PamGene International BV, The Netherlands.

Duchenne muscular dystrophy (DMD) is the most common and severe X-linked disease, with an incidence of 1 in 3,500 male births in China. Deletions and duplications are known to cause approximately two-thirds of DMD cases. Due to technical difficulties, screening for such mutations in the entire DMD gene is often not performed. Here, we present a technology that simplifies the quantitative analysis of copy number, enables the rapid analysis of over 100 genetic loci by a single tube reaction. Using a universal array employing address sequence tags immobilized on an aluminum-oxide substrate, along with a single multiplex ligation-PCR amplification for analysis, 96 samples can be analyzed in parallel in less than 1 hour. Using this method, we have screened 79 exons of the DMD gene for 44 control individuals and 237 DMD patients, and detected rearrangements in 76% of these patients, of which 92% showed deletions and 8% duplications. Most deletions observed occurred in the middle of the gene and 26% involved only one exon. The majority of the duplications were simple and contiguous duplications, although we detected two none-contiguous duplications among these patients. Our study demonstrated great potential of such technology for the rapid DNA diagnostics in clinical applications.

GABAergic dysfunction may modify clinical severity of Angelman syndrome. *S. Saitoh, K. Egawa, K. Hosoki, N. Asahina, H. Shiraishi* Dept Pediatrics, Hokkaido Univ Sch Medicine, Sapporo, Japan.

Angelman syndrome (AS) is a neurodevelopmental disorder caused by a defect of the imprinted *UBE3A* gene. Five molecular classes (maternal 15q11-q13 deletion, paternal uniparental disomy of chromosome 15, imprinting defects, *UBE3A* mutations, and unknown etiology) underlie the deficit of *UBE3A*, although AS patients with the deletion (AS-del) show more severe clinical phenotypes than those with other molecular classes (AS-non-del). Three $GABA_A$ receptor subunit genes (*GABRB3*, *GABRA5*, and *GABRG3*), which are not imprinted, are located in the deletion, and hemizygosity of these genes may contribute to more severe phenotypes in AS-del. *Gabrb3* heterozygous knock-out mice have epileptic discharges on EEG, similar to seizures in AS and raising the possibility of GABAergic dysfunction in AS. We therefore conducted a series of studies to evaluate what role the GABAergic system may play in AS. Eleven AS-del patients, three AS-non-del patients including one with an imprinting defect and two with a *UBE3A* mutation, and six epilepsy patients as controls participated in the study. We investigated primary somatosensory responses by measuring somatosensory evoked field in response to median nerve stimulation using magnetoencephalography. The N1m (first component) peak latency was significantly delayed in AS-del, but not in AS-non-del. This delay was partially restored by administration of clonazepam, which activates $GABA_A$ receptor function. Moreover, this unique somatosensory response pattern only in AS-del was reproduced by conventional somatosensory evoked potential, although peak latency delay differed between the two measurements. These findings establish GABAergic dysfunction in AS-del. Surprisingly, $GABA_A$ receptor expression studies in five AS-del and two AS-non-del patients by $[^{11}C]$flumazenil positron emission tomography indicated a higher $[^{11}C]$flumazenil binding potential in AS-del and AS-non-del than that in controls. These findings were inconsistent with previous reports, and we proposed that total $GABA_A$ receptor expression was not declined in AS. In conclusion, functional deficits of the GABAergic system are likely to modulate clinical severity in AS.

Identification of a novel imprinted transcription factor on human chromosome 7q32.3. *L. Parker-Katiraee[1,2], T. Yamada[1], S.N. Abu-Amero[3], G.E. Moore[3], M. Kaneda[4], H. Sasaki[4,5], K. Nakabayashi[1,4], S.W. Scherer[1,2]* 1) Dept. of Genetics & Genomic Biology, Hosp. for Sick Children, Toronto, Canada; 2) Dept. of Medical Genetics, Univ. of Toronto, Canada; 3) Inst. of Child Health, Univ. College London, UK; 4) Div. of Human Genetics, Dept. of Integrated Genetics, Nat. Inst. of Genetics, Research Org. of Info. and Systems; 5) Dept. of Genetics, School of Life Science, Graduate Univ. for Advanced Studies, Mishima, Japan.

Imprinted genes are expressed in a parent-of-origin specific manner. Aberrations in their expression have been associated with various developmental disorders, including Russell-Silver Syndrome (RSS). The imprinted cluster at 7q32.3, which harbours the paternally expressed *MEST*, is considered to be a prime candidate region for RSS. Here we describe the imprinting of *KLF14*, an intronless transcript located at 7q32.3 and a member of the Krüppel-like family of transcription factors. We show that the gene has monoallelic maternal expression in all embryonic and extra-embryonic tissues studied, in both human and mouse. We examine epigenetic modifications in the *KLF14* CpG island by sequencing bisulfite treated DNA from human fibroblast cell lines and murine embryos and find this region to be hypomethylated in both species. In addition, we perform chromatin-immunoprecipitation and find that the murine Klf14 CpG island lacks allele-specific histone modifications. Despite the absence of these defining features, our analysis of *Klf14* in offsprings from *Dnmt3a* conditional knockout mice reveals that the genes expression is dependent upon a maternally methylated region. We suggest that the differentially methylated region associated with *Mest* is a strong candidate as a primary germline control element regulating the expression of *Klf14*. Due to the intronless nature of *Klf14* and its homology to *Klf16*, we suggest that the gene is an ancient retrotransposed copy of *Klf16*. By sequence analysis in numerous mammalian species, we place the timing of this event after the divergence of Marsupialia, yet prior to the divergence of the Xenarthra superclade. Thus, *KLF14* is the first example of a maternally expressed protein-coding retrotransposed gene imprinted in both human and mouse.

Association of 5 region polymorphic markers of the Fibrillin-1 gene with systemic sclerosis and related pulmonary fibrosis in caucasian european patients. *Y. Allanore[1,2], J. Wipff[1,2], M. Giraud[3], H.J. Garchon[3], A. Kahan[2], C. Boileau[1]* 1) INSERM U 781, Necker hospital, Paris, France; 2) Rheumatology A, Cochin hospital, Paris, France; 3) INSERM U 580, Necker Hospital, Paris, France.

Introduction: A growing body of evidence suggests that systemic sclerosis (SSc) is a fibrillin disorder and associations with the fibrillin-1 gene (FBN1) were found in Choctaw and Japanese populations. Patients and Methods: First, we investigated 331 French subjects: 198 SSc and 133 controls with osteoarthritis. We then investigated three Italian cohorts from Florence (89 SSc/79 controls), Brescia (115/50) and Verona (62/24). We studied six FBN1 gene polymorphisms: one SNP in intron C and five microsatellite markers: D15S1028, MTS2, MTS3, D15S123 and D15S143. We used Arlequin, Cocaphase and Phase 2 software for statistical analysis. Results: All markers were in Hardy-Weinberg equilibrium. Two sets of markers were in linkage disequilibrium: one in the 5 (D15S1028, Intron C, MTS2, MTS3) and one in the 3 region (D15S123, D15S143). In the French cohort, the MTS3 146 allele was associated with SSc (p = 0.046), pulmonary fibrosis (p = 0.008) and related restrictive syndrome (p=0.04). We also detected an association between SSc and alleles 173 and 171 of marker D15S1028; respectively in SSc patients and controls, allele 173 was present in 7% versus 2% (p=0.03) and allele 171 in 4% versus 11% (p=0.04). In the Brescia cohort, D15S1028 179 allele was more frequent in SSc patients than in controls (20% vs 6%, p=0.005) and in SSc patients with pulmonary fibrosis (22% vs 6%, p=0.008). In the Verona cohort, we also observed a significant difference between SSc patients with pulmonary fibrosis and controls for the D15S1028 175 allele (17% vs 46%, p=0.03). Conclusion: These data show an association not only between the 5 region of the FBN1 gene and SSc but more importantly with pulmonary fibrosis in Caucasian European patients. These findings confirm the hypothesis of a key role for FBN-1 in the fibrotic process of this devastating disease.

A *Coch* knock-in mouse model for late-onset DFNA9 deafness. *N.G. Robertson[1], T.A. Sivakumaran[1,2], S.A. Hamaker[1], A.B.S. Giersch[1,2], C.C. Morton[1,2]* 1) Depts. OB/GYN & Pathology, Brigham & Women's Hospital; 2) Harvard Medical School, Boston, MA.

COCH, encoding cochlin, is expressed at high levels in the inner ear. Six familial *COCH* missense mutations have been found in the autosomal dominant late-onset deafness and vestibular disorder, DFNA9, showing abnormal eosinophilic deposits in the cochlear and vestibular labyrinths. To create a mouse model for DFNA9, we generated a mouse knock-in by introducing one of the *COCH* mutations (G88E). The targeting construct consists of ~5.6kb of 129/SvJae mouse *Coch* genomic sequence spanning introns 3 to 8. Positive and negative selection were performed through neomycin (*neo*) flanked by *loxP* sites in *Coch* intron 5, and thymidine kinase upstream of the *Coch* sequence, respectively. J1 embryonic stem (ES) cells were transfected with the *Coch* mutant construct and 240 ES cell clones were screened. Integrity of the recombination, *loxP* sites, the mutation, and exons were confirmed by PCR, Southern blots, and sequencing. A homologous recombinant ES cell clone was microinjected into C57BL/6 blastocysts, yielding several chimera that showed germline transmission of mutant *Coch*.

F1 mice heterozygous for the mutant allele were mated with EIIA-*cre* deletor mice for removal of *neo*, which interfered with efficient transcription or splicing of mutant *Coch*. After *neo* excision, RT/PCR of $Coch^{G88E/+}$ showed presence of the mutant *Coch* transcript. Immunohistochemistry on inner ears of $Coch^{G88E/G88E}$ mice showed strong immunostaining for cochlin, indicating the mutant protein is being successfully produced. For expression analysis, total RNA has been obtained from nine pairs of cochlea from each genotype to be used for analysis on Affymetrix expression chips as well as on cochlear-derived cDNA microarrays developed in our laboratory. Continued studies of these mice until advanced ages include auditory brainstem response (ABR) and vestibular testing, histopathology and immunohistochemistry, cochlear cDNA microarray and proteomic analyses. At 11 months of age, auditory and vestibular testing are within normal limits.

Linkage and mutational study in two families with Familial Hemiplegic Migraine. *P. Tarantino[1], E.V. De Marco[1], F. Bono[2], A. Gambardella[2], P. Forabosco[3], S. Carrideo[1], F. Annesi[1], D. Civitelli[1], F.E. Rocca[1], I.C. Cirò Candiano[1], G. Annesi[1]* 1) Inst Neurological Sciences, National Research Council, Mangone, (CS), Italy; 2) Inst of Neurology, University "Magna Graecia", Catanzaro, Italy; 3) Inst of Population Genetics, National Research Council, Alghero (SS),Italy.

Familial Hemiplegic Migraine (FHM) is a rare autosomal dominant subtype of migraine with aura, characterized by the occurrence of hemiplegia or hemiparesis during the attacks. Up to now, mutations in three genes have been shown to be causative for FHM: CACNA1A, ATP1A2, and the newly identified SCN1A, all coding proteins involved in regulating ionic flow through the membranes. The aim of this study was to investigate if these genes were associated to the disease in 2 FHM families from Calabria. FHM was diagnosed according to the criteria of the International Headache Society in 6 individuals (three in each family). DNA was made available from 20 members of the two families: 6 affected individuals, 1 obligate carrier and 13 unaffected individuals. All subjects were genotyped for 9 microsatellite markers to exclude linkage to CACNA1 and ATP1A2 by ABI 3130xl. Linkage analysis was performed by LINKAGE and GENEHUNTER. Sequencing analysis for mutation detection in SCN1A was performed on ABI 3130xl. The LOD score values obtained in the two FHM families allowed us to exclude linkage to CACNA1A and ATP1A2. Concerning the SCN1A gene, no mutations, including the already published one, have been identified in the 16 exons analyzed so far. Mutational screening on the 10 remaining exons is still in progress. To date, several mutations in the CACNA1 and ATP1A2 genes have been identified as causing FHM, but a consistent number of families do not show evidence of linkage to either of them. Very recently a mutation in the SCN1A gene was found in three European FHM families, but other families have been reported not to link to this gene. Our preliminary data led us to exclude both the linkage to CACNA1 and ATP1A2, and the presence of the already published mutation in SCN1A in our families. Further studies are needed to evaluate the role of SCN1A in FHM.

White Americans Beliefs on Genetic Contributions to Perceived Racial Differences in Athleticism: Impact on Prejudice Toward and Negative Stereotyping of Blacks. *E.M. Petty[1], J.P. Sheldon[2], T.E. Jayaratne[1]* 1) Univ Michigan, Ann Arbor, MI; 2) Univ Michigan, Dearborn, MI.

Individuals - in sports to science - have debated whether Blacks possess superior innate athletic abilities. The belief that Blacks are naturally athletic may reflect the large presence of talented Blacks in televised US sports and Blacks domination of Olympic sprinting events. National polls reveal that most Americans, especially Whites, believe that genetics underlies Blacks supposed athletic superiority. Such beliefs can serve as a legitimizing myth, supporting a White social dominance perspective. Historically, the Eugenics movement illustrated how beliefs in heritable racial differences led to stereotyping, prejudice and oppression. Recently, a negative stereotypical view of Whites having the brains and Blacks having the brawn has emerged in publicized discussions of athletics. We hypothesized that White Americans belief in a genetic difference for athleticism between Blacks and Whites would predict prejudice and negative stereotyping. To examine this, 600 self-identified Whites randomly selected from across America were asked about these issues during a telephone survey of genetic beliefs. Demographic information, including variables hypothesized to relate to Whites racial stereotyping and prejudice was obtained. Measures of their (1) belief in a genetic basis for race differences in athleticism, (2) level of racial prejudice, and (3) endorsement of negative racial stereotypes regarding intelligence and work ethic were analyzed. Supporting our hypothesis, Whites belief in a genetic race difference in athleticism, regardless of thier gender, predicted significantly higher levels of prejudice toward and negative stereotyping of Blacks. Thus, Whites praise of Blacks athletic superiority operates as backhanded compliment when it denigrates Blacks intellectual ability and work ethic. This suggests that a public better informed about genetics, race, and racial diversity may lead to more desirable social outcomes. Accurate reports of socially responsible research on, and decreased stereotyped and essentialist media portrayals of, genetics and race are needed.

MicroRNA expression profiling of lung cancer cell lines. *A. Pertsemlidis[1], Irnov[1], L. Du[1], J. Schageman[1], S. Goodson[2], J.M. Thompson[2], S. Hammond[2], L. Girard[3], M. Sato[3], J. Shay[3], A.F. Gazdar[3], J.D. Minna[3]* 1) McDermott Center for Human Growth and Development, UT Southwestern Medical Center, Dallas, TX; 2) Lineberger Comprehensive Cancer Center, School of Medicine, University of North Carolina, Chapel Hill, NC; 3) Hamon Center for Therapeutic Oncology Research, UT Southwestern Medical Center, Dallas, TX.

Using microRNA expression profiling of small cell lung cancer (SCLC) cell lines, non-small cell lung cancer (NSCLC) cell lines and immortalized human bronchial epithelial cells (HBECs), we have identified 35 microRNAs with patterns of expression that distinguish SCLC and NSCLCs, 10 microRNAs that distinguish HBECs from lung cancer cell lines, 3 microRNAs that correlate with drug sensitivity in NSCLC cell lines and 1 microRNA that correlates with progression in genetically manipulated HBECs. We have also found 3 microRNAs, miR-93, miR-98/let-7f and miR-197, which are over-expressed in SCLC cell lines relative to NSCLC cell lines and HBECs. Using a method of predicting gene targets of miRNA regulation developed in our lab, we predicted that all three miRNAs translationally repress FUS1/TUSC2, a lung cancer tumor suppressor gene located on human chromosome 3p21.3. Over-expression of FUS1 has been shown to lead to G1 arrest and growth inhibition of lung cancer cells, and to significant inhibition of tumor growth and progression in mouse models. Using an in vitro assay, we tested the hypothesis that these three microRNAs repress protein expression via interaction with a target site in the 3UTR of the TUSC2 transcript. The results show that miR-93, miR-98, and miR-197 inhibit luciferase expression by targeting the TUSC2 3UTR. miR-93, miR-98/let-7f and miR-197 are therefore negative regulators of FUS1, supporting a model in which aberrant over-expression any of the three miRNAs leads to the loss of FUS1 protein expression in SCLC. This suggests that the three miRNAs may be relevant in the development of lung cancer and may serve as effective diagnostic markers and therapeutic targets.

QTL fine-mapping reveals several discrete loci with small individual effects. *E.M. Smith[1], L. Martin[2], A. Kissebah[1], M. Olivier[1]* 1) Human and Molecular Genetics Center, Medical College of Wisconsin, Milwaukee, WI; 2) Children's Hospital Medical Center, Cincinnati, OH.

A previously identified quantitative trait locus (QTL) strongly linked to plasma triglyceride (LOD 3.7) and LDL levels (LOD 2.2) in subjects with the metabolic syndrome was selected for further investigation. A total of 1,048 single nucleotide polymorphisms (SNPs) were genotyped in an attempt to narrow the QTL, located on chromosome 7q36.

All non-synonymous SNPs in the region were genotyped in addition to tagSNPs selected based on the linkage disequilibrium (LD) patterns of the CEPH (Centre dEtude du Polymorphisme Humain) population of the HapMap (Phase 1 data). This resulted in a final overall density of one genotyped SNP every 4.6 kb. SNPs were genotyped in 1,560 samples on a custom-designed array using molecular inversion probe technology (Affymetrix).

One hundred of the 1,048 SNPs (9.5%) were determined to have marginal association with nominal p-values less than 0.05, a further nine (0.9%) had nominal p-values below 0.001. Of these 109 SNPs, 98 (89.9%) are located in regions of high LD ranging in size from 1.5 to 388 kb (average 35.8 kb). These can be condensed into six main regions of interest (size range 0.4 - 1.6 Mb) spread throughout the QTL interval, encompassing 14 known and six putative genes.

Individually, the associated SNPs are responsible for less than five percent of the overall linkage. However the number of loci identified indicates that the detected linkage is not caused by a single gene (or genetic locus.) Rather, it is more likely that the initial linkage analysis detected a cluster of loci of small individual effect that combine to create the observed overall effect on the plasma lipid profile.

The Mutation in the Renin Receptor (ATP6A2) Associated with X-linked Mental Retardation-Epilepsy (XMRE) Reduces ERK1/2 Activation by NGF in PC-12 Cells. *C.E. Schwartz, J. Norris, K.J. Franek* J.C. Self Research Institute, Greenwood Genetic Center, Greenwood, SC 29646.

The renin-angiotensin system (RAS) is essential for blood pressure control and water-electrolyte balance. The discovery of a mutation in the renin receptor (ATP6A2) in a family with X-linked mental retardation and epilepsy (XMRE; OMIN #300423) points to a novel role for the renin receptor. The neutral mutation (p.D107D) in the renin receptor (RER) resulted in an in-frame deletion of exon 4 (RERD4), due to an altered exon splice enhancer site (Ramser et al. HMG, 2005). In order to further characterize RERs function and potential role in brain development, we have transfected both wild-type and mutant RER cDNA into rat PC-12 pheochromocytoma cells. The RERwt was particularly localized to the tips of neural projections. However, RERD4 did not appear to accumulate at neurite tips. No other morphological differences between PC-12 cells expressing RERwt and RERD4 were evident. Nerve growth factor (NGF)-stimulated PC-12 cells expressing RERwt exhibited a nearly 100 percent increase in ERK1/2 phosphorylation compared to control cells (empty vector transfected PC-12 cells), whereas their RERD4 counterparts demonstrated approximately 30% lower phosphorylation levels relative to controls. Unstimulated PC-12 cells expressing RERwt and RERD4 were similar to the controls regarding phosphorylation of ERK1/2. Using differentially epitope-tagged RERwt and RERD4, we determined that RERD4 can dimerize with itself (RERD4-RERD4), as well as with wild type (RERD4-RERwt). Together, our data suggest that the RERD4 mutation associated with XMRE results in a form of renin receptor that may impair the ability of neurons to respond to NGF via the ERK1/2 pathway. These findings confirm the important role of the RER in cognitive function.

Combining trend tests for genome-wide association studies:Double trend tests. *K. Song[1], G. Zheng[2], R.C. Elston[3]*
1) GlaxoSmithKline, King of Prussia, PA; 2) National Heart, Lung and Blood Institue, Bethesda, MD; 3) Case Western Reserve University, Cleveland, OH.

To test marker-disease association in a case-control design using 100K-500K single-nucleotide polymorphisms (SNPs), a single marker analysis may be the first step. It provides insight into SNPs causing susceptibility to the disease or into further haplotype analysis. For genome-wide association studies with more than 100K SNPs (hypotheses), the significance level required in single marker analysis (single hypothesis) is less than 5 × 10-7. Such a significance level may not be achieved in a single stage analysis with the usual test statistics. We propose a procedure combining two trend tests, referred to as the double trend test (DTT), which is based on both the Hardy-Weinberg Disequilibrium trend test (HWDTT) and the Cochran-Armitage trend test (CATT), in a two-stage analysis. Our purpose is to screen SNPs in the first stage using the HWDTT and test in the second stage using the CATT a reduced number of SNPs that pass the screening stage. However, the features of our twostage analysis are (1) that the two trend test statistics are independent under the null hypothesis with Hardy-Weinberg equilibrium, so all samples are used in both stages to enhance the power in each stage, and (2) that it allows flexibility to choose the significance levels while ensuring asymptotic control over the overall Type I error rate. We discuss the optimal choice of the significance levels in both stages that ensures the asymptotic power of this two-stage analysis is at least as powerful as that using the CATT in a single-stage analysis. The results are illustrated with application to real data.

Homozygote Twinkle mutation in mitochondrial DNA depletion. *A. Rotig[1], D. Chretien[1], V. Serre[1], A. Slama[2], A. Munnich[1], E. Sarzi[1]* 1) INSERM U781, Hosp Necker, Paris, France; 2) Laboratoire de Biochimie 1, Hôpital Bicêtre, Le Kremlin-Bicêtre, France.

Twinkle is a mitochondrial 5-3 DNA helicase of particular importance for mitochondrial DNA (mtDNA) maintenance. Dominant Twinkle mutations have been reported in progressive external ophthalmoplegia with multiple mtDNA deletions (adPEO) whereas recessive Twinkle mutations are associated with infantile onset spinocerebellar ataxia (IOSCA). Because Twinkle controls mtDNA copy number, the Twinkle gene was regarded as a candidate gene in mtDNA depletion. We selected a series of 10 patients born to consanguineous parents and presenting with a severe mtDNA depletion of yet unknown origin and we studied the segregation of microsatellite markers flanking the Twinkle gene in these patients. Homozygosity of the flanking markers was found for two sibs belonging to the same family. They presented neonatal lactic acidosis, trunk hypotonia, seizures, cytolysis and cholestasis. A combined deficiency of complexes I, III and IV of the mitochondrial respiratory chain and as severe mtDNA depletion were found in their liver (8% of the normal mtDNA content). Homozygous mutation at a conserved position of the protein was found in the two patients (T457I). The similarity of Twinkle with the GP4D helicase of 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 mutation is located in the interface between two monomers of the hexameric enzyme, and most likely induces a local conformational change. This work reports the first description of a Twinkle mutation in mtDNA depletion in human.

Molecular evolution of the acyl-CoA dehydrogenase (ACAD) family. *Z. Swigonova, J. Vockley* Pediatrics, University of Pittsburgh School of Medicine, Children's Hospital of Pittsburgh, Pittsburgh, PA.

Mammalian ACADs constitute a family of flavoproteins that catalyze the ?,?-dehydrogenation of acyl-CoA thioesters to corresponding trans 2,3-enoyl-CoA products. Eleven members of this family have been described, however, the substrate specificity of two remains undetermined. Combining bioinformatic and phylogenetic approaches we investigated the evolutionary history of the ACADs with particular focus on substrate specificity prediction for the unknown enzymes. The origin of the ACAD family can be traced back more than 2 billion years ago to the origin of the Archaea, Bacteria, and Eukaryota. At least two primordial ACADs were already present at this time, one of which was the ancestor of glutaryl-CoA dehydrogenase. The emergence of the ACAD family is marked by several rounds of gene duplication at or early after the divergence of the three domains. The rise of the archaeal domain was accompanied by gene duplication early after its divergence producing ACADs specific to the archaeal lineage and by consecutive rounds of gene amplifications specific to extant archaeal taxa. This finding corresponds with known differences between the membrane lipids of Archaea and of Bacteria/Eukaryota. Unlike Eukaryota and Bacteria that have ester-linked fatty acids in lipids, Archaea have ether-linked lipids with C20-C40 isoprenoid (branched hydrocarbons) chains. Nevertheless, these long branched isoprenoid hydrocarbon fatty acids are not exclusive to Archaea but are also found to be prominent in basal Metazoa. Bacteria and Eukaryota emerged already equipped with 6-8 ACAD homologs and there were only occasional lineage-specific duplications towards extant taxa. Two additional duplications occurred in parallel before speciation of higher eukaryotes, particularly Coelomata, about 400-600 mya. The most parsimonious scenario suggests that the most ancestral ACAD had branched-chain specificity and that ACADs with straight-chain specificity derived later at the root of Bacteria and Eukaryota. Furthermore, our analysis suggests that the two undetermined ACADs will likely have specificity towards long branched-chain fatty acid substrates, particularly iso C-15 and C-17.

Incomplete medical follow up of birth defects patients in Bogotá Colombia. *I. Zarante, F. Suárez* Inst de Genetics Humana, Pontificia Univ Javeriana, Bogota, Colombia.

Government Birth defects surveillance is absent in Colombia. Latin American Collaborative Study of Congenital Malformations ECLAMC is the only program of epidemiological surveillance. Due to the absence of a structured system of genetics patients follow up, and the lack of medical genetics services, birth defects affected patients have an incomplete or missed diagnosis. Using the ECLAMC methodology during 2 years a total of 272 patients with major birth defects in 4 hospitals of Bogotá were detected. The actual health status of 225 patients (82.7%) is unknown. 10 patients (3.7%) died without a definitive diagnosis. A complete medical follow up and confirmed diagnosis after 6 months of birth defect detection in the new born, was accomplished in 37 patients (13%). During the first evaluation a syndromatic diagnostic was achieved in 24 cases (64.9%) and a new diagnosis was establish in 13 cases (35,1%) during the first visit 6 months later. 21 cases (56.8 %) had low birth weight and height, which remain below Percentile 5 in 18 cases after 6 moths. Most of the diagnosed syndromatic patients remain with low weight and height and there was a statistical difference in relation with the non syndromatic patients (p=0,005). Due to the nature of the disease in the new born, we expected that the clinical following were made at least once by a medical genetics specialists, but it only occurred in 18,9% of the cases (p=0,00001). All of the patients had the clinical indication of a karyotype but just in 8 cases (21.6%) were made. More than half of the patients (51.4%) showed clinical signs of developmental delay but only 5 had a specific therapy or treatment. None of the cases had genetic counseling. The diagnostic concordance between the prenatal diagnostic and the definitive diagnostic was insignificant: =0.053 agreement: 0.51 (95% Confidence Interval: -0.052, 0.157). These results shows the almost complete absence a good quality medical attention to the patients affected with birth defects in these population, and we firmly believe that this reflects the situation of the Colombian health system that ignores the families and patients with genetic diseases.

Telomeric associations in two cases of papillary renal cell carcinoma. *J. Tsai, B. Huang, M. Thangavelu, N. Qin, M. LeMieu, S. Lass* cytogenetics, Genzyme genetics, Corona, CA.

Telomere plays an important role in maintaining chromosome integrity and stability. Telomere association(TAS) is a rare phenomenon and has been reported in varioun solid tumors include renal cell carcinoma. TAS was proposed as one of the mechanisms of oncogenesis in these tumors. We present two cases of telomeric associations in papillary renal cell carcinoma. Multiple clonal numerical and structural rearrangements were observed in both cases, including gain and loss of chromosomes 7, 12, 17, and Y, frequent findings in papillary renal cell carcinoma. In addition, numerous TASs were observed. In the first case, TASs involved chromosomes 2, 4, 8, 12, 14, 15, 16, 17, 21, 22, and X were observed. In the second case, TASs were even more extensive, and involved chromosomes 1, 3, 7, 8, 9, 10, 16, 17, 19, 20, and 21. The majority of the associations were clonal and included chromosomes 16, 17, 19, and 20. A double telomeric association involves chromosomes 16, 19, and 21 was also observed. Overeall, twenty two different telomeric associations were observed in this case. The presence of multiple TASs, in addition to other chromosome abnormalities suggests chromosome instability and may be a sign of tumor progression.

Assessing the effect of missense variants in oligogenic disease. *N.A. Zaghloul, C.C. Leitch, N. Katsanis* Institute of Genetic Medicine, The Johns Hopkins University, Baltimore, MD.

A major challenge in human genetics is the assignment of pathogenicity to alleles. This problem is particularly poignant in oligogenic and complex traits, where the phenotype is the result of the combined effect of numerous, potentially mild alleles across several genes. Bardet-Biedl Syndrome (BBS) represents a useful oligogenic model, where mutations at a second locus can modify the penetrance and/or expressivity of the disorder. Numerous mutations in eleven genes have been identified in BBS patients, including 76 missense variants, some of which do not segregate with the disorder in the traditional Mandelian sense, suggesting that they are either benign polymorphisms or candidate modifiers. To determine the potential pathogenicity of each known missense variant, we capitalized on our recent development of BBS zebrafish models and assessed the ability of mutant bbs message to rescue the morphant phenotypes. Embryos treated with morpholinos alone showed a phenotype consistent with defects in convergence and extension, including shortened body axes, notochord defects, abnormal somites, and reduced brain size. Co-injection with wild type human mRNA rescued these phenotypes, whereas co-injection with mRNA bearing individual missense mutations produced a spectrum of effects. Most variants tested resulted in a partial rescue, suggesting that they are hypomorphic alleles, whereas some variants failed to rescue the phenotype and are therefore likely functional null alleles. Intriguingly, the introduction of some alleles recapitulated the morphant phenotype in the absence of morpholino, suggesting that these alleles might exert a dominant negative effect, including some alleles that have been associated with oligogenic inheritance. Although our approach is limited to the role of the BBS proteins during early development, this model potentially provides a powerful means to rapidly assess the effect of alleles for which genetic and computational data are inconclusive, a situation that is likely to represent an acute problem in oligogenic and complex traits.

Anterior versus posterior teeth hypodontia in families. *E. Severin, C. Albu, D.F. Albu* Dept Human Genetics, Carol Davila Univ Med Pharm, Bucharest, Romania.

Background: isolated hypodontia is one of the most common congenital anomaly affecting the tooth development of one or both dentitions.As a familial trait, hypodontia is found more commonly among individuals related with hypodontia patients than in the general population identifying it as a genetic disorder. Mutations in Pax9 gene are associated with posterior teeth hypodontia (permanent molars and second premolars). It is known that tooth agenesis does not represent a public health problem, but it may cause masticatory and speech dysfunctions or esthetic problems.**Objectives:** to describe the clinical phenotypes and modes of inheritance of hypodontia in families; to find evidence that mutations in Pax9 gene cause both anterior and posterior teeth agenesis.**Patients and Methods:** the sample consisted of 22 Caucasian patients from 15 families with hypodontia in two or three successive generations. They were evaluated by clinical and radiographic examinations.Blood samples were collected from 15 unrelated patients with hypodontia and 15 healthy control subjects and DNA was isolated and analyzed by PCR. **Results:** all patients and their affected relatives presented hypodontia of permanent teeth as an isolated trait. Three clinical phenotypes of hypodontia were described: missing anterior teeth (9 cases), missing posterior teeth (8 cases) and missing both anterior and posterior teeth (5 cases). Bilateral hypodontia appeared to be more frequent than unilateral absence of teeth. There were differences between right and left sides hypodontia. No significant sexual differences in the frequencies have been found. Hypodontia was inherited as an autosomal dominant condition with variable expression and incomplete penetrance. There were no clear correlations between the pattern of hypodontia and certain mutation of Pax9 gene. **Conclusions:**familial hypodontia is a genetic disorder with clinical variation; individuals within the same family would be expected to have the exact mutant genes and their different phenotypes could demonstrate the gene expression variation; anterior and posterior teeth hypodontia are two inherited conditions caused by different mutations.

A family-based association study of CYP17 and CYP19 gene polymorphisms with obesity phenotypes. *H. YAN*[1], *L.J. ZHAO*[3], *D.H. XIONG*[3], *R.R. RECKER*[3], *H.W. DENG*[1,2,3,4] 1) School of Life Science and Technology, Xi'an Jiaotong University, Xian, Shaanxi, 710049, China; 2) School of Medicine, University of Missouri- Kansas City, Kansas City, Missouri, 64108, USA; 3) Osteoporosis Research Center and Department of Biomedical Sciences, Creighton University, Omaha, Nebraska, 68131, USA; 4) College of Life Sciences, Hunan Normal University, Changsha, Hunan 410081, China.

Background: 17-Alpha-Hydroxylase (CYP17) and aromatase (CYP19) are two key biosynthesis enzymes of estrogen which is a critical regulator of adipogenesis and adipocyte development in humans. **Methods:** To investigate the association between CYP17 and CYP19 genes with obesity phenotypes, we performed a family-based association test (FBAT) in a large sample comprising 1,873 subjects from 405 Caucasian nuclear families. 7 single nucleotide polymorphisms (SNPs) in the CYP17 gene and 27 SNPs in the CYP19 gene were genotyped. Obesity phenotypes tested included obesity (OB, defined as BMI exceeds 30 kg/m^2), body mass index (BMI), fat mass, percentage fat mass (PFM), and lean mass, with the latter three measured by dual-energy X-ray absorptiometry (DXA). **Results:** Single markers and haplotypes (CYP17:1 block; CYP19: 5 blocks) were tested for associations. For the CYP17 gene, association was found between SNP1 (rs619824) and BMI (P value = 0.0386), between SNP5 (rs3740397) and both lean mass (P value = 0.0366) and BMI (P value = 0.0161), between SNP6 (rs6163) and both lean mass (P value = 0.0397) and BMI (P value = 0.0183), between haplotype CGCTCCA and both lean mass (P value = 0.0152) and BMI (P value = 0.0131), between haplotype AAAAGAA and both lean mass (P value = 0.0251) and BMI (P value = 0.0500). For the CYP19 gene, SNP 17 (rs2470176), SNP18 (rs730154) and SNP23 (rs1902584) showed association with BMI (P value = 0.0243, 0.0407 and 0.0117 respectively). Association was also observed between haplotype GGCAAAAA of block 4, containing SNP17 and SNP 18, with BMI (P value = 0.0168). **Conclusion:** In summary, our results suggest that both CYP17 and CYP19 genes are associated with obesity phenotypes in our study Caucasian population.

An extended angiotensinogen haplotype is associated with essential hypertension. *W.S. Watkins[1], W. Tolpinrud[1], S. Hunt[2], G.H. Williams[3], J-M. Lalouel[1], L.B. Jorde[1]* 1) Human Genetics, University of Utah, Salt Lake City, UT; 2) Department of Medicine, University of Utah, Salt Lake City, UT; 3) Brigham and Women's Hospital, Boston, MA.

Angiotensinogen (AGT) is the initial precursor of angiotensin II, a potent vasoconstrictor and regulator of blood pressure. Genetic variation at the angiotensinogen locus has been implicated in essential hypertension (EH). To further assess the genetic contribution of AGT variation to the hypertensive phenotype, we genotyped 24 SNPs (m.a.f. > 0.05) that span the promoter and exonic regions of the AGT locus in 372 hypertensive and 129 normotensive European-American subjects. Six SNPs were strongly associated with the hypertensive phenotype ($p < 0.01$). Four haplotype blocks were identified statistically in the 14 kb genomic region, and at least one haplotype in each block showed association with EH ($p < 0.05$). Interestingly, SNPs comprising one of the blocks were not associated with EH when tested individually, suggesting increased power to detect associations using haplotypes rather than individual polymorphisms. Combining the associated haplotypes from the 4 blocks revealed an extended haplotype present in 6% of controls and 11% of EH cases ($p < 0.017$). The extended haplotype was confirmed in one hypertensive individual homozygous for all 24 loci. This haplotype contains 6 promoter variants including the -1074T, -532T, -217A, -6A polymorphisms and the non-synonymous 4072C change (235T). No significant associations were found for African-American cases (59) and controls (21).

We also assessed the ability of HapMap tag SNPs to detect associations with EH. Seventeen SNPs were identified as tagging SNPs for Europeans using the HapMap. Nine of these were represented in our set of 24 SNPs identified from complete resequencing data and typed in our cases and controls. One tagging SNP, rs2004776, located less than 2 kb from -6A, was strongly associated with EH ($p < 0.001$). This result indicates that HapMap haplotype tagging SNPs could have successfully identified an association between AGT SNPs and essential hypertension in this case-control study.

C282Y and H63D Mutations in the HFE Gene, Are Not Rare In Jews of North African Ancestry In Israel. *O. Reish[1], V. Libman[1], G. Goltsman[2], I. Lorber[2], D. Chapman- Shimshoni[1]* 1) Genetics Institute, Assaf Harofeh Medical Center, Zerifin, Israel and The Sackelr School of Medicine, Tel Aviv University, Tel Aviv Isarel; 2) Department C of Internal Medicine, Assaf Harofeh Medical Center.

Hereditary Hemochromatosis (HHC) is one of the leading causes of iron overload due to increased absorption of dietary iron from the GI tract leading to iron accumulation and dysfunction of multiple organs. Only two mutations, C282Y and H63D in the HFE gene cover more than 95% of HHC chromosomes. About 60-90% of the patients are homozygotes for C282Y, while compound heterozygosity for C282Y/ H63D contributes to additional 3% of disease causing chromosomes. Despite being among the most common autosomal recessive diseases in Caucasians from North Europe, the disease has rarely been described in the Jewish population. We describe here an independent segregation of C282Y and H63D mutations in one family of Moroccan ancestry leading to 2 clinically affected patients with C282Y/ C282Y and one young, yet unaffected individual, with C282Y/ H63D genotype. We also screened 191 chromosomes derived from healthy North African Jews for the C282Y mutation and detected 4 mutated chromosomes. Screening for the H63D mutation showed 29 mutated out of 169 screened chromosomes. This preliminary study demonstrates gene frequency of 0.021 for the C282Y and 0.17 for the H63D, that correlate with carrier frequencies of 1/24 and 1/3 respectively. These findings may raise the possibility of under recognition of HHC among Jews of North African ancestry, and should increase the index of suspicion for this disease at least when initial clinical findings are detected.

Clinical and molecular studies of thirteen families with achromatopsia. *W. Wiszniewski, R.A. Lewis, J.R. Lupski*
Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.

Achromatopsia (ACH) or rod monochromacy is an autosomal recessive and genetically heterogeneous disease. It is characterized by a lack of color discrimination, poor visual acuity, photophobia and pendular nystagmus. Patients show absence of functioning cone photoreceptors in the retina in electroretinographic recordings while rod function is preserved. To date mutations in three genes have been reported including and subunits of the cyclic nucleotide-gated cation channel (CNGA3 and CNGB3 respectively) and cone photoreceptor transducin - GNAT2. The aim of this study was to determine the prevalence of mutations in ACH causing genes in a cohort of thirteen patients with both clinical and electroretinographic evidence for the disease. The patients were screened for mutations in each gene by direct sequencing of all coding exons. Mutations were detected in twelve out of thirteen families finding twenty two mutated alleles. Of the twelve ACH patients with detected mutations, nine had alterations in the CNGB3 gene, three in CNGA3 and none in GNAT2. The most frequent mutation - T383fs in CNGB3 was found in fifteen alleles. The analysis of SNPs within CNGB3 in unrelated patients with T383fs revealed transmission of a common haplotype. The high frequency of this mutation may reflect a founder effect. We identified three novel variants (R223G, G557R and L664F) in CNGBA and one (S435R) in CNGB3. We conclude that defects of these two genes are responsible for the vast majority of cases with complete achromatopsia.

Family Based Association Tests between sclerostin (*SOST*) gene and obesity phenotypes in a large sample. *L. WANG[1], L.J. ZHAO[3], D.H. XIONG[3], R.R. RECKER[3], H.W. DENG[1,2,3,4]* 1) Xi'an Jiaotong University, Xi'an, Shaanxi, 710049, China; 2) School of Medicine, University of Missouri- Kansas City, Kansas City, Missouri, 64108-2792, USA; 3) Osteoporosis Research Center and Department of Biomedical Sciences, Creighton University, Omaha, Nebraska, 68131, USA; 4) College of Life Sciences, Hunan Normal University, Changsha, Hunan 410081, China.

Introduction: Mesenchymal stem cells have the capacity to differentiate into osteoblasts and adipocytes. Such differentiations are triggered by cytokines and/or proteins. The secreted protein product of the *SOST* gene, sclerostin, is one of the regulation proteins.

Methods: To investigate the association between *SOST* polymorphisms with obesity, four SNPs of *SOST* gene were genotyped in 1,873 Caucasian subjects from 405 nuclear families. Five obesity related phenotypes, including obesity (defined as BMI exceed 30kg/m^2), fat mass, body mass index (BMI), lean mass and percentage of fat mass (PFM), were measured by dual-energy X-ray absorptiometry (DEXA). Linkage disequilibrium (LD) block structure of the *SOST* gene was examined by the program Haploview. A family-based association test (FBAT) was performed for the traits of obesity.

Results: Marginal significant association was found between SNP4 (rs1230393) and the three quantitative obesity-related phenotypes (fat mass, BMI and PFM). Haplotype A-G-G-G, with frequency of 0.32, showed significant association with fat mass, BMI and PFM (P = 0.0036, 0.0021, and 0.0036, respectively).

Conclusion: This study provides the first evidence that the genetic variation of *SOST* is associated with phenotypes of obesity.

Second Trimester Invasive Trophoblast Antigen (ITA) as a Down Syndrome Marker: A Prospective Cohort Study Using Fresh Serum Samples. *G. Palomaki[1], L. Neveux[1], C. Meier[2], P. Wyatt[3]* 1) Women & Infants Hospital, Providence, RI; 2) St. Michael's Hospital, Toronto, Canada; 3) York Central Hospital, Toronto, Canada.

Aim: Examine Down syndrome screening performance of second trimester ITA measurements, both alone and in combination with other biochemical markers. **Method:** For 1 year beginning in 11-2003, 20,346 women in Toronto provided a second trimester serum sample for Down syndrome screening (including 60% for an integrated test). Samples were tested for AFP, uE3 and hCG. ITA testing was performed on fresh samples with results converted to weight and race-corrected MoM levels, but not used clinically. 31 Down syndrome pregnancies were identified. **Results:** 18,462 women had ITA and triple markers, including 28 affected with Down syndrome. At a 5% false positive rate (FPR), 9, 8, 12 and 15 of the 28 cases were detected using AFP, uE3, hCG or ITA alone. The median MoM in Down syndrome pregnancies for hCG and ITA were 2.14 and 3.46. The logarithmic SD for hCG and ITA in unaffected pregnancies were 0.2252 and 0.2939 and 0.2678 and 0.3858 in Down syndrome pregnancies. Correlations between hCG and ITA were 0.801 and 0.868. At a fixed cut-off level for a 35 year old woman, the triple test with hCG or ITA detected 24 of 28 cases (86%, 95% CI 67% to 96%) with FPRs of 7.5% and 6.9%. When ITA and hCG were included with AFP and uE3, 23 of 28 (83%) cases were detected, with a 6.5% FPR. **Conclusions:** The observed performance of ITA and hCG in the second trimester (using fresh samples) is consistent with predictions from an earlier study of 45 cases, where sera were stored for 8 years, thawed and measured (Clin Chem 50:1804, 2004). The study predicted that 66% of cases can be detected by either version of the triple test. Using both ITA and hCG in a quadruple test improved detection to 76%. **Observed rates are higher than predicted. The reduced detection associated with including both markers is likely due to the small number of cases.** Based on these two datasets, ITA is a suitable replacement for hCG in second trimester screening, and screening is likely to be improved when both are measured and interpreted together with other second trimester markers.

Disclosing misattributed paternity: A survey of genetic counselors in the United States and Canada. *S.A. Zelenietz[1], C. Harrison[2], C. Trevors[1], C. Shuman[1], D. Chitayat[1, 3], P. McKeever[4]* 1) Genetics, HSC, Toronto, ON, Canada; 2) Department of Bioethics, The Hospital for Sick Children, Toronto, ON; 3) Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Toronto, ON; 4) Faculty of Nursing, University of Toronto, Toronto, ON.

The inadvertent discovery of misattributed paternity (MP) may lead to conflicts between professional obligations, ethical principles and the interests of the parties involved, thereby making it difficult to determine to whom to disclose this information. Previous studies have shown that genetic counselors (GCs) typically disclose MP to the female partner only; however, these reports have been narrow in scope, drawing conclusions from single case scenarios. The purpose of this study was to explore practice regarding the disclosure of MP, and to determine how GCs made this decision in diverse situations. GCs were surveyed using four case scenarios designed to simulate dilemmas in the disclosure of MP. Of the 273 survey respondents, 204 (75%) completed at least one case scenario. Respondents had been practicing for an average of 6.6 years, had seen an average of 1.6 cases of MP, and most (97.2%) indicated that their employer did not have a documented policy that governed the disclosure of MP. Overall, the results revealed little consensus concerning the disclosure of MP both within and between case scenarios. GCs chose to disclose MP to the female partner, male partner, child, or not to disclose. GCs considered factors including determination of the primary patient, the information included in the signed consent, the provision of accurate genetic counseling, the biological fathers right to know his carrier status, and the medical relevance of MP in medical decision-making. Non-maleficence was the most often cited ethical principle used in decision-making. The lack of consensus found in this study suggests that developing institutional guidelines and/or a professional position statement concerning the disclosure of MP would benefit GCs. Such policies could lead to the development of a decision-making framework which would serve to help guide GC practice.

A Robust Multiple Testing Method of Controlling the False Discovery Rate. *M. Rao, R. Chakraborty* Ctr Genome Information, Univ Cincinnati, Cincinnati, OH.

Currently multiple testing is almost a routine scenario in most statistical genetic applications. However, in most genomic studies, the individual tests are not necessarily independent, nor the joint distribution of their significance levels (p-values) can be parametrically specified. Data on genome-wide, or candidate gene based association studies, gene expression data on arrays of genes involving multiple networks (or pathways) are examples of such situations, where hundreds, if not thousands of tests, are required to be simultaneously performed, with no assumption on the joint distribution of their p-values. Standard Bonferroni-type of adjustment of multiplicity effect in multiple testing only controls the Family Wise Error Rate (FWER), but the False Discovery Rate (FDR) has gaining acceptance as the prime choice of error rate, which needs to be controlled. A step-up procedure of sequential inference, suggested recently, has an upper bound of FDR, based on the number of tests performed, proportion of true null hypotheses, and the over-all level of significance of the test procedure. In this research, we propose a step-down multiple testing procedure, built sequentially on the basis of the ordered p-values of the individual tests, and show that the upper bound of the FDR of the proposed test procedure is smaller than that of the step-up procedure suggested in the literature. Like the step-up procedure, the newly suggested step-down procedure also does not need any assumption regarding the joint distribution of p-values of the individual tests. Numerical illustrations of the efficiency of reduced FDR (i.e., ratio of upper bounds) are given for some choice of parameters (i.e., number of tests, proportion of true null hypotheses, etc.) that may commonly be encountered in genomic data analyses. (Research supported by US Public Health Service grant GM41399 from the US National Institutes of Health).

Transcriptional Profiling of Nicotine Dependence. *R. Philibert, G.-Y. Ryu, J.-G. Yoon, H. Sandhu, N. Hollenbeck, T. Gunter, A. Barkhurst, A. Madan* Psychiatry, University of Iowa, Iowa City, IA.

Transcriptional profiling has been used to identify gene expression patterns indicative of general medical illnesses such as atherosclerosis. However, whether these methods can identify common psychiatric disorders has not been established. To answer this question with respect to nicotine use, we used genome wide expression profiling (n=15) followed by real-time PCR (RT-PCR) (n=94) to examine gene expression patterns in lymphoblast derived RNA from 94 subjects in the Iowa Adoption Studies. As compared to controls without a history of smoking (n=9), 1163 of 29,0908 known genes were found to be differentially expressed two fold or more with false discovery rate of 5%. Of these, 579 genes were significantly up-regulated and 584 genes were significantly down-regulated. RT-PCR confirmation of three select RNA levels confirmed the validity of the overall profile and revealed highly significant relationships between 1) major depression, 2) nicotine use and 3) cannabis use. We conclude that the use of expression profiling may contribute significant insights into the biology of complex behavioral disorders and serve as a laboratory mechanism to confirm and extend clinical diagnosis of these disorders.

Analyzing and modeling dichotomous traits in large complex pedigrees. *C. Papachristou, C. Ober, M. Abney*
Human Genetics, University of Chicago, Chicago, IL.

Although it is believed that many common complex disorders have a genetic basis, attempts to unravel the transmission mechanism governing such traits have met with limited success. This shortcoming is partly attributed to genetic heterogeneity, trait loci of small contribution, and large contributions from environmental factors. It has been suggested that isolated founder populations, particularly ones with a large, known pedigree, may be advantageous for complex trait mapping. This is not only because of their extensive genealogical information, but also because a small number of founders is expected to reduce the number of alleles that contribute to susceptibility, and the homogeneity in lifestyle and uniformity of environment significantly attenuate many confounding effects. We are proposing a likelihood method for mapping dichotomous traits based on data from complex pedigrees. This method is an extension to that introduced by Abney et al. (2002) for mapping QTLs, which was developed in the context of linear mixed models. A natural extension of their method to accommodate binary traits can be easily found in the framework of the generalized linear mixed models (GLMMs) (McCulloch and Searle 2001). More specifically, our approach is based on a hierarchical model where we first assess the probability of each individual having the trait and then formulate a likelihood assuming conditional independence of individuals. The advantage of our formulation is that it easily incorporates information from pertinent covariates as fixed effects, as well as taking into account the correlation between related individuals that share genetic background through random effects. Due to the high dimensionality of the integration involved in the likelihood, exact computations are infeasible in most cases. Instead, a Monte Carlo expectation maximization (MCEM) algorithm is employed for obtaining the maximum likelihood estimates of the polygenic model parameters. We have developed a software package that efficiently implements our method and we are using it to analyze data from a 13-generation pedigree consisting of 1,653 Hutterites, focusing on the diabetes phenotype.

Defining Subphenotypes for Orofacial Clefts Based on Dental Development. *A. Letra[1,2], R. Menezes[1], J.M. Granjeiro[3], M.E. Cooper[1], M.L. Marazita[1], A.R. Vieira[1]* 1) Oral Biology and Center for Craniofacial and Dental Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Department of Biological Sciences, University of São Paulo, Bauru, SP, Brazil; 3) Department of Cellular and Molecular Biology, Fluminense Federal University, Niterói, RJ, Brazil.

Subjects with cleft lip/palate (CL/P) present more dental anomalies (DA) than controls. It may be an indication that DA are an extended phenotype of CL/P. 1,000 individuals, 500 with CL/P and 500 controls were examined to determine presence of DA outside the cleft area such as tooth agenesis (TA), microdontia, supernumerary teeth (ST), tooth impaction, tooth malposition, shape anomaly and transposition. Cleft individuals were divided according to cleft completeness or incompleteness and laterality. Cleft cases presented significantly more DA than controls (OR=8.72; 95%CI: 6.05-12.56). TA was most frequently observed affecting the left side in cases with complete unilateral right clefts (p=0.01). Contrarily, cases with unilateral left clefts presented more TA on the right side (p=0.01). Bilateral TA occurred more commonly in cases with bilateral clefts (p=0.04). TA affecting upper teeth was more common to unilateral right clefts (p=0.02). Left lateral incisors were most frequently absent in cases with unilateral right clefts (p=0.00001). The opposite was true for right lateral incisors, which were most frequently absent in cases of unilateral left clefts (p=0.007). Cases with complete unilateral right clefts presented more microdontia of upper left teeth (p=0.00001). As for ST, most were observed in cases with unilateral left clefts (p=0.002) with no significant differences when comparing side of the ST. Lower canines are more commonly rotated (lingual surface facing buccal) than any other type of teeth in cleft cases (p=0.00001). The consistent presence of DA on the opposite side of unilateral clefts with preferential TA of the lateral incisor could mean an unsuccessful bilateral cleft, and, moreover, the genes that contribute to laterality of the cleft may be different resulting in alternate phenotypes for DA also. Supported by NIH grants R21-DE016718, R01-DE016148, P50-DE016215, and CAPES BEX341305-5, Brazil.

Detecting Disease-Gene Association in the Presence of Misclassification of Control Subjects. *R. Seiver, M. Rao, X. Lei, R. Chakraborty* Ctr Genome Information, University of Cincinnati, Cincinnati, OH.

Population based case-control designs have gained popularity in recent years for disease-gene association studies. Though case-control study designs have been used for a long time in epidemiological research, for several diseases precise methods of control selection is hampered by lack of consent of subjects or invasiveness of tests for determining affection status of subjects. When controls are selected based on less rigid criteria of determining affection status, or for late onset of diseases, some fraction of recorded controls may indeed be affected. In this research, we derive analytical formulae to predict the extent of dilution of the strength of disease-gene association (odds ratio, OR) in the presence of misclassification errors in controls. We obtained the maximum likelihood estimate of OR in the presence of misclassification, and developed algorithms to determine the extent to which power of detecting significant OR is reduced in the presence of misclassification error. Effect of misclassification on diluting the strength of OR appears to be insensitive to the prevalence of allele/genotypes in controls. For example, with 10% misclassification rate in controls, OR reduces from 1.5 to approximately 1.43 for a prevalence rate in the range of 1 to 20 percent in the controls. Of course, with increasing rate of misclassification, OR gets closer to one monotonically, consequently reducing the power of detecting its significance. A user-friendly web-based software is developed for systematic evaluation of reduction of power for any specified fraction of control subjects that are truly affected. (Research supported by US Public Health Service grant GM41399 and a Summer Undergraduate Research Training grant (REU) from the US National Institutes of Health).

Genetic evaluation of the serotonergic system in Chronic Fatigue Syndrome. *A.K. Smith, M.S. Rajeevan* Centers for Disease Control & Prevention, Atlanta, GA.

Chronic Fatigue Syndrome (CFS) is a significant public health problem of unknown etiology. Central nervous system abnormalities, particularly hyperactivity in the serotonergic (5HT) and hypoactivity in the hypothalamic pituitary adrenal axis (HPA) systems, have been implicated in the pathogenesis of CFS. Since alterations in 5HT signaling may result in altered HPA activity, polymorphisms were examined in genes of the serotonergic system to identify putative risk factors for CFS and potential mechanisms for altered serotonin or HPA systems. A total of 79 polymorphisms in 14 genes related to serotonin synthesis (*TPH2*), signaling (*HTR1A, HTR1E, HTR2A, HTR2B, HTR2C, HTR3A, HTR3B, HTR4, HTR5A, HTR6,* and *HTR7*), transport (*SLC6A4*), and catabolism (*MAOA*) were examined in 137 subjects (40 CFS, 55 with insufficient fatigue, and 42 non-fatigued controls) derived from a study in Wichita, Kansas. Subjects participated in a clinical evaluation where exclusionary conditions were identified and assessment of functional impairment, fatigue and symptoms was performed. Three polymorphisms located in the 5HT receptor subtype *HTR2A* (rs6311, rs6313, and rs1923884) were associated with CFS when compared to non-fatigued controls (p=0.0065-0.015). Consistent associations were observed between *HTR2A* variants and quantitative measures of functional impairment (p=0.0031-0.040), fatigue (p=0.0037-0.047) and symptoms (p=0.017-0.022) in all subjects. No haplotype effects or gene-gene interactions were observed. The most compelling association was with rs6311 (-1438 G/A). Functional studies of rs6311 report increased promoter activity with the A allele relative to the G allele. *In silico* analysis of the variants revealed that the rs6311 A allele creates a consensus binding site for Th1/E47, a transcription factor expressed in the brain and implicated in nervous system development. These results suggest enhanced activity of HTR2A in CFS subjects carrying the rs6311 A allele and supports the hypothesis that alterations in the serotonergic or HPA axis systems are involved in the development or progression of chronic fatigue. Future studies will be needed to verify Th1/E47 binding at rs6311 and to replicate these findings in a larger group of subjects.

Large-scale sequence variation detection in diploid samples using Affymetrix arrays. *A.B. Sparks, N. Patil, F. Collin, S. Walsh* Affymetrix, Santa Clara, CA.

Large-scale sequencing projects require increasingly rapid, accurate, and cost-effective methods for variant detection. Affymetrix next-generation GeneChip CustomSeq Resequencing Arrays employ higher density 8m X 8m features, improved sample prep methods, and improved base calling algorithms to enable analysis of up to 300kb of unique DNA sequence on a single chip. To demonstrate the performance of these arrays in detecting SNPs in diploid samples, we designed a chip to query 300kb of contiguous sequence within an ENCODE interval on chromosome 4. A 250kb region was amplified from each of 16 CEPH DNA samples using 25 long range PCR amplicons, and the resulting 16 sets of PCR products were pooled, fragmented, labeled, and hybridized to arrays. The arrays were stained and scanned, and the intensity data from the 16 scans was analyzed using the GSEQ 4.0 base calling software package, set to detect heterozygous as well as homozygous variants. The array-derived base calls were compared to reference capillary sequence/genotyping data obtained from the ENCODE project for the same 250kb interval in the same 16 CEPH samples. The array call rates across the 16 samples ranged from 95% to 98% (average of 96.5%), whereas the capillary sequence coverage ranged from 67% to 81% (average of 74%). Average call rates remained above 90% for probe GC compositions ranging up to 70%. Overall array base call accuracy (as compared to the reference dataset) was found to be 99.95%. The homozygous variant detection rate was 96.9%, and the heterozygous variant detection rate was 89.1%. Approximately 4 in 10,000 non-variant positions were identified as variant in the array-derived base call data. To reduce this false-positive rate, we developed a set of post-GSEQ filters, which together increased the overall accuracy from 99.95% to 99.98% and decreased the rate of false positives from 4 in 10kbp to 1.6 in 10kbp, while only decreasing the overall call rate from 96.5% to 96.0%. To demonstrate the utility of arrays for querying discontinuous sequences, we have designed a kinase exon resequencing chip. Sample prep methods and performance data for this array will be presented.

Identification of genetic epistasis models within large SNP datasets. *D.A. Ross[1], J. Shaw[2], G. Shaw[2], J.H. Moore[3], B. White[3], J. Sninsky[1], M. Frome[2]* 1) Celera Diagnostics, Alameda, CA; 2) Focus Biology, San Jose, CA; 3) Dartmouth College, NH.

Causation in complex disease is manifested as a spectrum of genetic and environmental effects. The genetic effects range from rare events with large effect to common polymorphisms with modest to large effects. Systems biology experiments suggest that proteins/RNA interact as modules to perform tasks; SNPs effecting gene function could have epistatic effects within or between modules that would lead to variation in epidemiological causes of the disorder. Statistical methods are being explored to identify these epistatic interactions. The problem is in three parts. 1. Can the SNPs in a model be extracted from a large dataset with high specificity? In a real dataset there are false positives that do not replicate in additional datasets; it is therefore important that the model SNPs be extracted consistently and the metric used to rank the SNPs be robust. 2. Can the model SNPs be extracted when increasing numbers of noise SNPs are added? This requires a parallel computation algorithm for large numbers of SNPs. Using either a functional genome scan of 30K SNPs or a positional genome scan of 500K SNPs should not impact specificity and occur with acceptable runtimes. 3. Can the original model be regenerated given the cut-off used in part 1? We have used the suite of data mining techniques in FOCUS Biologys FOCUS Discovery SNP analysis software and evaluated the search for two genetic models. The epistatic models investigated included four SNPs. In model A, two of the SNPs have a dominant-dominant interaction (M27)(Li and Reich Human Heredity(2001)) and two SNPs lacking main effects (M170). Model B has two pairs of SNPs interacting as M170. All SNPs are in HWE. Heritability, in the broad sense, was examined at 0.4, 0.2, 0.1 and 0.05 with ten replicates for each model type and level of heritability. Under all levels of heritability, FOCUS Discovery successfully identified the SNPs at high sensitivity and specificity, even with Model B, down to a heritability of 0.05, the 4 SNPs were identified in the top 3%. Evaluation of the models with 300 to 300K noise SNPs will be presented.

Likelihood ratio tests of association for X-linked QTL in family-based designs. *L. Zhang[1,2], R.W. Morris[2,3], R.-H. Chung[1,2], E.R. Martin[2], Y.-J. Li[2]* 1) Bioinformatics, NCSU, Raleigh, NC; 2) Center for Human Genetics, DUMC, Durham, N.C; 3) Anesthesiology, DUMC, Durham, N.C.

Although genetic association methods for detecting quantitative trait loci (QTL) in family-based designs have been well developed for autosomes, comparable methods for X-linked QTL have received little attention. Here we develop a test for genetic association using X-linked markers in nuclear families with multiple offspring and possibly missing parental genotype information. We extend the model of Abecasis et al. (2000) by modifying the scheme for scoring marker genotypes in the sexes, and by incorporating variance components developed by Kent et al (2005). We show that the fixed effect within family remains a direct estimator of additive genetic value conditional on sex. Restricted Maximum Likelihood (REML) and Fisher Scoring method are applied to calculate variances of the random genetic or environmental effects. The means of the fixed effects between and within families are estimated through General Least-Squares Estimation. In addition, we introduce a parameter for dosage compensation, which can be used to model X-inactivation in females. Simulation results show correct type I error rates and that estimates of the genetic effect of marker alleles is unbiased and equal that of QTL alleles when linkage disequilibrium between the marker and QTL is maximum. Power is illustrated for a variety of nuclear family structures and genetic models. The loss of efficiency with missing parental genotypes can be offset by additional sibling genotype information. As an example in real data, we present analysis of monoamine oxidase genes (MAOA and MAOB) on the X chromosome using age of onset of Parkinson disease as a quantitative trait.

Subtelomeric chip: Novel assay for detection of subtelomeric and genome wide aberrations. *G.A. Toruner, D. Streck, J.J. Dermody, M.N. Schwalb* Center for Human and Molecular Genetics, UMDNJ - NJ Medical School Newark, NJ.

Global developmental delay (GDD) and mental retardation (MR) are important child heath issues. According to various estimates, the prevalence of GDD/MR is between 1-10%. During the genetic evaluation of GDD/MR, in addition to history and physical examination, laboratory tests such as karyotyping, subtelomeric FISH, mutation analysis of FMR1 and MECP2 genes are performed. The total yield of these diagnostic tests is only 10%. Microarray comparative genomic hybridization (CGH) has been developed to detect deletions or duplications in very small segments of chromosomes and the use of this technology is expected to increase the diagnostic yield. The major limitation of the current BAC based array technologies employed in some clinical diagnostic labs, however, is the low resolution (~1MB) of the chip and having not enough coverage in the subtelomeric regions. Our aims were to design a novel array-CGH chip with high-density coverage in the subtelomeric regions and to conduct a pilot study in previously clinically tested specimens for the evaluation of the novel array. For this purpose, we used Human Genome CGH Microarray 44B chip (Agilent), which contains 42K test probes and 2K control probes, as the template for the novel design. Using e-array 4.0 (Agilent), one third of the probes were randomly removed from the chip and replaced by 14,000 subtelomeric probes (probes at most 1MB away from telomeres) The average density of the probe coverage is 3 KB in subtelomeric regions and 125 KB genome wide. Currently, eighteen clinical samples were tested (including subtelomeric aberrations and other microdeletion syndromes) and the concordance rate between chip results and conventional lab testing is 100%. The results of the pilot study are highly encouraging and the analysis of additional samples are still undergoing.

A small molecule enhances RNA interference and promotes the biogenesis of microRNAs. *G. Shan[1], J. Zhang[2], K. Szulwach[1], Y. Qin[1], R. Duan[1], H. Ju[1], A. Chan[1], C. He[2], P. Jin[1]* 1) Department of Human Genetics, Emory University, Atlanta, GA; 2) Department of Chemistry, University of Chicago.

RNA interference (RNAi) is a well-conserved and universal mechanism that uses small noncoding RNAs to silence gene expression post-transcriptionally. The endogenous small RNAs, called microRNAs (miRNAs), are processed from hairpin precursors and could shape diverse cellular pathways. Since small double-stranded RNAs, called small interfering RNAs (siRNAs), could induce sequence-specific mRNA degradation when introduced into cells, RNAi has become attractive for functional genomics and human therapeutics. Although the major components in the RNAi pathway have been identified, however, the regulation of this pathway remains largely unknown. Here we have developed a novel cell-base assay to monitor the activity of RNAi pathway and taken a small-molecule-based approach to dissect the RNAi pathway. Through screening a chemical library with diversified compounds, we have identified a small molecule (RNAi-E) that could enhance siRNA-mediated mRNA degradation as well as miRNA-mediated translational suppression. Furthermore, RNAi-E could promote the biogenesis of endogenous miRNAs. We identified TRBP as the potential protein target of RNAi-E. Application of RNAi-E could enhance the knocking-down efficiency of both siRNA and shRNA as well as reduce the dosage of siRNA duplex at least 5-fold. We also demonstrated that RNAi-E could function in animal models including C. elegans and mice. The identification of RNAi-E not only helps further understand the modulation of the RNAi pathway, but also facilitates the development of siRNAs as therapeutic reagents.

Combined saposin C and D mutations in mice affect processing of prosaposin and lead to a severe neurological phenotype with predominant glucosylceramide and hydroxy ceramide accumulation. *Y. Sun[1], M. Zamzow[1], H. Ran[1], B. Quinn[1], W.P. Witte[2], G.A. Grabowski[1]* 1) The Division and Program in Human Genetics; 2) Division of Pediatric Pathology, Children's Hospital Research Foundation, and University of Cincinnati College of Medicine, Department of Pediatrics, Cincinnati, OH.

Saposins (A, B, C and D) are ~80 amino acid glycosphingolipids activator proteins that derive from their precursor, prosaposin. In both humans and mice, prosaposin/saposin deficiencies lead to severe neurological deficits. Deficiencies of individual saposins have been reported in only a few humans, thus limiting the availability of the patient samples for physiological function analysis. Here, the generation of saposin C and saposin D combined deficiencies (CD-/-) in mice were produced by introducing mutations into critical cysteines of each saposin. The mutations of saposin C and D influenced the processing of prosaposin, and led to increases of prosaposin and decreases of saposin A and B cellular levels. The CD-/- mice developed a severe neurological phenotype and showed ataxia, kyphotic posturing and hind limb paralysis. They had an extended life span (~56 days) relative to prosaposin null mice (~30 days). Loss of Purkinje cells was evident after 6 weeks. The storage cells were in spinal cord, brain and dorsal root ganglion. There were marked accumulations of glucosylceramides and hydroxy ceramides. Only slight increases of lactosylceramide (LacCer) were present in liver suggesting that saposin B compensates for saposin C in LacCer degradation in specific tissues. In addition, the deficiency of saposin C in CD-/- mice resulted in decreases of GCase activity and protein. This CD saposin mutant mouse provides a model to explore the in vivo function of saposin(s) in glycosphingolipid metabolism and lysosomal storage diseases.

Spatiotemporal patterns of Bex1/2 expression in developmental mouse brains. *J.Z. Yang[1], W. Ju[2], H. H. Zhu[1], J. Shen[2], G.Y. Wen[2], C.P. Corbo[2], R. Zhong[1], Y.Z. Zhang[1], J.H. Zou[1], 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.

The Bex (brain expressed X-linked) is a gene family comprised of Bex1-6, named originally with the characteristics of highly expressed in brain and localized at chromosome X. Among Bex1-6, Bex1 and Bex2 share a high homologue in their primary sequences and were characterized to interact with an olfactory marker protein (OMP). However, biological features of Bex1/Bex2 involved in brain development are yet unknown. ThereforeJun, we have undertaken a study to investigate the protein expression and localization of Bex1/Bex2 in the mouse developmental brains. Our results showed a spatiotemporal expression pattern of Bex1/Bex2 at different stages of mouse developmental brains. Bex1/Bex2 were expressed comprehensively in E15 and P0 mouse brain, non-diffusely located in specified encephalic region and nucleus groups. High expression was observed in olfactory system, hypothalamus and limbic system such as hippocampus, cingulum and amygdaloid nucleus. Subcellular localization in human SY5Y neuroblastoma cell line indicates Bex1/Bex2 are cytoplasm protein with a few distributions in the neuronal nuclei, which can be confirmed by electronic immunogold stain. The spatiotemporal expression patterns suggested Bex1/Bex2 might have played some functional roles in the neuron differentiation and brain development.

X-linked and autosomal gene expression in human tissues: Male and female comparisons. *Z. Talebizadeh, S.D. Simon, M.G. Butler* Childrens Mercy Hospital and University of Missouri-Kansas City, Kansas City, MO.

Dosage compensation in mammalian females occurs in early development and results in inactivation of one X chromosome leading to equality of X-linked gene products between male and female. X chromosome genes, in particular, are of interest for their involvement in neurodevelopmental disorders such as mental retardation and autism where unequal gender prevalence exists, but not all X-linked genes are inactivated. *In vitro* assays using somatic cell hybrids or human fibroblasts suggest that 25% of X-linked genes escape inactivation at least to some degree but *in vitro* results may not reflect what happens *in vivo*. Therefore, we analyzed the female/male (F/M) gene fold expression ratio from 11 different tissues of an existing *in vivo* microarray database including 14 males and 10 females for 299 X-linked and 7,795 autosomal genes to determine the percentage of X-linked genes compared with autosomal genes that are overexpressed (or underexpressed) in females. On average 5.1 and 4.9% of genes showed higher expression (F/M gene fold ratio 1.5) in females compared with 7.4 and 7.9% in males, respectively for X-linked and autosomal genes. More variations were seen in gene expression from the cerebrum and adrenal gland compared with other tissues studied. A trend was found for F/M gene fold ratios greater than 1.5 for several X-linked genes indicating overexpression in females among multiple tissues. Only nine (3%) of the 299 X-linked genes showed overexpression in females in at least 3 of the 11 tissues studied. Of the 9 genes, 6 were located on the short arm and 3 on the long arm of the X chromosome. Six (*HDHD1A*, *PNPLA4*, *AP1S2*, *ZFX*, *UTX*, and *RPS4X*) of the 9 genes were previously reported to escape X inactivation. However, in general, no consistent pattern was seen for the expression of X-linked genes between *in vitro* and *in vivo* systems. This study indicates that factors other than the X inactivation process (e.g., sex hormone influence, tissue specificity, monoallelic expression) may impact on expression of X-linked genes resulting in an overall similar gender expression for both X-linked and autosomal genes.

Comprehensive and highly efficient molecular diagnostics of autosomal dominant polycystic kidney disease (ADPKD) by mutation screening. *S. Rossetti[1], M.B. Consugar[1], A.B. Chapman[2], V.E. Torres[1], J.J. Grantham[3], L.M. Guay-Woodford[4], C.M. Myers[5], J.P. Miller[6], P.C. Harris[1] for CRISP Consortium* 1) Nephrology, Mayo Clinic, Rochester, MN; 2) Nephrology, Emory Univ., Atlanta, GA; 3) Kidney Inst., Kansas Univ., Kansas, KA; 4) Nephrology, Univ. of Alabama, Birmingham, AL; 5) NIDDK, Bethesda, MD; 6) Biostatistics, Washington Univ., St. Louis, MO.

ADPKD is a common inherited kidney disease characterized by progressively enlarging kidney cysts leading to kidney failure. ADPKD is genetically heterogeneous, *PKD1* accounting for ~85% and *PKD2* for 15% of cases. Diagnostics of ADPKD is mainly performed by renal imaging techniques, but molecular diagnostics plays a role particularly in young, pre-symptomatic individuals, where the imaging data may be inconclusive. We have screened for *PKD1/2* mutations in a large and very well characterized clinical population (the CRISP population) by DHPLC, direct sequencing, and screening for large deletions. We have developed an algorithm to predict the pathogenicity of missense and atypical splicing changes. This included the chemical difference, evolutionary conservation in a multi-sequence alignment, scoring of potential splice variants, and population data (analysis in pedigrees and normal controls). Using this comprehensive screening approach on the 202 probands, mutations were determined in 181 (90% final detection rate). *PKD1* accounted for 155 mutations (86%), and *PKD2* for 26 (14%). For the *PKD1* dataset, 103 (66%) were truncating mutations (either frameshift-50, nonsense-37, or splice-16), 43 (28%) aminoacid substitutions and 9 (16%) small in-frame deletions. For the *PKD2* dataset, 22 (84%) were truncating, and only 4 (16%) aminoacid substitutions or small in-frame deletions. Most of these mutations were private, but 36 changes (20%) were recurrent in apparently unrelated pedigrees. This study has shown the utility of molecular diagnostics in ADPKD with a high overall detection rate, 2/3 of all mutations being truncating, and employing a careful evaluation of other variants for pathogenicity. Molecular diagnostics of this disorder is likely to become very important as therapies are developed and clear diagnoses are required in young individuals.

KABUKI SYNDROME WITH CENANI-LENZ SYNDACTYLY: CASE REPORT. *A.B.A. Perez, N.L.M. Sobreira, M.C.P. Cernach, D. Brunoni* Centro de Genetica Medica, Unifesp - EPM, Sao Paulo, São Paulo, Brazil.

Cenani-Lenz Syndactyly (CLS) is characterized by phalanges with a disorganized aspect, syndactyly and oligodactyly, metacarpal and carpal fusion, radioulnar synostosis, mesomelic shortening of the upper members, luxation of the radial head and feet with a variety of anomalies. There are no defined craniofacial characteristics. Drohm et al.(1976), Verma et al.(1976) and Seven et al.(2000) described large ears. Temtamy et al.(2003) described a broad forehead, downward slanting palpebral fissures, hypertelorism, small and prominent philtrum, malar hypoplasia and curved upper lip, long palpebral fissures, discrete eversion of the lower lid, and high-arched palate. Elliott (2004) described a boy with non-classical Cenani-Lenz syndactyly, with syndactyly and oligodactyly of the fingers and a phenotype consistent with Kabuki Syndrome. The pattern of inheritance is autosomal recessive. Kabuki Syndrome (KS) has a sporadic occurrence and presents limb alterations such as: persistence of pads, brachydactyly, short medial phalanx of the fifth finger, cutaneous syndactyly of the toes and polydactyly. Wessels et al. (2000) evaluated 300 patients with KS, 71% of which had dental anomalies. These are not common in patients with CLS. We report the case of a 6-year-old female patient, the fifth child of a non-consanguineous and healthy couple. On physical examination, the findings were: downslanting palpebral fissures, eversion of the lower lids, long eyelashes, sparse medially flared eyebrows, depressed nasal root, anteverted nostrils, long philtrum, thin upper lip, persistence of pads, spatulated fingers, clinodactyly of fifth fingers. The patients feet presented bilateral syndactyly of the first and second and of the fourth and fifth toes and hypoplasia of the third toe. The patient presents recurrent infections and normal NPMD. Karyotype: 46,XX. The genes causing CLS and KS are unknown and their elucidation may determine to which extent they are genetically related. Meanwhile, a careful evaluation of the craniofacial characteristics of patients with CLS is necessary, in order to correctly characterize this association.

A likelihood approach to defining homozygous tracts using high-density SNP genotypes. *J.P. Struewing, R.J. Clifford, L.H.M. Pereira, M.A. Pineda, C.E. Fasola, H. Zhang, R.P. Finney, J. Zhang, K.H. Buetow* Lab of Population Genetics, NCI/NIH, Bethesda, MD.

Current high-density genotyping platforms allow one to identify chromosomal regions in which a given individual is homozygous, representing possible autozygous segments inherited identical by descent from a common ancestor. We defined homozygous tracts by extension of the likelihood based method of Broman & Weber (AJHG 1999) originally applied to short tandem repeat polymorphisms. A log likelihood odds (lod) score was calculated at each SNP position, contrasting the likelihood of observing the genotype assuming the surrounding segment is autozygous with the likelihood if it is not autozygous. The method accounts for population-specific allele frequencies as well as for genotyping error and mutation. Since SNPs are not independent, the lod score was adjusted using a log-transformation of a recombination measure (in cM/kbp, from the Oxford HapMapI r16c.1 UCSC tract) at each SNP position. We used Affymetrix 500K SNP chip data (490K autosomal SNPs, mean 1 SNP per 5.8kb) for the 4 HapMap populations, and for 12 unrelated, anonymous Bedouin DNA samples. For every run of two or more consecutive homozygous SNP sites in an individual, the adjusted lod scores were summed and a homozygous tract was defined when the score reached 20. Using this threshold, the median number of tracts per subject ranged from 22-66 in the 5 population groups, with median lengths of 567kb to 893kb and SNP density within tracts of 1 per 5.0kb to 6.0kb. Bedouin subjects had a significantly greater proportion of their genome within homozygous tracts (median 2.5%) compared to the other populations (0.6% to 1.0%). Among HapMap populations, 3 JPT subjects and one CEU subject had significantly greater homozygous tracts (2.9% or higher), while 7 Bedouin subjects had this level of homozygosity. We are developing methods to identify genomic regions that are homozygous more frequently in cases than controls. If performed in isolated populations, particularly those with consanguinity, such methods may be a powerful strategy for mapping susceptibility genes for complex traits, especially autosomal recessive alleles.

Genome-wide linkage scan of blood pressure in the Beaver Dam Eye Study: confirmation of multiple loci and identification of novel loci. *C. Xing[1], B.E.K. Klein[2], R. Klein[2], K.E. Klein[2], S.K. Iyengar[1]* 1) Epidemiology & Biostatistics, Case Western Reserve Univ, Cleveland, OH; 2) Department of Ophthalmology and Visual Sciences, University of Wisconsin Medical School, Madison, WI.

Blood pressure, as a specific predictor of cardiovascular disease, may contribute to 50% of the global cardiovascular disease epidemic. By understanding the hypertension predisposing genes we may gain insights into novel pathophysiological mechanisms and potential therapeutic targets. The Beaver Dam Eye Study (BDES) is a population-based cohort study of age-related eye diseases, and therefore, it gives us a particular opportunity to study the genetic effect of blood pressure. In the current study we aim to identify the genetic regions / genes predisposing susceptibility to essential hypertension by genome-wide linkage scans. At the time of census 4,926 subjects between 43 and 86 years of age participated in the baseline examination from 1988 to 1990; 602 pedigrees containing at least two eligible participants were then constructed. We already performed a genome-wide scan for systolic blood pressure (SBP) and diastolic blood pressure (DBP) employing the Haseman-Elson regression approach on 1049 sib pairs with the 10-centiMorgan density microsatellite markers map, and identified 15 regions with linkage signals, including novel evidence at 1q24, 2q35, 4q32, 8p21, 10q26, 13q13, and 16p12 (P 0.01) and replications at 2p25, 3q26, 4p15, 8q24, 15q26, 16q12, 17q25, and 20q11 (P 0.05). Currently the Center for Inherited Disease Research is genome-widely genotyping dense single nucleotide polymorphisms (SNPs). We anticipate that with more accurate inheritance information we can localize the hypertension disposing genes by both linkage and association approaches, the results of which will be presented at the meeting.

Anonychia-Onychodystrophy with Hipoplasia or Absence of Distal Phalanges (Cooks Syndome). Report of the first case in México. *E.J. Ramirez-Lizardo[1,2], N.O. Dávalos-Rodríguez[3], S.E. Totsuka-Sutto[1], E.G. Cardona-Muñoz[4]* 1) Instituto de Genética Humana Dr. Enrique Corona Rivera CUCS, Universidad de Guadalajara,Guadalajara,Jalisco, México, elizardo@cucs.udg.mx; 2) Departamento de Genética, Instituto Jaliscience de Cirugía Reconstructiva, Secretaria de Salud Jalisco; 3) Dpto. Genética, Instituto de Seguridad y Servicios Sociales de los Trabajadores del Estado; 4) Unidad de Investigación Cardiovascular, CUCS, Universidad de Guadalajara, Guadalajara, Jalisco México.

The anonychia is defined as the absence of all or part of one or several nails. Anonychia-onychodystrophy with hipoplasia or absence of distal phalanges (OMIM % 106995) or Cooks syndrome is a rare disorder autosomal dominant described in 11 cases in the literature. It is characterized by onycodystrophy, anonychia, brachydactyly of the fifth finger and digitalization of the thumbs, with absence or hypoplasia of the distal phalanges of the hands and feet. The proposita, 6 month old, was born after a 40-week gestation. She was the result of the first pregnancy of healthy and unrelated parents. The father was 17 and the mother was 18 years of age at the time of birth, the history family reveled similar clinical findings in her father. At physical examination showed normal weight and heigh, flat face with prominent eyes, small nose, depressed nasal bridge, high and narrow palate, low-set ears, short neck. Limbs were normal with normal finger and fingernails in hands, on feet bilateral anonychia on 4th toes and bilateral dystrophic toenails on 3rd and 5th toes. The radiological examination was normal in our case and her father showed hypoplasia of the distal phalanges of the feet. The present case show a family with anonychia-onychodystrophy support on the clinical date of partial anonychia and onychodystrophy in the proposita and hypoplasia of the distal phalanges of the feet in her father, this is a condition with autosomal dominant inheritance by the male-to-male transmission observed in 2 families, one reported by Cooks in 1985 and the other by Nevi in 1995 with seven and four affected individuals respectively. To our knowledge this is the first case in Mexico.

Discontinuities and unsynapsed regions in meiotic chromosomes have a trans effect on meiotic recombination of some chromosomes in normal human males. *F. Sun[1, 2], M. Oliver-Bonet[1, 2], T. Liehr[3], H. Starke[3], E. Ko[2], A. Rademaker[4], R.H. Martin[1, 2]* 1) Medical Genetics, University of Calgary, Calgary, Canada; 2) Genetics, Alberta Children's Hospital, Calgary, Canada; 3) Institute of Human Genetics and Anthropology, Jena, Germany; 4) Preventive Medicine, Northwestern University Medical School, Chicago, USA.

During meiosis, homologous chromosome pairing is essential for subsequent meiotic recombination (crossover). Discontinuous chromosome regions (gaps) or unsynapsed chromosome regions (splits) in the synaptonemal complex (SC) indicate anomalies in chromosome synapsis. Gaps and splits were most frequently observed in the heterochromatic regions of chromosome 9, and we have previously demonstrated that gaps and splits significantly altered the distribution of MLH1 recombination foci on the SC 9. Here, recently-developed immunofluorescence techniques (using antibodies against SC proteins and the crossover-associated MLH1 protein) were combined with fluorescence in situ hybridization (using centromere-specific DNA probes) to examine the effect of gaps/splits on meiotic recombination patterns in chromosomes other than chromosome 9 during the pachytene stage of meiotic prophase from three normal human males. Gaps and splits significantly altered the number of MLH1 foci on some SCs. In 64 analyzed cells with a gapped SC 9, the frequency of MLH1 foci in SCs 5 and 10 and in SC arms 10q, 11p and 16q was decreased compared to 168 cells analyzed in controls. In 24 analyzed cells with splits (unsynapsed regions) in SC 9, there was a significant reduction in MLH1 focus frequency for SC 10q and the whole bivalent. The positioning of MLH1 foci in other SCs in cells with gapped/split SC 9 was not altered. These studies suggest that gaps and splits not only have a cis effect, but can also have a trans effect on meiotic recombination distribution in humans.

Familial high myopia in a Polish population. *M. Rydzanicz[1], A. Frajdenberg[2], M. Podfigurna-Musielak[3], S.M. Leal[4], K. Pecold[2], B.A. Bejjani[5,6], M. Gajecka[1,5]* 1) Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland; 2) Department of Ophthalmology, Marcinkowski University of Medical Sciences, Poznan, Poland; 3) Department of Ophthalmology, Leszno Hospital, Leszno, Poland; 4) Baylor College of Medicine, Houston, TX; 5) Washington State University, Spokane, WA; 6) Sacred Heart Medical Center, Spokane, WA.

Myopia is the most common of all ocular conditions. Although high myopia (myopia in excess of - 6.0 diopters [D]) is far rarer than mild/moderate myopia, the importance of high myopia is significant because the development of high myopia involves anterior-posterior enlargement of the eye, abnormal changes in the eye and frequent detachment of the retina. The etiology of myopia is not known. Both genetic and environmental factors seem to play a role. In high myopia, genetic factors appear to play a predominant one. To date, we have examined, collected blood and purified DNA from 199 individuals from 32 unrelated high myopia families in Poland. The preliminary genotyping was conducted in 23 families. Prior to proceeding with the targeting genotyping, linkage to markers for Stickler syndrome types I, II and III, Marfan syndrome and juvenile glaucoma were tested. Next, we examined previously proposed familial high myopia loci as a first step before embarking on a genome-wide screen aimed at mapping and cloning gene(s) responsible for high myopia in these Polish families. Genotyping of high-myopia-associated loci [18p11.31 (MYP2), 12q21-23 (MYP3), 7q36 (MYP4), 17q21-23 (MYP5), 2q37, 4q22-4q27 (MYP11), and 10q21.1 were performed. Additionally we performed genotyping to test the linkage for mild/moderate myopia established in Ashkenazi Jewish families [22q12 (MYP6] and to the *PAX6* gene region reported for dizygotic twins [11p13 (MYP7)]. Linkage to Stickler syndrome, Marfan syndrome and juvenile glaucoma loci were excluded. Genotyping with well-spaced polymorphic markers of high myopia associated loci revealed no evidence of linkage to any of the candidate genes. A genome-wide screen is in progress.

OMOSDYSPLASIA: REPORT OF A CASE IN SIBS AND REVIEW OF THE LITERATURE. *N.L.M. Sobreira, A.B.A. Perez, M.C.P. Cernach, D. Brunoni* Centro de Genética Médica, UNIFESP-EPM, São Paulo, São Paulo, Brazil.

Omodysplasia is a skeletal dysplasia with neonatal manifestation, characterized by severe micromelia with shortening of the humerus and femur, radioulnar diastasis of the elbows and facial characteristics. The limb shortening is mainly rhizomelic and involves both the upper and lower limbs. Less common characteristics include NPMDR, congenital cardiopathy and neonatal death. Less than 20 cases are described in the literature and, based on the multiple cases of affected siblings and on the high frequency of consanguinity, there is little doubt about its autosomal recessive inheritance. We report the case of two siblings, the children of young and consanguineous parents. The first case is a 7-year-old girl; her weight was 2400g at birth, when the rhizomelic shortening of her limbs was noted and the diagnosis of achondroplasia was suggested. The patients face displays no phenotypic deviations, she presents rhizomelic shortening of the limbs with extension limitation of elbows and knees, her hands and feet are normal, NPMD is normal, her height is 89.2cm (P2)her upper/lower segment ratio is 1.87 (VR-1.06), span 75cm, CP is 49cm (P2), and the echocardiogram showed persistence of the arterial channel and dilation of the pulmonary stem. The second case is a 5-year-old boy, who had rhizomelic shortening of the limbs noted at birth, and the diagnosis of achondroplasia was suggested. He does not display facial phenotypic deviations, but he has rhizomelic shortening of the limbs with extension limitation of elbows and knees, normal hands and feet, normal NPMD, height of 91.5cm (P2), an upper/lower segment ratio of 1.40 (VR-1.19), span of 67cm, CP of 49cm (P2), and the echocardiogram showed a bicuspid aortic valve. The X-ray study of both patients showed a shortening of the humerus, radioulnar diastasis at the level of the elbows, shortening of radius and ulna, shortening of the femur with a rounded and deviated proximal portion (clubbing). These clinical-radiological characteristics are typical of omodysplasia, and our cases reinforce the autosomal recessive inheritance pattern, whose typical presentation is the involvement of the upper and lower limbs.

Genetics of obesity and its dietary interactions. *L. Perusse* Dept. Preventive Medicine, Laval University, Ste Foy, PQ, Canada.

The yearly updates of the obesity gene map published over the past 10 years provide a good indication of the progress accomplished in the past decade in the search for genes and DNA sequence variations associated with human obesity. According to the most recent version of the map, a total of 426 studies involving 127 different candidate genes have reported positive associations with obesity phenotypes. Despite the large number of genes potentially influencing obesity, only a small fraction of them have been the object of replications in at least five studies. The difficulty to replicate findings of association studies in obesity could be partly explained by the failure of most studies to consider the presence of gene-environment interactions in determining the risk of obesity. This presentation will review the role of genetic factors in obesity and provide an overview of the scientific evidence supporting the key role of gene-diet interactions in the susceptibility to obesity and related metabolic complications. A review of the genes involved in interactive effects with dietary factors will also be presented. Finally, the implications of nutrigenetics in the prevention and treatment of obesity will be discussed.

Genetic Variants in ADIPOR2 are Strongly Associated with Triglyceride and Adiponectin levels in Mexican Americans. *D.K. Richardson[1], J. Schneider[2], J. Blangero[2], M.P. Stern[1], R.A. DeFronzo[1], R. Duggirala[2], C.P. Jenkinson[1]* 1) Department of Medicine, University of Texas Health Science Center at San Antonio, TX; 2) Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX.

Adiponectin is an adipocyte-derived hormone implicated in energy homeostasis, and insulin resistance. Two receptors for adiponectin have been identified (ADIPOR1 and ADIPOR2) both of which are abundantly expressed in skeletal muscle and liver. We genotyped single nucleotide polymorphisms (SNPs) in the ADIPOR2 gene in Mexican American subjects (N=439) from the San Antonio Family Diabetes Study, and performed association analysis of insulin resistance syndrome-related traits. Of 38 SNPs genotyped, 24 were polymorphic in this population. The average proportion of shared variation between SNPs, measured by the pairwise correlation was 0.31 (range 0-0.99). Of these 24 SNPs, 14 were significantly associated with fasting plasma triglyceride and 10 with adiponectin levels ($P<0.05$). Most minor alleles were associated with decreased triglyceride and increased adiponectin levels. Notably, 4 SNPs (rs10848569, rs929434, rs3809266 and rs12342) were in high pairwise linkage disequilibrium ($r2=0.99$) and were strongly associated with triglyceride levels ($P=0.00029$, 0.00016, 0.00027 and 0.00021). After adjusting for the effects of BMI and HOMA-%S on triglyceride concentrations, the association significance increased to $P=0.000060$ for SNP rs929434. Bayesian Quantitative Trait Nucleotide analysis was used to examine all possible models of gene action. SNP rs929434 provided the strongest statistical evidence of an effect on triglyceride concentrations. Haplotype information did not improve the association. The ADIPOR2 locus is close to chromosome 12p13.31 where we reported linkage of triglyceride levels. Linkage analysis conditional on rs929434 decreased the LOD score from 1.85 to 0.98. Thus, 47% of the evidence for linkage, at 12p13.31, was explained by rs929434. In summary, these results provide strong evidence for association of several genetic variants in ADIPOR2 with triglyceride and adiponectin levels in Mexican Americans.

Narrowing in on the genotype-phenotype correlation in 1q terminal deletion syndrome. *N. Scribanu[1], J. Meck[2], C. Kozma[1], J. Blancato[3], L. Matyakhina[2]* 1) Pediatrics, Georgetown University Children's Medical Center, Washington, DC; 2) Obstetrics & Gynecology, Georgetown University Hospital, Washington, DC; 3) Oncology, Georgetown University Medical Center, Washington, DC.

To date, 30 cases of 1q terminal deletion have been reported, the majority of which are deleted from 1q42 or q43 to the terminus (q44). G-banding of lymphocyte chromosomes from a 2-month-old female patient demonstrated an apparent terminal deletion at 1q44. The abnormal chromosome 1 had a significantly diminished signal using a 1q subtelomere FISH probe (Vysis). The remainder of that signal was not noted on any other chromosome, ruling out translocation of the 1q material. This probe is within 300 kb of 1q terminus suggesting that the deletion of 1q is confined to only a portion of 1q44. The baby was delivered at term by C-section to a 35-year-old G1P1 following a pregnancy complicated by IUGR. All growth parameters were below the 5th percentile. Multiple congenital abnormalities were noted including brachycephaly, retromicrognathia, a single umbilical artery, posterior cleft palate, congenital heart defects, bilateral postaxial polydactyly of the lower extremities, and severe unilateral calcaneovalgus deformity. Also noted were a unilateral single transverse palmar crease, mild bilateral clinodactyly, and index finger overlapping the third finger. The baby had symmetrical failure to thrive with increased extensor tone, exaggerated DTR, a high-pitched cry, and mottled skin. Brain MRI revealed pachygyria. To better characterize this chromosome abnormality, a complete subtelome FISH panel is being performed because of the possibility that a portion of another subtelomere has been translocated to 1q terminus. BAC mapping to refine the breakpoint and assist in regional gene identification are in progress. Furthermore, to determine de novo vs. inherited status of the structural abnormality, parental karyotyping and FISH are planned. The phenotype of our patient is strikingly similar to that of patients with larger 1q deletions. Based on our FISH findings and reference databases, it appears that the phenotype of terminal 1q deletion is primarily due to haploinsufficiency of fewer than 8 genes.

Exploring the Reversibility of the Smith-Magenis Syndrome (SMS) Phenotype. *K. Walz[1], J. Molina[1], A. Cardenas[1], P. Carmona[1], J.I. Young[1], J.R. Lupski[2]* 1) Centro de Estudios Cientificos, Valdivia, X Region, Chile; 2) Human Molecular Genetics, Baylor College of Medicine, Houston, Texas, USA.

SMS is a contiguous gene syndrome (CGS) associated with a microdeletion of ~4 Mb within chromosome 17 band p11.2. The clinical phenotype includes craniofacial abnormalities, brachydactyly, self injurious behavior, sleep abnormalities, seizures and mental retardation. Mutations in RAI1 (one of 19 genes present in the deleted region) have been identified as causative of SMS, and seem to be responsible for most of the phenotypes present in SMS. Human chromosome 17p11.2 is syntenic to the 32-34 cM region of murine chromosome 11. By chromosome engineering we have generated a mouse model for SMS, Df(11)17/+, that carries a 3 Mb deletion and presents craniofacial abnormalities, seizures, obesity, alterations in circadian rhythm and hypoactivity. The objective of this work is to restore the normal gene dosage of Rai1 at an appropriate time to study if it is possible to prevent the development of at least some of the phenotypes seen in the mouse model for SMS. For this purpose we are generating mice that bear an inducible wild-type Rai1 allele in a mutant Df(11)17/+ 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 will then cross the Rai1 transgenic mice with those expressing tTA under Rai1 promoter regulation. For this purpose we have generated a modified BAC, by recombineering, expressing the tetracycline-inhibitable transcription factor (tTA) under the control of Rai1 specific promoter sequences (BAC-tTA). Both transgenes, EGFP-pBi-Rai1-HA and BAC-tTA are being injected, and double transgenic mice will be mated in the Df(11)17/+ mice genetic background to be analyzed. (Supported by NIH FIRCA TW007536-01 to KW -and FONDECYT No. 1061067, Chile to KW).

Practical solution for population stratification in genome-wide association scans and targeted studies. *A.L. Price[1,2], N.J. Patterson[2], R.M. Plenge[2,3], M.E. Weinblatt[3], N.A. Shadick[3], J. Butler[1,4], C.D. Campbell[1,4], A. Ramos[1,4], A. Ruiz-Linares[5], J.N. Hirschhorn[1,2,4], D. Reich[1,2]* 1) Harvard Medical School; 2) Broad Institute of MIT & Harvard; 3) Brigham & Women's Hospital, Boston; 4) Children's Hospital, Boston; 5) University College London.

Population stratification-allele frequency differences between cases and controls due to systematic ancestry differences-can cause spurious associations in disease association studies. Stratification is a concern both in genome-wide scans of hundreds of thousands of markers, and in targeted studies of selected markers. Here, we introduce a new method and software to correct for stratification and describe a new resource, 160 markers highly informative for within-Europe ancestry, which can be used to correct for stratification in targeted studies on European Americans. Our method, EIGENSTRAT, uses principal components analysis to explicitly model ancestry differences between cases and controls. This approach has significant advantages over Genomic Control, whose uniform correction for all markers is insufficient at markers that are unusually differentiated in frequency across populations, and Structured Association, which runs too slowly to be practical on large data sets. Furthermore, simulations show that EIGENSTRAT retains higher power to detect true associations than either of those methods. The method provides an effective correction for stratification in genome-wide scans, as we demonstrate on a real data set of 488 European American samples.

Correcting for stratification is an even greater challenge in targeted studies, due to the limited number of markers for inferring ancestry. A solution is to genotype additional ancestry informative markers. We designed a set of 160 markers highly informative for within-Europe ancestry, which we further validated using 300 samples from Italy, Spain, Sweden, England and Poland. We genotyped these markers in 368 European American samples discordant for the height phenotype, in which stratification had previously been shown to cause a spurious association. Using these markers, EIGENSTRAT successfully corrects for stratification.

HNF-1 mutation search in families of the Polish Nationwide Registry of MODY. *J. Skupien[1], M.T. Malecki[1], T. Klupa[1], S. Gorczynska-Kosiorz[2], D.K. Moczulski[2], J. Sieradzki[1]* 1) Department and Chair of Metabolic Diseases, Jagiellonian University Medical College, Krakow, Poland; 2) Department of Internal Medicine, Diabetology and Nephrology, Silesian School of Medicine, Zabrze, Poland.

Introduction: Maturity-onset diabetes of the young (MODY) is an autosomal dominant form of diabetes characterised by beta-cell defect and early age of diagnosis. The most frequent MODY3 subtype is caused by mutations in the HNF-1 gene. **Aim:** To determine the frequency of diabetes due to mutations in the HNF-1 gene in MODY families from the Polish Nationwide Registry and to provide its clinical characteristics. **Methods:** We identified 37 families with the early onset, autosomal dominant form of diabetes that meet the criteria of MODY. The 10 exons and promoter region of the gene were screened for sequence differences by direct sequencing of DNA from the probands of these families. **Results:** So far, during a screening of probands from MODY families of Polish origin, we identified twelve mutations. They segregated with diabetes in the families where they were identified. In eight probands, previously described mutations were found. Among them there were three missense mutations (R131Q, R271W, and P447L), three were frameshift mutations (S225fsdelC, P291fsinsC, and P378fsdelT), and one was the cryptic splice acceptor site mutation IVS7nt-6G>A resulting in skipping exon 7 and the premature termination of translation. In addition, we found one previously reported nonsense mutation R171X. Moreover, we found four novel mutations. There were two missense mutations (S249P and N257T). In another proband, we identified a splice mutation IVS2nt+1G>A. Interestingly, another novel mutation was a duplication of a 7 base pair motif (GGGTTGG) in the 5-UTR of the gene, between -164 and -165 base pairs upstream from ATG. This family was characterized by a severe phenotype with no response to sulphonylurea in some mutation carriers. **Conclusion:** MODY3 constitutes about one third of the Polish Nationwide Registry. In addition, the Pro291fsinsC mutation was encountered only once in our group, while it is the most frequent mutation, constituting a hot-spot in other well characterised European and Asian populations.

Classical Sotos syndrome with a Mutation in the NSD1 gene in a Two Generation Family. *AhmadS. Teebi, ReganE. Klatt, Tawfeg. Ben-Omran* Division of Clinical and Metabolic Genetics, Hospital for Sick Children, University of Toronto, Toronto, Canada University of Toronto,, toronto, on, Canada.

Sotos syndrome (OMIM#117550) is a well recognized overgrowth syndrome characterized by a typical facial gestalt, pre- and post-natal excessive growth including macrocephaly, advanced bone age, and variable degrees of developmental delays. Other clinical manifestations include neonatal jaundice, seizures, scoliosis, strabismus, conductive hearing loss, congenital heart disease, renal anomalies, and behavioral problems. Mutations and deletions of NSD1 (the nuclear-receptor-binding SET-domain-containing protein 1) at 5q35 are associated with Sotos syndrome. A few cases of parent-to-child transmission of Sotos syndrome have been published so far with only 15 familial cases have documented NSD1 mutations. Here, we describe the clinical and molecular findings in a family (father and 2 children) with autosomal dominant segregation of classical Sotos syndrome. This family will further contribute to our understanding of genotype-phenotype correlation and the scope of intrafamilial variability in Sotos syndrome. Familial examples offer more precise genetic counseling including risk figures and long term prognosis.

CHARGE syndrome: genotype-phenotype correlations and mutational hot-spots at the CHD7 locus. *D. Sanlaville[1,2], S. Audollent[1], G. Goudefroye[1], J. Amiel[1], V. Abadie[3], C. Baumann[4], H. Dollfus[5], D. Genevieve[1], C. Thauvin[6], S. Marlin[7], A. Toutain[8], C. Cruaud[9], H. Etchevers[1], J. Weissenbach[9], A. Munnich[1], T. Attié-Bitach[1], S. Lyonnet[1]* 1) Dept de Génétique and INSERM U781 , Hôpital Necker Enfants Malades, Paris, France; 2) Service de Cytogénétique, Hospices Civils de Lyon, Hôpital Edouard Herriot, Lyon; 3) Service de Pédiatrie générale, Hôpital Necker-Enfants Malades, Paris; 4) Service de Génétique, Hôpital Robert Debré, Paris; 5) Service de Génétique, Hôpital de Hautepierre, Strasbourg; 6) Centre de Génétique, Hôpital d'Enfants, Dijon; 7) Service de Génétique, Hôpital Trousseau, Paris; 8) Service de Génétique, Hôpital Bretonneau, Tours; 9) Genoscope and CNRS UMR8030, Evry, France.

Introduction: CHARGE syndrome is a dominantly inherited multiple congenital anomalies (MCA) syndrome defined by ocular coloboma (C), heart disease (H), choanal atresia (A), retarded growth and/or anomalies of the central nervous system (R), genito-urinary defects and/or hypogonadism (G), ear anomalies and/or deafness (E). Recently, *CHD7* gene mutations have been identified in 55 to 60 % of CHARGE patients. **Methods:** We studied, by direct sequencing, a series of 164 cases in whom the diagnosis was suspected based on the criteria proposed by Pagon or Blake. **Results:** The diagnosis was confirmed in 91 cases (55 %) by the identification of a *CHD7* heterozygous mutation. We identified 29 (32 %) stop codon mutations, 38 (42 %) frameshift mutations leading a premature codon stop, 19 splice site mutations (18 %) and 8 missense mutations (9 %). More half of mutations were identified in 6/38 exons (exons 2, 10, 21, 26, and 36), whereas no mutation could be identified in 11/38 of the 38 exons of *CHD7* gene. **Discussion:** Our data support that almost only truncation mutation at the CHD7 locus result in full-blown CHARGE syndrome. However, some apparently milder missense mutations were observed in milder cases, sometimes with dominant inheritance and variable expression suggesting a careful genetic counselling. Interestingly 60 % of mutations were localized in only 6/38 exons. Our results allowed us to propose a strategy to screen mutations in *CHD7* gene.

The Development of Genomic SELEX for the Identification of Direct Transcriptional Targets of Pax3, FKHR and the Oncogenic Fusion Protein Pax3-FKHR. *A. Sidhu, K.E. Johanson, R.J. Scioneaux, A.D. Hollenbach*
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Alveolar rhabdomyosarcoma (ARMS, MIM 268220) is a soft tissue tumour, arising primarily in the trunk and extremities of adolescents and young adults. The four year overall survival rate is less than 30% for ARMS, with a lower incidence of survival in patients with metastases and bone marrow involvement. ARMS is an aggressive malignancy most commonly characterized by a t(2;13) (q35;q14) chromosomal translocation, which results in the fusion of two transcription factors important for myogenesis, Pax3 and FKHR. The resulting oncogenic fusion protein, Pax3-FKHR, is a potent transcriptional activator and functions as a dominant acting oncogene. Thus, screening for target genes of Pax3, Pax3-FKHR, and FKHR would be of great significance in determining the molecular basis of this disease and its clinical outcome. Therefore, the long term goal of this work is to identify the direct transcriptional targets of Pax3 and FKHR and to determine how gene expression differs in the presence of Pax3-FKHR. We will address this goal with the development of a Genomic SELEX technique, through a modification of the standard SELEX, in order to identify promoter elements directly bound and regulated by Pax3 and FKHR. We will then confirm, identify, and evaluate the promoter elements that are bound by Pax3 and FKHR. Lastly, we will determine how Pax3-FKHR alters the expression of the identified genes in the physiologically relevant mouse primary myoblasts. The identification of physiologically relevant genes whose expression is altered by the presence of Pax3-FKHR will allow us to elucidate the cellular pathways that contribute to ARMS tumor progression. The knowledge of these aberrantly expressed genes will provide us with critical information that will allow the development of novel therapies for the treatment of ARMS.

Increased *Hprt* deletions in *Blm* hypomorphic mice *in vivo* are mediated by illegitimate recombination. *I.V. Tereshchenko[1], Y. Chen[1], L.D. McDaniel[2], R.A. Schultz[2], J.A. Tischfield[1], C. Shao[1]* 1) Genetics Department, LSB, Rutgers University, Piscataway, NJ; 2) University of Texas Southwestern Medical Center at Dallas, Dallas, TX.

BS (Blooms syndrome) is an autosomal recessive disease with the phenotype of growth retardation, sunlight sensitivity and a predisposition to cancer. BS is caused by mutations in BLM, a RecQ DNA helicases that has both DNA-stimulated ATPase and ATP-dependent DNA helicase activities. Homozygous null Blm mutation is embryonic lethal in the mouse. To study the role of BLM in the maintenance of genomic integrity in vivo, we characterized the spontaneous Hprt mutations in T cells and fibroblasts in Blm hypomorphic mice. The Blm hypomorphic mice carry a null allele (L) and a hypomorphic allele containing insertion of an extra exon (T). The Hprt mutants were detected as cell colonies resistant to 6-thioguanine (6-TG). We found that the frequency of Hprt mutants was increased about ten fold in T cells of Blm hypomorphic mice (L/T) when compared to T/+ mice, but to a lesser extent in fibroblasts. Multiplex PCR of Hprt exons indicates that many of the mutations were caused by deletions of one or more exons. Sequencing analysis of break junctions revealed that deletions were flanked by short stretches of homologous sequences, indicating that illegitimate recombination is responsible for the deletions. Thus, in addition to increased chromosomal instability and sister chromatid exchanges, deletions from illegitimate recombination are also elevated when BLM is reduced.

Generation and characterization of Elovl4 Y270X knockin mouse. *Z. Tong, D. Gibbs, S. Kamaya, H. Chen, Z. Yang, C. Wang, D.J. Cameron, Y. Chen, A. Praggastis, E. Pearson, K. Howes, K. Zhang* Moran Eye Center, Department of Ophthalmology, University of Utah, Salt Lake City, UT. 84112.

Purpose: ELOVL4 was first identified as a disease-causing gene in Stargardt macular dystrophy. To date, three ELOVL4 mutations have been identified including Y270X, all of which result in truncated proteins. However, the specific role of ELOVL4 in photoreceptors and the degenerative events caused by dominant ELOVL4 mutations are not well understood. The purpose of this study is to generate Y270X knockin mice which will provide a mouse model to examine the dominant pathogenic mode of degeneration in human STGD3 patients. Methods: A mouse Elovl4 knockin targeting construct was generated containing a neo cassette flanked by loxP sites, and a Y270X mutation and HA tagged Elovl4 fragment introduced into exon 6. For gene targeting, the targeting vector was electroporated into mouse embryonic stem (ES) cells derived from 129. Clones in which homologous recombination resulted in targeted replacement of exon 6 were identified by PCR and by Southern blotting. Chimeric mice will be bred with wild type C57Bl/6 mice for germline transmission of the recombinant Elovl4 gene. Results: Twenty-one positive recombinant ES cell clones have been identified. Injections have yielded eight chimeric mice so far. Chimeric mice are currently being mated with C57Bl/6 mice to generate heterozygous knockin mouse. Conclusions: We have generated chimeric mice which will be used to generate the Elovl4 Y270X knockin mutation. Characterization of retinal phenotypes associated with Y270X knockin mutation will be presented. These mice will provide an important animal model for examining autosomal dominant macular degeneration in STGD patients.

Myotonic Dystrophy 1: CUG repeats alter phosphorylation-dependent functions of CUG triplet repeat binding protein, CUGBP1. *L.T. Timchenko[1], C.H. Huichalaf[1], K. Sakai[1], H. Nguyen[1], E. Salisbury[2], N.A. Timchenko[2]* 1) Medicine, Section of Cardiovascular Sciences, Baylor College of Medicine, Houston, TX; 2) Huffington Center on Aging and Department of Pathology, Baylor College of Medicine, Houston, TX.

Myotonic Dystrophy 1 (DM1) is a multisystemic disease associated with skeletal muscle weakness, progressive wasting and myotonia. The most severe and deadly form of disease, congenital DM1, is characterized by a delay of muscle development and differentiation. DM1 is caused by an expansion of polymorphic CTG repeats in the 3 UTR in DMPK gene. Major symptoms of DM1 are mediated by the mutant RNA CUG repeats which affect two RNA-binding proteins, CUGBP1 and MBNL. In DM1 patients, CUGBP1 levels are increased due to stabilization of CUGBP1 by CUG repeats. Examination of animal models showed that the unscheduled elevation of CUGBP1 is toxic and might be a major cause of DM1 pathology. In transgenic mice, the elevation of CUGBP1 results in a significant delay of muscle development and myofiber fusion. Our data and observations from other labs suggest that CUGBP1 plays a significant role in several aspects of RNA metabolism including splicing, translation and mRNA stability. Such diverse functions of CUGBP1 in RNA metabolism suggest that CUGBP1 might regulate a large number of mRNAs. We have found that the RNA binding activity of CUGBP1 and protein-protein interactions of CUGBP1 are regulated by specific phosphorylation at different residues within CUGBP1 molecule. In normal cells, biological activities of CUGBP1 are differently regulated in proliferating and in differentiating muscle cells. Examination of phosphorylation status of CUGBP1 in DM1 muscle cells supports hypothesis that, in addition to elevation of CUGBP1, CUG repeats also change signal transduction pathways which phosphorylate CUGBP1. We suggest that a synergistic effect of these alterations dramatically changes processing of multiple mRNAs in DM1 tissues.

3D morphometric analysis of the craniofacial complex in first degree relatives of individuals with orofacial clefts.
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Numerous studies have described altered patterns of craniofacial form in the unaffected relatives (URs) of those with oral clefts. Results from these studies have been highly variable and have failed to provide a reliable method for discriminating at-risk relatives from controls. In the present study, we compare craniofacial shape between a sample of URs (33 females; 14 males) from CL/P multiplex families and an equal number of age/sex matched controls. A total of 16 x,y,z facial coordinates derived from 3D photogrammetry were analyzed via Euclidean Distance Matrix Analysis (EDMA), while 14 additional linear distances from direct anthropometry were analyzed via t-tests. Variables identified as significantly different (p .10 from EDMA; .05 from t-tests) were then entered into a discriminant function analysis. All analyses were carried out for each sex separately. Female URs demonstrated increased facial width, midface reduction and lateral displacement of the alar cartilage. A single discriminant function was derived (canonical correlation = .43; p = .01) which correctly classified 70% of female URs and 73% of female controls. Male URs demonstrated increased facial and cranial base width, increased lower facial height and decreased upper facial height. Again, a single discriminant function was derived (canonical correlation = .79; p < .001) which correctly classified 86% of male URs and 93% of male controls. In both males and females, facial width contributed most to the discriminant function (loading > .60). These results suggest that the craniofacial shape differences characterizing URs are largely sex-specific and perhaps more pronounced in males. Importantly, the pattern of relative-control differences observed in both sexes is in broad agreement with previous findings from both humans and animal models. Although preliminary, these results suggest that a relatively simple phenotypic assessment may provide a tool for the identification of at-risk individuals within CL/P multiplex families. NIH grants R01-DE016148 and P50-DE016215.

High throughput mutation screen of human ion channel genes in episodic neurological disorders. *J.F. Poulin[1], R. Lafrenière[1], M. Simoneau[1], N. Gupta[1], F. Lafrenière[1], K. Boisvert[1], A. Anton[1], S. McLaughlan-Edwards[1], G. Ebers[2], Z. Cader[3], J. Sequeiros[4], J.M. Pereira Monteiro[5], G. Turecki[6], M. Alda[6], P. Grof[7], S. Chouinard[8], B. Brais[8], P. Cossette[8], G. A. Rouleau[1,8]* 1) Emerillon Therapeutics, Inc., Montreal, Quebec, Canada; 2) Wellcome Trust Centre for Human Genetics, Oxford, UK; 3) Oxford University, Oxford, UK; 4) UNIgene, IBMC, University of Porto, Porto, Portugal; 5) Hospital Santo António, Porto, Portugal; 6) McGill University, Montreal, Canada; 7) University of Ottawa, Ottawa, Canada; 8) Universite de Montreal, Montreal, Canada.

Common neurological conditions such as epilepsy and migraine are episodic disorders showing significant heritability. Mutations in several ion channel genes have been identified as the genetic basis of these disorders in some patients. Additional diseases such as bipolar disorder, essential tremor and Tourette syndrome share some aspects of episodic disorders, and may be caused by dysfunction of ion channel genes. In an effort to identify relevant drug targets for these neurological disorders, we have initiated a high throughput mutation detection screen of brain-expressed human ion channel genes. To date, we have screened 140 ion channel genes in a panel of 368 unrelated patient samples using dHPLC and have identified a total of 1266 different variants. Sequence variants are prioritized and genotyped in additional cohorts of patients and controls to determine allelic frequencies. Statistical analyses (Fishers Exact test, Hardy-Weinberg equilibrium test, etc) are then automatically performed on each genotyped variant to test any possible association with a particular disease. Infrequent variants are genotyped in extended pedigrees to test for co-segregation of the variant with affected family members. These genotype data allow us to better assess the possible relevance of these variants for each of the diseases studied. The most interesting candidate genes/variants will be presented, including preliminary biochemical validation data. Identification of genetic factors causing these neurological disorders will benefit patients and families through better molecular diagnosis and improved therapeutics.

Simulating association studies: a data-based resampling method for candidate regions or whole genome scans. *F. Zou[1], H. Huang[1], X. Guan[2], K. Gamiel[2], C. Jeffries[2,3], W.T. Barry[1], F. Pardo-Manuel[4], P.F. Sullivan[4], K.C. Wilhelmsen[4], F.A. Wright[1]* 1) Department of Biostatistics, University of North Carolina, Chapel Hill; 2) Renaissance Computing Institute,UNC Chapel Hill; 3) School of Pharmacy,UNC Chapel Hill; 4) Department of Genetics,UNC Chapel Hill.

Reductions in genotyping costs have heightened interest in performing whole genome association scans and in the fine mapping of candidate regions. Improvements in study design and analytic techniques will require the simulation of datasets with realistic patterns of linkage disequilibrium and allele frequencies for typed SNPs. We describe a general approach to simulate genotyped datasets for standard case-control or affected child trio data, by resampling from existing phased datasets. The approach allows for considerable flexibility in disease models, potentially involving a large number of interacting loci. The method is most applicable for diseases caused by common variants that have not been under strong selection, a class specifically targeted by the International HapMap project. Using Phase I/II HapMap CEU data as a testbed for our approach, we have implemented the approach in HAP-SAMPLE, a web-based simulation tool. With HAP-SAMPLE, we can perform simulations that effectively mimic data from whole-genome platforms. For example, 94.4% of the SNPs on the Affymetrix 100K platform are among the Phase I/II HapMap CEU data. Further, as an intermediate step, we developed a simple phasing approach suitable for genome-wide phasing of parent-child trios. Our phasing algorithm has competitively low error rates in comparison with the official HapMap phased version, and is so fast that the entire Phase II dataset can be phased in a few hours on a single PC.

CNS Tumors, Melanosis and More: A Lesson in the Value of Research and Longitudinal Genetic Counseling. *K. Zbuk, A. Shealy, C. Eng* Genomic Medicine Institute, Cleveland Clinic Foundation, Cleveland, OH.

We often counsel based on case reports or rare case series in uncommon syndromes. These reports are usually based on data obtained at a single snapshot in time. This case family illustrates the importance of longitudinal patient follow-up and participation in research. In 2003, the proband, a 12yo female, was referred for a posterior fossa paraganglioma (PGL). Her father and paternal uncle had hemangiomas (pedal and hepatic, respectively) and a paternal first cousin once-removed reported a brain tumor of unknown type. We offered *SDHD* and *VHL* gene testing; both were negative. Consequently, we requested further medical records on the relative's brain tumor. However, the family was lost to follow up until this year (2006). The patient was recently diagnosed with primary acquired melanosis of the conjunctiva and cornea and was referred back to our Center for Personalized Genetic Healthcare. Updated family history revealed that the brain tumor in the first cousin once-removed was reportedly a chordoma. While this could be a chordoma, chordomas could be histologically mistaken for PGLs. The patient is currently being tested for *SDHB* and *SDHC* mutations on a research protocol that additionally allows DNA to be stored for 20 years to be used in any relevant future research. We are now able to consider several new differential diagnoses. NF1 is a consideration given that the patient has 3 cafe-au-lait spots, as does the mother who also has axillary freckling, and the history of paragangliomas, melanosis, and cutaneous and liver hemangiomas. Posterior fossa neuroectodermal tumors are reminiscent of a syndrome characterized by *SNF5* mutations. Finally, if the chordoma was a misdiagnosed PGL, consideration is given to *SDHC*-related PGL syndrome, which is enriched for head and neck PGLs. While *SDHB* is in the differential, it is less likely than *SDHC* because the former is enriched for abdominal, extra-adrenal PGL. Since the chordoma was diagnosed in adulthood, tuberous sclerosis complex is unlikely. This case illustrates the value of following complicated families over time and aggresively utilizing research studies which ultimately enhance clinical care.

First report of two siblings with SMS due to maternal mosaicism. *A.C.M. Smith[1,2], B.A. Pletcher[3], J. Spilka[2], J. Blancato[2], J. Meck[2]* 1) SMS Research Unit, OCD/NHGRI/NIH, Bethesda, MD; 2) Georgetown Univ Medical Center, Washington, DC; 3) New Jersey Medical School, Newark, NJ.

Smith-Magenis syndrome (SMS) is a clinically recognized MCA/MR syndrome associated with interstitial deletion of chromosome 17p11.2 in the majority of cases. The deletion usually occurs de novo. Only one case of SMS with transmission from a partially affected mosaic mother has been reported (Zori et al.,1993). We report a 2nd family with SMS due to maternal mosaicism that, to our knowledge, represents the first report of SMS recurrence in siblings. Case Report: The 37y proband (P) is the youngest and his 54y sister (S) the oldest in a sibship of 9 that includes 2 miscarriages & 5 healthy siblings. Both carried a diagnosis of Down syndrome (DS) since infancy. Cytogenetic confirmation of DS was not pursued until adulthood, when the DS "diagnosis" was challenged. Both siblings were confirmed to have SMS (del 17p11.2). Parental karyotyping on lymphocytes confirmed normal chromosomes in the father; however, the mother was mosaic for the deletion [31/50 (62%) cells with del 17p11.2]. Record review and recent genetic evaluation of P and S confirm psychomotor and speech delays, moderate/severe MR, self-injurious and maladaptive behaviors, sleep disturbances, and many physical findings classically seen in SMS. S also has Type 2 diabetes, hypothyroidism, seizures, and bipolar disease. Their 80y mother in her youth was cognitively normal with a sociable personality and loved attention, but also had behavior and emotional problems (i.e., immaturity, poor self-esteem) with aggressive mood swings (bipolar); she is currently being evaluated for early signs of dementia. Summary: This family illustrates several points for discussion and genetic counseling: 1) Delays in diagnosis and suspicion of DS in infancy are common in SMS emphasizing the need to consider cytogenetic testing; 2) parental chromosome studies are indicated in all newly diagnosed cases of SMS; 3) parental somatic/germline mosaicism for del 17p11.2 may be more common than originally thought with implications for genetic counseling; and 4) del 17p11.2 mosaicism may be associated with a milder phenotype than classic SMS.

Evidence for Association of Interferon Regulatory Factor 2 (IRF2) with Systemic Lupus Erythematosus (SLE).

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SLE is a complex autoimmune disease characterized by dyrsregulation of both innate and adaptive immune responses. Using linkage analysis of intermediate phenotypes defined by autoantibody production in SLE multiplex families, we have mapped a locus related to anti-Ro/SSA and anti-La/SSB to chromosome 4q35.1. This region has been previously implicated through linkage and association studies in psoriasis, atopic dermatitis, multiple sclerosis, and certain clinical subsets of SLE patients with dermatological manifestations, suggesting the presence of a gene involved in multiple autoimmune diseases. Evaluation of potential candidate genes within the 4q35.1 region indicated that IRF2 is a strong candidate gene for SLE. Numerous lines of evidence suggest that pathways influenced by interferons (IFNs) are dysregulated in multiple autoimmune diseases, including SLE. IRF2 plays a repressive role in transcriptional regulation of IFN-inducible genes, and numerous functional studies are consistent with the hypothesis that altered function or expression levels of IRF2 may lead to dysregulation of IFN-inducible pathways as observed in SLE. In order to test for genetic association with SLE, we genotyped over 400 Caucasian SLE pedigrees and assessed association of 16 haplotype tag single nucleotide polymorphisms (SNPs) across IRF2 using family-based association (TDT and PDT) analysis (mean r2=0.6). We also genotyped a SNP previously reported to be associated with psoriasis and atopic dermatitis in our collection of 184 sib-pair and 185 trio SLE pedigrees for TDT and PDT analysis, as well as in 894 controls for a case-control study. We found significant evidence for association of six SNPs in IRF2 with SLE and in subsets of SLE patients with dermatological manifestations ($p<0.05$). Our studies support the hypothesis that IRF2 may predispose to SLE by altering transcriptional regulation of IFN-inducible genes and that disease-associated variants are enriched in patients with certain dermatological manifestations.

Differential influences on maximum likelihood (ACE-A) and intraclass correlation (IC-A) estimators of additive genetic variance from twin studies. *M.T. Stillitano[1], E. Byrne[2], C.J. Williams[3], J.C. Christian[1]* 1) Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN; 2) Trinity College Dublin; 3) Department of Statistics, University of Idaho, Moscow, ID.

In an attempt to define differences between the two estimators, simulation studies were carried out using 1,000 sets of 50 MZ and 50 DZ twins, with 0.55 additive genetic variance and 0.45 random environmental variance.

The ACE-A and IC-A estimators had a sample correlation of 0.83. In addition, regression analyses of the estimators showed that the total variance of DZ twins (DZTV) explained 13% of the variance in ACE-A estimates. The DZTV was negatively correlated with the IC-A estimator (r = -0.23) and positively correlated with the ACE-A estimator (r = 0.13), resulting in a differential effect on the two estimators.

Because the ACE-A model controls within pair DZ variance, as DZ variance increases there is an expected increase in the DZ covariance, which results in a decrease in the difference between MZ and DZ covariances and a decrease in the ACE-A value of the estimator. In contrast, in increasing DZTV, not influenced by control of the within DZ variance, when the IC-A is calculated results in a decrease in the DZ intraclass correlation, accounting for the differential effect on the ACE-A and IC-A estimates.

The twins were divided into three classes based upon DZTV standard deviation (SD); (MEDIUM = DZTV SD -0.5 to 0.5 from the mean; LOW = greater than 0.5 below the mean; HIGH = greater than 0.5 above the mean). The ACE-A estimator found 52, 54, and 56% significant results in the three classes (LOW, MEDIUM, and HIGH respectively), while the IC-A estimator found 62, 50, and 40% significant results, respectively. This indicates that control of the within pair DZ variance results in the ACE-A estimator being less influenced by random variation in the DZTV than the IC-A estimator.

The Mouse Genome Informatics Database (MGI): Biological data mining in the 21st century. *B.A. Richards-Smith, J.A. Blake, C.J. Bult, J. Kadin, M. Ringwald, J.T. Eppig* The Jackson Laboratory, Bar Harbor, ME.

The Mouse Genome Informatics Database (MGI, http://www.informatics.jax.org/) is a comprehensive, integrated information resource for the genetics, genomics and biology of the mouse. At the core of MGI are DNA and protein sequence data, descriptions of genes/gene products and their functions, orthologous gene relationships between mouse and other mammals, and genetic and physical mapping data. Integrated with this core are other data. Phenotypes are described for nearly 16,000 alleles, including spontaneous, mutagen-induced, targeted and conditional mutations, transgenes, and 2,900 QTL. Association of parallel mouse and human data aids genotype-to-phenotype comparisons and relates mouse mutants or QTL to human disease. An extensive and growing catalog of mouse SNPs can aid in mapping disease-associated mutations and localizing QTL. The database may be queried for gene-, tissue- or developmental stage-specific expression patterns.

MGI's utility has recently been enhanced by a number of additions and modifications. Perhaps most obvious is the representation of phenotype data; each phenotypic detail now is annotated to a term from the Mammalian Phenotype (MP) ontology, a hierarchically structured vocabulary. Online Mendelian Inheritance in Man (OMIM) is used to make similarity assertions between mouse models and human diseases. These and additional controlled vocabularies (ontologies) for anatomy, molecular functions, biological processes and cellular components (the latter three comprising the Gene Ontology, GO) permit querying the database simultaneously for any combination of these parameters. Such queries also may incorporate expression, sequence, polymorphism and/or mapping data. For example, one might ask for any gene of which there are mutations that cause deafness and whose product is associated with the plasma membrane and has signal transduction activity. To search for modifiers mapping to a particular genomic region, one might display SNPs in that region. Use of ontologies in annotating phenotypes, diseases, alleles and genes support data mining *via* such complex queries. Supported by NIH grants HG00330, HG02273, HD33745, CA89713.

Association of Innate Immune Response Candidate Genes in Primary Sjögrens Syndrome. *D.C. Patel, P.S. Ramos, S.M. Rao, E.S. Emamian, L.M. Tobon, T.W. Behrens, P.M. Gaffney, A.J.W. Huang, N.L. Rhodus, B. Segal, K.L. Moser*
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Sjögrens Syndrome (SS) is one of the most prevalent inflammatory autoimmune diseases, characterized by an infiltration and focal accumulation of lymphocytes in exocrine glands. The underlying etiology is thought to be complex, involving multiple genes and influenced by environmental factors. Microarray analysis of primary SS patients has shown dysregulation of expression levels for genes involved in several key biological pathways, including a prominent signature defined by genes known to be inducible by Type I interferons. In this study, we chose candidate genes related to pathways that are dysregulated in SS and tested them for genetic association using a case-control cohort. We evaluated interleukin 1 receptor antagonist (IL1RN), the 2-prime,5-prime oligoadenylate synthetase (OAS) cluster, Toll-like receptor 2 (TLR2) and 52-kD Ro/SSA (TRIM21/Ro52). Single nucleotide polymorphisms (SNPs) covering at least 80% of the total variation across each gene were genotyped in 98 Caucasian primary SS cases and 175 Caucasian controls. We found at least one variant in each candidate gene whose genotype frequencies were significantly different between cases and controls ($p < 0.05$). Association with TRIM21/Ro52 was found with a variant in the mRNA/UTR (p-value = 0.0004) and a second SNP in intron 2 ($p = 0.0027$). In the OAS cluster, we observed evidence of association with an upstream SNP ($p = 0.0176$) in OAS1, and an intronic SNP in OAS3 ($p = 0.0065$). We also observed evidence of association of a variant in the mRNA/UTR region of TLR2 ($p = 0.0013$) and a variant upstream of IL1RN ($p = 0.036$). Our studies support the hypothesis that dysregulated gene expression levels of these genes may be due to underlying genetic variants that are associated with primary SS. Further investigation of these genes for a role in this complex disease is warranted.

Linkage studies in autosomal dominant lateral temporal lobe epilepsy: searching for the alternative locus. *F.R. Torres, N.S. Ferreira, R. Secolin, M.C. Gonsales, E. Kobayashi, L.A.C. Sardinha, F. Cendes, I. Lopes - Cendes* Dept Medical Genetics, UNICAMP, Campinas, Brazil.

The objective of this study was to localize the major *locus* causing autosomal dominant lateral temporal lobe epilepsy (ADLTLE) in a kindred with no mutations in the *LGI1* gene and no linkage to chromosome (ch) 10q. We studied 23 affected individuals from a single family segregating ADLTLE. In addition, all 23 patients had high resolution MRI scans. As an initial strategy we chose the candidate gene approach. A total of 42 family members were genotyped for 15 polymorphic dinucleotide repeat markers flanking the *LGI2*, *LGI3*, *LGI4* and *MASS1* genes. Two-point lod scores (Z) were calculated using the LINKAGE package. Auditory auras were reported by 18 of 23 patients (78%). *Deja-vu* phenomena, associated to auditory auras, were reported by 13/18 (72%). Three individuals had only isolated frequent *deja-vu* episodes, and two individuals had only generalized tonic clonic seizures. MRI scans of all 23 patients in this kindred were normal and two-point lod scores were significantly negative ($Z < -2.00$) for all 15 markers tested. Most affected individuals in this kindred have *deja-vu* phenomena suggesting involvement of mesial aspects of temporal lobe structures as well. This clinical characteristic is not a common feature in ADLTLE families with mutations in the *LGI1 gene*. In addition, we found that mutations in the *LGI2*, *LGI3*, *LGI4* and *MASS1* genes are not causing ADLTLE in the family studied. A wide genome search is under way in order to identify the causative gene in this kindred.

Genetic Analysis of Osteoarthritis. *B.H. Reck[1], A. Philip[1], M. Chiano[2], U. Atif[3]* 1) Genetics Analysis, GlaxoSmithKline, RTP, NC; 2) Genetics Analysis, GlaxoSmithKline, UK; 3) Medical Genetics, GlaxoSmithKline, RTP, NC.

Osteoarthritis (OA) is the most common cause of musculoskeletal disability in most developed countries. The site that causes the greatest burden of disability in public health terms is the knee, which is estimated to result in pain and loss of function in 10% to 15% of men and women aged over 45 years. The etiology and pathogenesis of the condition, however, remain largely unknown. It is more common in and has been strongly associated with several environmental risk factors including obesity, previous injury, meniscectomy, and other physical and metabolic factors. Although environmental factors have traditionally been thought to be the main influences, there are few data to support this. Between 39% and 65% of Osteoarthritis in the general population can be attributed to genetic factors. Identification of the genes concerned could have a large impact on the disease in terms of prevention and new therapeutic approaches. GlaxoSmithKline has undertaken a series of genetic studies on Osteoarthritis which consist of a combination of linkage and association analysis on multiple OA phenotypes. An initial whole-genome scan identified broad chromosomal regions which were possibly linked to OA. A Linkage Refinement analysis performed in these regions produced two peaks, one on chromosome 9 and one on chromosome 18, meriting further investigation. An association analysis was performed on these regions using a dense set of SNP markers, and association was seen in these regions for a number of SNPs using different phenotypes. Although the high density association analysis suggests some genes which may be involved in OA susceptibility, a replication analysis that is currently being planned will be necessary to confirm these findings. We will describe this project from the original scan through the high density association analysis, focusing on the patient collection studies, OA radiographic phenotypes investigated and methods implemented. The objective of this study is to gain increased understanding of osteoarthritis, leading to the identification of novel susceptibility genes and drug target pathways.

Association study between asthma and candidate genes identified by microarrays. *K. Tremblay[1,2], M. Lemire[3], A. Montpetit[3], D. Daley[4], AJ. Sandford[4], PD. Paré[4], TJ. Hudson[3], C. Laprise[1,5]* 1) University of Montreal Community Genomic Medicine, Chicoutimi, Canada; 2) Laval University, Quebec, Canada; 3) McGill University and Genome Quebec Innovation Centre, Montreal, Canada; 4) University of British Columbia, Vancouver, Canada; 5) Université du Québec à Chicoutimi, Chicoutimi, Canada.

RATIONALE: Asthma is a heterogeneous complex trait. To date, knowledge about its genetic risk factors remain largely unknown. Microarrays allowed us to identify new or known genes relevant to asthma. We selected 9 differentially expressed genes (SFRP1, CX3CR1, IL2RB, IL7R, CXCL12, TNFRSF7, NOS2A, CD14, ALOX15) and 8 candidate genes based upon the biological plausibility of inflammation and immunity pathways (STAT6, MS4A2, IL1B, IL4, IL4R, IL6, IL13, IL15). **AIM**: To conduct a candidate gene association study in a founder population sample from Northeastern Quebec. **METHODS**: TagSNPs were selected from the HapMap database with minor allele frequency .10 and an r2.80 for each gene (mean of 10 tagSNPs/gene). Asthma was defined used the American Thoracic Societys criteria and 1139 individuals (n=260 families) were included. Genotyping was performed using Illumina SNP chip (AllerGen network). Genetic analyses were done using the TDT as implemented in the FBAT program. **RESULTS**: Additive and dominant models were used to test for association with asthma and related phenotypes. In the additive model, analyses showed positive association with IL13 only (asthma, p=0.007). In dominant model, analyses show positive associations for three common alleles of SFRP1 (IgE, p0.004), two common alleles of CX3CR1 (asthma and atopy, p0.002), two common alleles of IL2RB (atopy and airway hyperresponsiveness, p0.005), one minor allele of CXCL12 (IgE, p=0.009) and for one minor allele of IL13 (asthma, p=0.006). **CONCLUSIONS**: These results await further replication studies and refined statistics. Indeed, we currently working on haplotype analyses and models that allow for gene-gene and gene-environment interactions. Nevertheless, our preliminary results show a potential benefit to use microarrays in the selection of candidate genes for asthma association studies.

Development of Clinical Molecular Genetics Testing for Primary Ciliary Dyskinesia. *M.A. Zariwala, M. Langley, M.W. Leigh, J. Booker, M.R. Knowles, K. Weck* Dept Pathology/Lab Medicine, Medicine, Pediatrics, University of North Carolina-Chapel Hill, Chapel Hill, NC-27599.

Primary ciliary dyskinesia (PCD) is a rare, autosomal recessive genetic disorder associated with abnormalities in the structure and function of cilia of the respiratory tract and flagella of sperm. Phenotype includes oto-sinu-pulmonary disease, situs inversus (50% of patients) and male infertility. Diagnosis has relied upon compatible clinical phenotype and tests such as ultrastructural studies of cilia, nasal nitric oxide and ciliary beat frequency measurements. Recent work (Hornef et al, 2006 and our unpublished data) has identified mutations in two ciliary outer dynein arm genes (*DNAI1* and *DNAH5*) in approximately 38% of PCD patients. Due to the large size, full gene sequencing may prove to be cumbersome. Although allelic heterogeneity has been noted, there are eight exons where mutation clusters reside including exons 34, 50, 63, 76, 77 of *DNAH5* and exons 1, 16 and 17 of *DNAI1*. We have developed a sequenced based clinical molecular genetics panel for PCD. We designed primers specific for each of the targeted eight exons such that PCR and sequencing reactions could be run in parallel. Using positive controls for each of the known mutations, the UNC Molecular Genetics Laboratory devised and validated sequencing assays using consistent conditions that would be robust in a clinical setting. Final assay conditions were determined based on the quality of sequence data in multiple runs. Eight exons with a total of 18 different mutations were analyzed with complete concordance between the data from research and the clinical labs. In conclusion, we have developed and validated a robust clinical sequencing assay with consistent reaction conditions that will aid in the diagnosis of PCD. Based on our previous experience, this strategy will allow the identification of a causative mutation on at least one allele in approximately 23% of well-characterized PCD patients. Ours is the first CLIA and CAP-approved laboratory to offer genetic testing for PCD on a clinical basis. This abstract was funded in part by GCRC#00046, MO1 RR00046-42, 1 RO1 HL071798, 5 U54 RR019480, NIH/ORD CETT.

An Association Analysis of Cannabinoid Receptor 1 Gene with Bipolar Disorder. *J. Shi, J.A. Badner, E.S. Gershon, C. Liu* Dept Psychiatry, Univ Chicago, Chicago, IL.

Several lines of evidence implicate cannabinoid receptor 1 gene (CNR1) as a candidate for bipolar disorder (BD). First, CNR1 is mapped to chromosome 6q14-q15, a potential BD linked region. Second, cannabinoid receptors in the central nervous system regulate mood and anxiety. Blockade of these receptors engenders antidepressant-like neurochemical changes and behavioural effects consistent with antidepressant/antistress activity in rodents. Third, increased CNR1 levels were found in the hippocampus and prefrontal cortex of the post-mortem brains of patients with major depression. Finally, polymorphisms of CNR1 were reported to be associated with schizophrenia, substance addiction/abuse, and depression in patients with Parkinson's disease. The present study analyzes four tag SNPs in the CNR1 gene in 240 Caucasian BD families, which comprise the Clinical Neurogenetics (CNG) pedigrees with 45 samples (7 trios and 6 quads) and the National Institute of Mental Health (NIMH) Genetics Initiative for Bipolar Disorder waves I-IV with 868 samples (40 trios and 187 quads). Neither allelic nor haplotypic association with BD is found in our study (Sib-Transmission/Disequilibrium test for allelic association: rs12720071, $P = 0.64$; rs806368, $P = 0.92$; rs1049353, $P = 0.69$; rs806370, $P = 1$; TDTPHASE for haplotype consisting of rs12720071, rs806368 and rs1049353: global $P = 0.45$). Our sample has 80% power at $P < 0.01$ to detect an odds ratio (OR) > 1.6 for allele frequency of 0.1 and an OR > 1.4 for allele frequency of 0.5, under a multiplicative model. Thus it is unlikely that the CNR1 gene plays a major role in the susceptibility to bipolar disorder.

Ovotestes and XY sex reversal in a female with an interstitial 9q33.3-q34.1 deletion encompassing NR5A1 and LMX1B. *S. Schlaubitz[1, 9], S. Yatsenko[1], L. Vissers[3], L. Smith[4, 5], K. Keller[5], D.A. Scott[1], W.W. Cai[1], W. Reardon[6], O. Abdul-Rahman[7], E. Lammer[8], E. Magenis[4], J. Veltman[3], P. Stankiewicz[1], B. Zabel[8], B. Lee[1, 2]* 1) Dept. of Mol. & Human Genetics, Baylor College of Medicine, Houston, TX; 2) HHMI, Baylor College of Medicine Houston, TX; 3) Dept. of Human Genetics, NCMLS, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands; 4) Sect. of Medical Genetics & Mol. Medicine, Children's Mercy Hospital, Kansas City, MO; 5) Oregon Health & Science University, Dept. of Mol.& Medical Genetics, Portland, OR; 6) Our Lady's Hospital for Sick Children Crumlin, Dublin, Ireland; 7) Division of Medical Genetics, Dept. of Preventive Medicine, University of Mississippi, Jackson, MS; 8) Childrens Hospital Research Institute, Oakland, CA; 9) Childrens Hospital, University of Mainz, Mainz, Germany.

Genitopatellar syndrome (GPS [MIM 606170]) is a rare multiple anomaly syndrome characterized by renal and genital anomalies, congenital contractures, aplastic/ hypoplastic and often displaced patellae, dysmorphic facial features, and mental retardation. Reported individuals have significant delays in gross motor development and expressive language. Because animal models have shown that limb and genitourinary development share common genetic pathways, we collected blood from seven GPS patients to perform a systematic molecular analyses. This involved mutation screening of the coding regions of WNT4, WNT7A, TBX4 and LMX1B, CGH using a high density BAC array consisting of 32,447 clones, and FISH. We found a microdeletion of chromosome 9q that includes LMX1B and NR5A1 in a child who has features of GPS along with true hermaphroditism and a 46,XY karyotype. This microdeletion, causing haploinsuffiency of LMX1B and NR5A1, was not seen in other patients with GPS by FISH. LMX1B is essential in dorso-ventral patterning of the developing limb and is mutated in nail patella syndrome, while NR5A1 is required for male sexual differentiation and is mutated in different forms of XY sex reversal. These data suggest that the etiology of GPS is heterogeneous and may include microdeletions associated with haploinsufficiency of LMX1B and NR5A1.

Identifying the Molecular Basis of Morphological Variation in Beaks. *K. Powder[1], S. Brugmann[2], J. Helms[2], M. Lovett[1]* 1) Washington University, St Louis, MO; 2) Stanford University, Stanford, CA.

Avian beaks show extreme species-specific variability in morphology, though they develop from the same primordial structures. Understanding the molecular and developmental bases of these variations enhances our insights into evolution and provides a unique model for investigating human craniofacial disorders. In both humans and birds, cranial neural crest cells are the primary source of mesenchyme for the frontonasal prominence; previous work has shown that these cells contain species-specific patterning information. To determine the molecular basis of avian craniofacial patterning, we measured changes in gene expression between cranial neural crest cells from the frontonasal prominence of three bird species (chickens, quails, and ducks) at two different developmental stages. Samples were dissected before (HH20) and after (HH25) morphological distinctions between the species are evident. A total of 55 comparative hybridizations were conducted on a custom microarray, which contains oligonucleotides designed for cross-species comparisons of most transcription factors plus a wide range of signaling pathways. Our data indicate that by HH20, prior to any overt morphological differences, cranial neural crest cells have established a species-specific expression profile and this profile is largely maintained, with very few changes, to HH25 when morphological differences are evident. Over 280 genes were found to be differentially expressed (>2-fold with p-values < 0.05) between the three species, with fold changes ranging from a 30-fold up-regulation of ZNF334 to a 17-fold down-regulation of ZNF2 in duck versus chicken. Of the seventy genes that exhibited the largest changes, ten percent are known components of WNT signaling and another ten percent are parts of the TGF/BMP signaling pathways. Several of the other genes have also been previously implicated in human craniofacial morphogenesis (e.g. OSR1, OSR2, SATB2, and Jagged2). This set of genes provides a list of valuable candidates to investigate the molecular mechanisms that lead to species-specific craniofacial form, and also underlie a number of human craniofacial anomalies.

Glucocerebrosidase Mutations in Sporadic Parkinson Disease. *E. Sidransky[1], M.J. Eblan[1], U. Gutti[1], B. Stubblefield[1], O. Goker Alpan[1], A.B. Singleton[2]* 1) Molec Neurogen,MGB/NHGRI/NIH, Bethesda, MD; 2) Molecular Genetic Unit NIA,NIH< Bethesda, MD.

Background: An association between glucocerebrosidase, the enzyme deficient in Gaucher disease, and the synucleinopathies has been suggested both by the development of parkinsonism in Gaucher probands and carriers and by the presence of mutations in the gene for glucocerebrosidase (GBA) in different series of subjects with synucleinopathies. An open access Parkinson repository was used to better establish the incidence of GBA mutation in cohorts with sporadic Parkinson disease (PD). Methods: The glucocerebrosidase gene (GBA) was sequenced in samples collected from 92 Caucasian Parkinson disease patients from the United States and 92 Chinese Parkinson disease patients from Taiwan and in 184 clinically screened controls, matched for age and ethnicity. Findings: Among the Caucasian cohort, 5.4% of subjects carried mutations in GBA, while there were no carriers among the matched controls. The frequency of GBA mutations among the Chinese PD probands was 4.3%, in contrast to 1.1% in Chinese controls. Mutant alleles identified included the known mutations N370S (3 cases), L444P, D409H, T323I and three novel mutations, R262H, L174P and Q497R. Conclusion: These results, ascertained in two ethnically diverse cohorts collected in a standardized and clinically rigorous fashion by an open access Parkinson disease repository, and screened by direct sequencing of GBA, demonstrate that GBA mutations are encountered in subjects with sporadic PD at a higher frequency than most known PD genes. These findings suggest that the presence of this mutant lysosomal protein contributes to the neurodegenerative process, perhaps via enhanced protein aggregation.

Candidate gene medical sequencing: Fast identification of causitive variant candidates in genetically heterogeneous cardiac disease. *S.E. Scherer[1], T. Xu[2], Z. Yang[2], N.E. Bowles[2], J.A. Towbin[2], G. Weinstock[1], R.A. Gibbs[1]* 1) Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX; 2) Cardiac Genetics, Dept. of Pediatrics, Baylor College of Medicine, Houston, TX.

We applied candidate gene medical sequencing to the cardiac disease, Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) to define the scope of gene variation and highlight potential functional pathways leading to the observed phenotype. ARVD/C is characterized by sudden cardiac death and associated with nine genetic loci. Four genes have been linked to the defect, two of which encode proteins present in cardiac desmosomes and adherens junctions. Five desmosome genes as well as genes encoding sarcomeric, sarcolemma-sarcomere link and ion channel proteins were sequenced across 234 ARVD/C registry patients, including 146 unrelated probands and 250 healthy, ethnic matched controls. We identified 47 heterozygous variants in the desmosome genes while observing no variants in non-desmosome genes and low frequency in controls. Kindred analysis of PKP2 results suggested that the incomplete penetrance observed in this disease might be due in part to compound and/or digenic heterozygosity. Preliminary functional analyses are underway. These studies demonstrate the power and speed of candidate gene medical sequencing over traditional approaches in defining variant frequency and functional pathways involved in heterogeneous disease phenotypes.

Mutations in FGF Signaling Pathway Genes Contribute to Cleft Lip and Palate. *B.M. Riley[1], M.A. Mansilla[1], L. Raffensperger[1], B. Maher[2], M.L. Marazita[2], M. Mohammadi[3], J.C. Murray[1]* 1) Dept Pediatrics, U of Iowa; 2) Ctr for Craniofacial and Dental Genetics, U of Pittsburgh; 3) Dept Pharmacology, NYU School of Medicine.

Nonsyndromic cleft lip and palate (NSCLP) is a complex birth defect resulting from a combination of genetic and environmental factors. We hypothesize that members of the fibroblast growth factor (FGF) signaling pathway family contribute to NSCLP. Genotyping of SNPs in the FGF and FGFR candidate genes found significant P-value associations between NSCLP and FGF3, FGF7, FGF10, FGF18, and FGFR1 and the FGF3-FGF4 haplotype. Gene x gene interaction data supports interactions between members of the FGF family, such as FGF3/FGF18 and FGF10/FGF18, but also interactions with other NSCLP candidate genes. Medical resequencing of the coding regions of these genes in 184 NSCLP patients identified 7 novel mutations: FGFR1 M369I, E467K, R609X; FGFR2 R84S, D138N; FGFR3 V329I; and FGF8 D73H. Structural modeling of these proteins has elucidated possible functional roles for several of the mutations. Modeling of the D73H mutation indicates reduced biological activity due to disruption of FGF8 protein conformation and critical ligand-receptor binding interactions. These findings predict D73H to be a loss-of-function mutation. The E467K mutation may influence recruitment and phosphorylation of downstream signaling molecules due to its close proximity to Y463, one of FGFR1's autophosphorylation sites. Models of the V329I mutation predict that this mutation should lead to loss-of-function. Specifically, the mutation should destabilize the tertiary folding of the Ig domain 3, resulting in incomplete processing and retention of the mutated FGFR3 in intracellular compartments. These 7 point mutations may contribute to as much as 3-6% of isolated cleft lip and palate. Together with data from IRF6(12%); FOXE1, GLI2, MSX2, SKI, SATB2, and SPRY2(6%); and MSX1(2%), these genes can account for 23-26% of isolated cleft cases and have a significant impact on the clinical diagnosis and genetic counseling of CLP, in addition to providing further evidence that the FGF signaling pathway is important in lip and palate development.

RETINITIS PIGMENTOSA IN COLOMBIA: CLINICAL AND MOLECULAR STUDIES. *L. Urrego[1], N. Gelvez[1], S. Florez[2], D. Medina[2], M. Tamayo[1, 2]* 1) Instituto de Genética Humana, Bogotá, Colombia; 2) Fundación Oftalmológica Nacional, Bogotá, Colombia.

The objective of this study was identify mutations in Rhodopsin, Peripherin and NRL genes. We performed clinical and molecular studies in 115 unrelated Colombian individuals with Retinitis Pigmentosa (RP). A complete ocular examination was performed with ophthalmological exams including ERG, visual field, fluoresceinic angiography and photos of funduscopy. Genomic DNA was amplified by PCR, followed by SSCP-HA analysis and automatic sequencing. Among 115 affected we confirmed Autosomal Recessive inheritance in the 40.9% (47/115), Autosomal Dominant in 9.5% (11/115), recessive X-linked in 3.5% (4/115) and sporadic cases in 46.1% (53/115). The SSCP-HA analysis showed 14 different migration patterns in 85 RP individuals. By sequencing, 6 different polymorphisms were identified, three of them are already reported (Gly338Asp, Lys310Arg and Glu304Gln) and the others (24037 Ins G, Ala351Ala and Thr278Thr), are no reported yet in the literature. The frequency of Gly338Asp was 48.7% (56/115), Lys310Arg 26.9% (31/115), Glu304Gln 13.9% (16/115), 24037 Ins G 41.7% (48/115), Ala351Ala 5.2% (65/115) and Thr278Thr 0.87% (1/115). Although our statistical analysis are not conclusive, the severity of the disease seems to be higher than expected by several mechanism of inheritance. On the other hand, the presence of some polymorphisms could be related with the tendency to present an earlier onset of the disease.

Genotype-phenotype correlations for sub-microscopic copy number variants. *F. Zahir[1], A. Baross[2], A.D. Delaney[2], P. Eydoux[3], H.V. Firth[4], W.T. Gibson[1], S. Langlois[1], H. Martin[4], M. Marra[1,2], R.M. Pettett[5], A-C. Thuresson[6], J. Vermeesch[7], L. Willat[4], SL. Yong[1], J.M. Friedman[1]* 1) Dept Med Genet, Univ BC; 2) BC Genome Sci Ctr; 3) Dept Path & Lab Med, C&W Hosp (1-3 Vancouver, Canada); 4) Dept Med Genet, Addenbrookes Hosp; 5) Wellcome Trust Sanger Ins (4&5 Cambridge, UK); 6) Uppsala Univ Child Hosp, Sweden; 7) Univ Hosp, Ctr Hum-Genet, Leuven, Belgium.

Mental Retardation (MR) is idiopathic in at least half of all cases. Potentially pathogenic de novo, submicroscopic gains/losses of genomic material (copy number variants-CNVs) were identified using Affymetrix GeneChip 100K Whole Genome Sampling Analysis in 10 of 100 children with idiopathic MR. Genotype-phenotype correlations were performed in these 10 cases to establish pathogenicity. Five cases have CNVs with genomic coordinates that overlap with other cases in DECIPHER (http://decipher.sanger.ac.uk), an international database of pathogenic CNVs detected by array genome hybridization. Two of our cases and DECIPHER #CAM126 show overlapping deletions of 14q11.2. These cases exhibit similar facial features: widely-spaced eyes, prominent epicanthic folds, very short nose with flat bridge, long philtrum, prominent Cupids bow, full lower lip, and unusual auricle malformation. The genomic overlap region covers only 3 annotated genes, which can be considered good candidate MR genes. Another child with a 3.6 Mb, 16p13.3 duplication, has a striking phenotypic resemblance to reported cases with cytogenetically apparent duplications of the same region, and with a larger duplication in DECIPHER #CGH237, indicating the critical genomic region lies in our patient's lesion. Other cases show a deletion 22q12 matching DECIPHER #CHG758 and a deletion 7p21 matching DECIPHER #UPP969. The fact that half of our de novo CNVs show overlap with others reported in DECIPHER supports the pathogenicity of these lesions for MR. High resolution Array Genome Hybridization permits identification of new chromosomal abnormality syndromes and provides insight into copy-number-sensitive genes affecting embryonic brain development.

Thirty percent of *LMNA* cardiomyopathy families manifest complex disease in which some mutation negative family members are phenotype positive. *S. Parks[1], J. Kushner[1], D. Nauman[1], D. Burgess[1], S. Ludwigsen[1], A. Peterson[1], D. Li[1], P. Jakobs[1], M. Litt[2], R. Hershberger[1]* 1) Divison of Cardiovascular Medicine, Oregon Health & Science University, Portland, OR; 2) Department of Molecular and Medical Genetics, Oregon Health & Science University, Portland, OR.

As many as one half of all cases of idiopathic dilated cardiomyopathy (IDC) may be familial dilated cardiomyopathy (FDC), which is thought to be caused by rarely occurring, single base mutations in any of several different genes. The lamin A/C gene (*LMNA*), known to cause progeria, AD Emery-Driefuss muscular dystrophy, lipodystrophy and other disorders, is also a well known cause of FDC. To evaluate the risk of lamin A/C cardiomyopathy in patients with FDC versus those with non-familial IDC, blood was collected from 324 probands, of whom 138 had IDC and 186 had FDC. Genomic DNA was PCR amplified and sequenced for nucleotide alterations in *LMNA*. Likely disease causing mutations were followed up by evaluating additional family members, when possible.

LMNA mutations were observed in 20 of the 324 probands (6.2%), including 6 of 138 (4.4%) with IDC and 14 of 186 (7.5%) with FDC. Conduction system disease, which is commonly observed in *LMNA* cardiomyopathy, was evident in 19 of the 20 probands (95%). Unexpectedly, we found that in 6 of the 20 *LMNA* cases, (30%) there was at least one additional family member with dilated cardiomyopathy who did not carry the *LMNA* mutation, a condition we refer to as complex disease. This suggests that an additional factor, such as a second disease gene, is involved, and indicates that genetic dilated cardiomyopathy may have a more complex basis than previously appreciated. These findings challenge disease models suggesting that a mutation in a single gene is generally sufficient to explain FDC. The implications are profound for providers and patients, especially in the interpretation of clinical molecular genetic results, as neither a negative nor a positive *LMNA* sequence finding may provide a definitive explanation. We suggest extreme caution in the interpretation of clinical genetic testing results for lamin A/C cardiomyopathy.

A worldwide survey of linkage disequilibrium and haplotype variation in the human genome. *J.K. Pritchard[1], D.F. Conrad[1], G. Coop[1], M. Jakobsson[2], X. Wen[1], J.D. Wall[3], N.A. Rosenberg[2]* 1) Department of Human Genetics, University of Chicago, Chicago, IL; 2) Department of Human Genetics, University of Michigan, Ann Arbor, MI; 3) Department of Biological Sciences, University of Southern California, Los Angeles, CA.

Although recent genome-wide surveys have provided high-resolution information about haplotype structure in a small number of human populations, little is known about the extent to which these populations are representative of global haplotype diversity. We report haplotype structure across 12 megabases of DNA sequence in a sample of 927 individuals from 52 human populations. The geographic distribution of haplotypes strongly reflects human history, with a loss of haplotype diversity as distance increases from our species ancestral range in Africa. Although there is dramatic variation across populations in the extent of linkage disequilibrium (LD), considerable sharing of haplotype structure exists, and the locations of inferred recombination hotspots generally match across diverse human populations. The four samples studied by the International HapMap Project contain the majority of haplotypes found in most populations: averaging across the 52 populations, 83% of common haplotypes in a population are also common in the most similar HapMap sample. Consequently, although the efficiency of tag-SNPs based on the HapMap is reduced in low-LD African populations and in Eurasian populations distant from those studied in the HapMap, the HapMap will be helpful for the design of genome-wide association mapping studies in nearly all human populations.

A mutation of -Actin responsible for a new neurological syndrome associated with dystonia and deafness alters actin dynamics and mitochondrial bioenergetics. *V. Procaccio[1], G. Salazar[2], S. Ono[2], M. Styers[2], M. Gearing[2], A. Davila[1], R. Jimenez[1], J. Juncos[2], C. Gutekunst[2], G. Meroni[3], B. Fontanella[3], E. Sontag[4], J.M. Sontag[4], V. Faundez[2], B. Wainer[2]* 1) Center for Molecular and Mitochondrial Medicine, Univ California, Irvine; 2) Departments of Cell Biology and Pathology, Laboratory Medicine and Neurology, Emory Univ School of Medicine, Atlanta; 3) Telethon Institute of Genetics and Medicine, Naples; 4) Department of Pathology, Univ of Texas Southwestern Medical School, Dallas.

Actin is a major cytoskeletal protein in neurons, and the dynamics of its assembly affect many aspects of cell motility and membrane turnover, representing a major ATP-consuming process in cells. Actin protein is one of the most highly conserved proteins. Actin is co-transported with actin-binding proteins, including ADF and cofilin, which are essential for rapid turnover of actin filaments in vivo. An extensive actin aggregation in the brains of identical twins with generalized dystonia and deafness associated with multiple developmental abnormalities was previously reported. We recently found a missense mutation in a non-muscle form of the actin gene, -actin, resulting in an arginine to tryptophan substitution at position 183 in a highly conserved domain of the protein. Cellular studies of a probands lymphoblastoid cell line showed altered actin depolymerization dynamics in response to latrunculin A, an actin momomer sequestering drug. In addition, DNA microarray studies of mitochondrial gene expression using our Mitochip and real-time quantitative PCR in brain tissues showed increased expression of several genes including actin, cofilin, genes involved in apoptosis and mitochondrial reactive oxygen species (ROS) scavenging enzymes in response to increased mitochondrial ROS production. Expression of genes involved in mitochondrial ATP production was down-regulated in brain cortex. Actin turnover requires ATP consumption and actin depolymerisation is the rate limiting step for actin turnover. Hence the actin mutation may affect the integrity and function of the ATP-binding site, altering energy production and increasing free radical generation.

Angiotensinogen gene polymophism predicts blood pressure response to an angiotensin converting enzyme inhibitors therapy. *X. Su[1], L. Lee[2], X. Li[1], J. Lu[2], Y. Hu[2], S. Zhan[2], W. Cao[2], ling. Mei[1], YM. Tang[1], R. Krauss[3], J. Rotter[1], H. Yang[1]* 1) Medical Genetics, Cedars-Siniai Medical Center, Los Angeles, CA; 2) Department of Epidemiology, School of Public Health, Peking University Health Science Center, Beijing, China; 3) Children's Hospital Oakland Research Institute, Oakland, CA.

Bacground-Angiotensinogen(AGT), one of the major structural candidate genes in the Renin-Angiotensin-Aldosterone System pathway, was evaluated for its association with BP response to ACEI therapy in a large sample of Chinese hypertensives by utilizing a haplotype approach in a two-stage design. Methods and Results-1447 hypertensives ascertained from a Chinese community-based screening for hypertension and underwent the benazepril treatment as sole therapy for 3 years were randomly assigned into two groups, an exploratory set(n=733) and a confirmatory set(n=714). Four SNPs, -6 A/G, T207M, M268T and C11537A, were selected based on the TagSNP approach and previous reports. In the exploratory set, all 4 common haplotypes and SNPs were analyzed. Haplotype 2 (H2) carriers had a borderline significant increase in diastolic BP(DBP) reduction than those without H2, p=0.086 after adjusting for baseline BP, gender, age, kidney function and dose of benazepril received. Among the four SNPs, C11537A in the 3 untranslated region(3-UTR)of the AGT gene was significantly associated with DBP response to ACEI therapy(P=0.028). The A allele carrier had a greater DBP reduction than CC homozygotes. Since the H2 is the only common haplotype contained the A allele at C11537A suggests that the observed borderline H2 association is likely driven by A allele in the SNP. In the confirmatory set, we observed a significant association (p=0.018) after adjusting for the same covariates. Electrophoretic mobility shift assay demonstrated that this SNP located on AP4 specific binding site and that the binding affinity was significantly higher with the C than the A allele. Conclusion-We identified that a SNP in 3-UTR of the AGT gene is associated with DBP response to an ACEI therapy and suggest its potential function might be related to its effect on AP4 binding.

Development of High Multiplex TaqMan MicroRNA Assays. *R. Tan, C. Chen, K. Guegler* Applied Biosystems, Foster City, CA.

MicroRNAs (miRNAs) are endogenous small non-coding RNAs averaging ~22- nucleotides. MicroRNAs down-regulate gene expression by inducing endonucleotic cleavage or translational repression of specific target mRNAs, and play important regulatory roles in many cellular processes. More than 1,000 miRNAs have been identified, and the number of discovered miRNAs is still growing. The explosion of interests in miRNAs has created a demand for experimental tools to identify, quantify and profile miRNAs. A stem-loop RT-based TaqMan miRNA assay has been developed at Applied Biosystems for miRNA quantification. Recently, we have optimized the condition of the RT-based high multiplex TaqMan miRNA assay. To further improve the rate of throughput, we have developed a ligation-based full plex TaqMan miRNA assay, in which all miRNAs can be multiplexed together to perform ligation, digestion and reverse transcription in a single tube, followed by individual real-time PCR. The relative quantification results of ligation-based assay are similar with those of RT-based assay. Both RT-based and ligation-based high multiplex TaqMan miRNA assays have provided powerful tools to profile miRNAs sensitively and cost effectively in high through put manner.

Detection of genomic copy number variations (CNV) using the SNPlex genotyping system. *M. Wenz[1], F. de la Vega[1], R. Koehler[1], A. Tobler[1], T. Hemming Karlsen[2], A. Franke[3], S. Schreiber[3], J. Hampe[3]* 1) Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA, USA 94404; 2) Institute of Immunology, Rikshospital et University Hospital, N-0027 Oslo, Norway; 3) Institute for Clinical Molecular Biology, Christian-Albrechts-University, Schittenhelmstr. 12, 24105 Kiel/Germany.

Genome copy number variations (CNVs) are far more frequent than originally expected, and many of them affect gene copy numbers. Several genetic disorders are the result of CNVs, however because of technical limitations, the extent to which such contribute to phenotypic variations is still poorly understood. We recently introduced the SNPlex Genotyping System to address the need for accurate genotyping data, high sample throughput, study design flexibility, and cost efficiency. The system uses oligonucleotide ligation/polymerase chain reaction (OLA/PCR) and capillary electrophoresis (CE) to analyze single nucleotide polymorphism (SNP) genotypes (Tobler et al. J. Biomol. Tech. 16(4), 2005). Here we demonstrate the feasibility of an adaptation of the SNPlex Genotyping System, to analyze CNVs by comparing the intensity ratios of OLA reactions in test and reference regions. We will present the copy number analysis of DNAs with known chromosomal duplications. Specifically, we studied 88 genomic DNAs, 7 of which contained duplications of the chromosomes 9, 13, 18 or X. On each duplicated chromosome we analyzed at least 10 test OLA reactions, and differences in intensity ratios confirmed all known chromosomal duplications. The assay further identified male (XY) and female (XX) DNA samples due to their copy number difference of the X chromosome. In addition to identifying known chromosomal duplications, we analyzed copy numbers changes of the RCCX module of the human MHC complement gene cluster.

A mouse model for Sjögren-Larsson syndrome produced by targeted knockout of the ALDH3A2 gene encoding fatty aldehyde dehydrogenase. *W.B. Rizzo[1], G. Carney[1], J. Bridger[1], R. Spieker[1], L. Bunnell[1], L. Spaulding[2], J.A. Stribley[2], J.M. Salbaum[2]* 1) Dept Pediatrics; 2) Dept Genetics, Anatomy and Cell Biology, Univ Nebraska Med Ctr, Omaha, NE.

Sjögren-Larsson syndrome (SLS) is an inherited neurocutaneous disorder characterized by ichthyosis, mental retardation and spasticity. The disease is caused by mutations in the ALDH3A2 gene encoding fatty aldehyde dehydrogenase (FALDH), an enzyme that catalyzes the oxidation of aliphatic aldehydes derived from metabolism of fatty alcohol and other lipids. To investigate the biochemical pathogenesis of SLS and explore therapeutic options, we produced an FALDH-deficient mouse strain by gene targeting methods that introduced a mutation in the ALDH3A2 gene that completely abolishes enzyme activity. ALDH3A2$^{-/-}$ mice showed undetectable (<0.05% of wild-type) FALDH activity in liver using octadecanal as substrate, but variable amounts of residual enzyme activity in intestine (7 3%), brain (35 12%) and skin (60 5%) probably due to other tissue-specific aldehyde dehydrogenase enzymes. Heterozygous mice expressed a partial FALDH deficiency in liver (42 8 % of the wild-type). Fatty alcohol (hexadecanol and octadecanol) levels in tissues from the ALDH3A2$^{-/-}$ mice were increased in liver (7-fold), skin (3-fold) and brain (11-fold) compared to wild-type animals. The mutant mice were fertile and appeared to have a normal lifespan. The clinical phenotype of the ALDH3A2$^{-/-}$ mice was variable on a mixed 129 genetic background, suggesting the presence of modifier genes. Approximately one-fourth of the mutant mice developed ichthyosis at 6-12 months of age, whereas a small number of mutant mice, all products of homozygous crosses, showed congenital ichthyosis. Some mice displayed self-mutilation with loss of ear tissue due to excessive scratching that resembles the pruritus seen in SLS patients. ALDH3A2$^{-/-}$ mice performed poorly on Rotorod testing compared to wild-type mice. This animal model of SLS should prove valuable for elucidating the biochemical pathogenesis of the disease, exploring modifier genes and developing new therapeutic approaches.

Bivariate Linkage Analysis Identified Genomic Regions for Body Fat Mass and Bone Mineral Density. *P. Xiao*[1], *Z.H. Tang*[2], *H. Shen*[3], *H.W. Deng*[3] 1) Osteoporosis Research Center, Creighton University, Omaha, NE, USA; 2) Laboratory of Molecular and Statistical Genetics, College of Life Sciences, Hunan Normal University, Changsha, Hunan, P. R. China; 3) Departments of Orthopedic Surgery and Basic Medical Science, School of Medicine, University of Missouri-Kansas City, Kansas City, MO, USA.

Body fat mass (BFM) is a predictor of bone mineral density (BMD). It was also suggested that BFM and BMD are highly correlated. However, knowledge on specific genes influencing both BFM and BMD is limited. To identify genomic regions affecting both BFM and BMD, we performed a bivariate linkage analysis involving 4,126 subjects from 451 families on BFM and BMD at spine, hip and wrist, respectively. The highest LOD score of 2.69 was achieved on 7q21 for BFM and spine BMD. Another interesting suggestive linkage was detected on 6q27 for both BFM and spine BMD (LOD = 2.42) and BFM and hip BMD (LOD = 2.30). In addition, suggestive evidence was also found on 2q32 for BFM and hip BMD, and 6p22 and 11p13 for BFM and wrist BMD. Our results suggest that there may be pleiotropic genes on chromosome 2, 6, 7, and 11 for BFM and BMD at different skeletal sites.

Gene-environment interactions between maternal stress and Factor 5 polymorphisms on preterm delivery. *H.-J. Tsai[1,2], Y. Yu[1,2], X. Wang[1,2]* 1) The Mary Ann and J. Milburn Smith Child Health Research Program, Children's Memorial Hospital and Children's Memorial Research Center, Chicago, IL; 2) Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL.

Preterm delivery (PTD, < 37 weeks of gestation) is a major public health concern and a leading cause of infant mortality and morbidity. PTD has been recognized as a complex trait, but the etiology is still unclear. Previous results have suggested that genetic effects, environmental factors and gene-environment interactions may play an important role in PTD. However, only very few studies have examined genetic effects, gene-gene and gene-environment interactions. In this study, we genotyped seven single nucleotide polymorphisms (SNPs) of Factor 5 gene in 337 mothers with PTD and 527 mothers with term deliveries in multiple racial groups at the Boston Medical Center. Of these, 4 SNP variants resulted in non-synonymous amino acid changes. We only carried out association tests for 4 SNPs with major allele frequencies more than 10%. We adjusted maternal age, race, education, marital status, parity, passive smoking, and infant gender in all analyses. Significant genotype associations of PTD were observed with two SNPs 6 and 7 (p=0.04 and 0.02, separately). Locations of SNP 6 and 7 are in an intronic region and His107Asp, respectively. Moreover, we examined genetic effect of SNPs 6 and 7 in PTD stratified by lifetime stress and stress during pregnancy, respectively. We found that SNP 7 was significantly associated with PTD among women with low-to-medium, but not high lifetime stress (p=0.01 and 0.92, separately). Similarly, significant association between SNP 7 and PTD was only observed among women with low-to-medium stress during pregnancy (p=0.01). Our results have demonstrated gene-environment interactions between maternal stress and Factor 5 genetic variants on PTD. We are planning to examine whether F5 SNPs are associated with PTD related traits such as low birth weights, and account for potential confounding effect due to population stratification in future analyses.

A new estimator for locus-specific genetic effect from case-control data based on generalized linear model. *G. Zhang[1], R. Chakraborty[1], L. Jin[2,3,1]* 1) Center for Genome Information, University of Cincinnati, Cincinnati, OH; 2) School of Life Sciences, Fudan University, Shanghai, China; 3) CAS-MPG Partner Institute of Computational Biology, Shanghai, China.

Genetic architecture underlying complex disease is governed by loci that have quantitative genetic effects. Their locus-specific effects can be stated in terms of amounts of variation; i.e., the fraction of the phenotypic variation attributable to certain DNA variant(s) between individuals. However, for binary traits, the continuous liability is a latent variable, which makes the variation attributable to certain DNA variant(s) cannot be readily calculated. Instead, the most widely used measures for genetic contribution or effective size of genetic variant(s) are based on penetrance, such as genotypic relative risk, odds ratio or heritability of penetrance. These measures evaluate the outcomes of a complex disease as zero or one, which neglect the underlying gradation of some attribute related to the disease causation. Furthermore, these measures fail to reflect the intrinsic effects of disease allele frequency or disease prevalence. Consequently, these measures are not directly comparable between different diseases, or even between different genetic variants of a same disease. In this presentation, we develop a method to estimate locus-specific genetic effect of certain genetic variant(s) from case-control data based on a generalized linear model (GLM). The proposed estimator is less sensitive to allele frequency or disease prevalence. We show analytically that the estimator is proportional to the Wald statistic, which brings another favorable property of it - it can serve as a direct index for the power of association study. We evaluate the performance of the Wald test on the proposed estimator and show that the power is greater or equal to those of the traditional ^{2} or Hotelling's T^2 tests. In addition, this estimator can be extended to multi-locus or haplotype settings for estimating the genetic effect of a haplotype block. Finally, the potential usage of this estimator to study genetic epistasis as departure from additivity of variance explained by single-locus effects is discussed.

The Prader Willi-like phenotype in Fragile X syndrome. *F. Tassone[1,2], S.T. Nowicki[2], M-F. Croquette[3], P.J. Hagerman[1], R.J. Hagerman[2]* 1) Department of Biochemistry and Molecular Medicine, University of California, Davis, School of Medicine, Davis, CA, 95616, USA; 2) M.I.N.D. Institute, UC Davis, Sacramento, 95817, CA, USA; 3) Department of Neuropediatrics, Centre Hospitalier Regional et Universitarie de Lille, France.

Fragile X syndrome (FXS) is a single-gene disorder with a broad spectrum of involvement and a strong association with autism. The spectrum of involvement in fragile X is likely related to molecular variations both within the FMR1 gene, within the genes that FMR1 protein (FMRP) regulates and in other background genes in addition to environmental factors. Indeed FMRP is a translational regulator, whom absence leads to dysregulation of a number of other genes. FMRP binds to additional proteins, including CYFIP1 and CYFIP2, in carrying out its role as a transporter and regulator of translation of mRNAs. CYFIP1, which has been recently mapped to the critical region of 15q (Prader Willi/Angelman region)is an important protein that interacts with Rac1 in an activity dependent manner and acts as a link between two processes that underlie synaptic remodeling, including the cytoskeleton reorganization regulated by Rac1 and control of local protein translation via FMRP. A subgroup of patients with FXS has a Prader-Willi phenotype (PWP), which includes severe hyperphagia, obesity, hypogonadism. Here, we describe a group of 13 of these patients who present with severe hyperphagia, severe behavioral problems but most striklingly a high rate of autism compared to individuals with FXS only. In addition, our preliminary molecular studies demonstrate a significant dysregulation of CYFIP1, at both RNA and protein level, in the majority of patients with FXS and PWP suggesting that abnormal expression of CYFIP1 and/or contiguous genes may be associated with autism and with the PWP in fragile X syndrome. Since synaptic remodeling problems are seen in subgroups of patients with autism, particularly those with newly discovered X-linked MR genes, the PWP in FXS may be a model disorder for understanding the link between FXS and other forms of autism.

Association testing of candidate genes in the anabolic neuropeptide pathway with obesity among the Samoans of Polynesia. *D.T. Smelser[1], G. Sun[1], R. Kaushal[1], P. Pal[1], S. Viali[2], J. Tufa[3], R. Chakraborty[1], D.E. Weeks[4,5], S.T. McGarvey[6], R. Deka[1]* 1) Center for Genome Information, Department of Environmental Health, University of Cincinnati, Cincinnati,OH; 2) Department of Health, Government of Samoa, Apia, Samoa; 3) Department of Health, American Samoa Government, Pago Pago, American Samoa; 4) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 5) Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 6) International Health Institute, Brown University, Providence, RI.

The Samoans of Polynesia have a high prevalence of obesity (84% in women and 78% in men in American Samoa and 59% in women and 29% in men in Samoa). Samoans living in the independent nation of Samoa lead a traditional lifestyle, while American Samoans in the US territory have taken on a Western way of life. We have tested for association of variants in eight genes in the anabolic neuropeptide pathway with obesity in this population. This pathway stimulates appetite, increases feeding behavior and decreases metabolic rates, leading to overeating and obesity. DNA analysis was performed on a total of 991 samples (456 American Samoans and 535 Samoans) using a total of 45 tagging SNPs covering eight genes (AGRP, GAL, GHRL, INS, LEP, NPY, PMCH, RETN). All SNPs were found to be in Hardy Weinberg equilibrium. We found significant differences in allele frequencies at two SNPs in NPY when comparing the two populations. We found significant associations with BMI (adjusted for age and sex) in the American Samoan population for the following genes and SNPs: LEP rs3828942 (p=0.03), PMCH rs7973796 (p=0.01), PMCH rs10860845 (p=0.01) and PMCH rs10507144 (p=0.03). For the Samoan population, significant associations with BMI were seen in GHRL rs1642977 (p=0.05) and GHRL rs26310 (p=0.05). The American Samoans and Samoans are genetically similar, so the difference in the results is likely due to gene-environment interactions brought on by the variance in lifestyles between the populations. Further haplotype analysis is currently being conducted in this study.
This research is supported by NIH R01 DK59642.

Families with multiple types of Idiopathic Interstitial Pneumonia (IIP) have decreased survival. *A. Wise[1,2], M. Steele[1,2], M. Speer[2], J. Loyd[3], K. Brown[4,5], A. Herron[1], L. Burch[1], M. Schwarz[4,5], D. Schwartz[1,2]* 1) NIEHS, Research Triangle Park, NC; 2) Duke University, Durham, NC; 3) Vanderbilt University School of Medicine, Nashville, TN; 4) National Jewish Medical and Research Center, Denver, CO; 5) University of Colorado Health Sciences Center, Denver, CO.

Previously, it was thought that non-Usual Interstitial Pneumonia (UIP) forms of IIP were inflammatory and would either resolve or eventually evolve into the more fibrotic UIP phenotype. Thus, families with multiple types of IIP (heterogeneous) were thought to have the same underlying genetic basis as families with only UIP (homogeneous). To determine whether the consistency of IIP within families with Familial Interstitial Pneumonia (FIP) defined a specific phenotype, we evaluated the type of IIP within 146 families with two or more cases of IIP. Among these families, 74 of the families presented as homogeneous, while 72 exhibited a heterogeneous phenotype. Current age, age at diagnosis, age at death, mortality, sex, dyspnea, vital capacity, Dl_{CO}, smoking status, and for smokers, pack years and cigarettes per day smoked; were evaluated in affected individuals. Surprisingly, survival was decreased in the heterogeneous (47%) compared to the homogeneous (61%) families ($p<0.01$). In order to assure that this difference was not due solely to the different IIP diagnoses present in the heterogeneous families, the UIP affected individuals only from the heterogeneous families were compared to the individuals from the homogeneous families. Decreased survival in the heterogeneous families was also seen in the UIP only individuals (39% vs. 61%, $p<0.001$). Additionally, the mean age of death was significantly lower ($p<0.01$) in both heterogeneous (64 +/-12) and UIP only heterogeneous individuals (64 +/-12) when compared to individuals with UIP from homogeneous families (69 +/-10). Therefore, homogeneous and heterogeneous families may, in fact, represent two distinct phenotypic groups with heterogeneous families presenting with more aggressive disease. This difference in disease progression may also indicate genetic heterogeneity in FIP with variable genetic susceptibility leading to different disease outcomes.

A method for screening susceptibility genes and modeling gene-gene and gene-environmental interactions in large-scale candidate gene association studies. *N. Tanaka[1], K. Tokunaga[2], T. Miki[3]* 1) Dept Clinical Bioinformatics, Grad Sch of Medicine,Univ Tokyo, Tokyo, Japan; 2) Dept Human Genetics, Grad Sch of Medicine, Univ of Tokyo,Tokyo,Japan; 3) Dept Geriatric Medicine, Ehime Univ Sch of Medicine, Ehime, Japan.

Virtually any common human disease results from the genetic susceptibility factors and modifiable environmental factors. Then a large number of candidate genes can be subjected to whole genome candidate gene association studies with various diseases. Nevertheless, it is often the case that the candidate genes for further analysis are selected based on statistical analyses on disease associations for single genes. Recently several approaches have been proposed to identify gene-gene interactions. However, these approaches may be inadequate for identifying high-order interactions in the absence of the gene effects of which marginal effects are large enough to be detectable from high dimensional data, and generally do not permit us to control for potential confounders. Furthermore the interaction effects of most common disease are assumed to be non-linear. We propose here a novel screening method for case-control association studies to select candidate genes, which is composed of combined resampling methods and semiparametric additive mixed modeling. The proposed heuristic approach can handle a large number of inputs and take into account both categorical and continuous variables. We evaluate the performance of the procedures through simulation studies and illustrate their application with data from an extensive multi-institutional case-control association study on hypertension in Japanese. The proposed method provides a flexible statistical framework for controlling potential confounders and screening combinations of multiple genes and environmental factors that are associated with the disease onset.

Novel mutation in the triple helical coding domain of the *COL1A1* gene of type I collagen (R780L) causes a mild form of osteogenesis imperfecta (OI). *G. Sun, D. Chen, P. Byers* Dept. of Pathology, Univ Washington, Seattle, WA.

OI is a clinically and genetically heterogeneous disorder characterized primarily by osseous fragility. The clinical range is from very mild to lethal in the perinatal period. About 90% of affected individuals are heterozygous for mutations in one of the type I collagen genes, COL1A1 and COL1A2. Most mutations result in substitution for any one of the 338 glycine residues in the triple helical domain (Gly-Xaa-Yaa)338, alterations at splice sites, frameshifts with premature termination codons, and mutations that affect assembly of the trimer of the procollagen molecule. In a woman with a mild form of OI (25th percentile in height, blue sclerae, modest number of fractures, and some bowing of the tibias) we identified a unique mutation, c.2873G>T, that resulted in substitution of the arginine at position 780 of the triple helical domain (970 of the chain from the initiator methionine) by leucine (R780L), a Y-position substitution. Sequence of the full length of the cDNA from both genes revealed no additional mutations. Neither of her parents was affected. She had one pregnancy with the same mutation that had a large encephalocele as well as evidence of bony abnormalities. A healthy infant who did not receive the mutant allele was born subsequently. Cultured dermal fibroblasts from the proband produced some normal type I procollagen molecules and others that had been overmodified amino terminal to the region of the mutation. The mutation was cloned and expressed in the context of a full length cDNA and the expressed protein had a mobility shift that reflected the charge change. Of the 31 leucine residues in the triple helical domains of the pro1(I) and pro2(I) chains of type I procollagen only one occurs in the Y-position. Substitution experiments in peptides suggest that leucine may destabilize the helix when in the Y-position, and may slow helix propagation in a local region. This is one of a very small class of mutations in the X or Y-positions that leads to OI. Both overmodification and disruption of interactions with other matrix molecules may be important in producing bone abnormalities.

Cluster analysis of extended Y-STR haplotypes leads to discovery of a large and widespread subclade of Y Haplogroup J2. *B.E. Schrack[1], T.W. Athey[2], J.F. Wilson[3]* 1) College of Information Studies, University of Maryland, College Park, MD; 2) Retired, Brookeville, MD; 3) Public Health Sciences, University of Edinburgh, Scotland.

Cluster analysis of extended Y-STR haplotypes within Y Haplogroup J2 has led to the identification of a new subclade of J2 that is widespread, and substantial in size. The new subclade is defined by a deletion that includes part of the Y-STR marker, DYS445. The presence of the deletion is revealed when testing of DYS445 results in apparent repeat values of six, or rarely seven, instead of the values of 11 or 12 typically found in J2. Testing of Y-SNPs within haplogroup J2 in a selected group of subjects has shown that the new subclade is within Haplogroup J2a1 (as defined by Sengupta et al, Am J Hum Genet, 78:202-221, 2006). We have named the clade J2a1k. J2a1k represents approximately one-fifth of all J2 Y chromosomes in northwest Europe. An analysis of the geographic origins of members of the subclade shows that J2a1k is widely distributed within the range of Haplogroup J2 in Europe and Turkey.

Development of a high-throughput assay for the identification of small molecule inhibitors of human galactokinase. *K.J. Wierenga, K. Lai* Dr John T Macdonald Fndn Ctr Med Gen, Univ Miami, Miami, FL.

Introduction. Classic galactosemia (OMIM 230400) is caused by deficiency of galactose-1-phosphate uridylyltransferase (GALT; EC 2.7.7.12), resulting in the unique accumulation of galactose-1-phosphate (gal-1-p). Due to endogenous production of galactose adherence to galactose-free diet does not prevent long-term sequelae, which include developmental delay, ataxia, speech dyspraxia and premature ovarian failure. Since galactokinase deficiency (OMIM 230200) is a comparatively benign condition not associated with complications other than cataracts, we hypothesized that an inhibitory molecule of galactokinase (GALK, EC 2.7.1.6) activity will reduce conversion of endogenously produced galactose to gal-1-p, and potentially prevent long-term sequelae in patients with classic galactosemia. **Materials/Methods.** Human galactokinase was purified using Nickel affinity chromatography on a lysate from E. coli strain BL21 (DE3) (Novagen) over-expressing the human GALK gene (cDNA clone obtained from I.M.A.G.E. consortium, ID: 3501788). We used PlateMate Plus High Throughput 384-Channel Automated Pipetting System (Matrix Technologies Corporation) and EnVision Multilabel Plate Reader (PerkinElmer) to miniaturize the GALK assay: galactose + ATP gal-1-p + ADP. The ATP not consumed during the reaction was measured using Kinase-Glo Luminescent Kinase Assay (Promega): ATP + luciferin luminescence. Z factor was used to assess the suitability for high-throughput screening (HTS) of kinase inhibitors. Inhibition of GALK was tested using the ATP-analog adenosine 5-O-(3-thio)triphosphate (ATPS). **Results.** The optimized assay contained 0.15g GALK, 5mM $MgCl_2$, 60mM NaCl, 20mM HEPES, 30mM galactose, 500mM DTT, 0.5% DMSO and 5M ATP. Assay volume per well was 30L prior to adding Kinase-Glo. Z score was 0.91. There was a 96-fold difference in luminescence between this assay, and a similar assay without GALK. An assay with added ATPS revealed increasing inhibition of GALK activity with increasing concentration of ATPS. **Conclusions.** We established a miniaturized assay for HTS of inhibitors of GALK activity using 0.15g GALK and 5M ATP. The assay identified inhibition by ATPS.

Analysis of Transcriptomes: A Genomics Multimedia Module. *M.I. Roche[1,2], D.J. Lofland[1]* 1) Instructional Media Group, Morehead Planetarium and Science Center, UNC, Chapel Hill, NC; 2) Department of Pediatrics, UNC, Chapel Hill, NC.

Analysis of Transcriptomes is an engaging, inquiry-based module designed for advanced high school students and undergraduates. The module begins by posing the paradoxical question: how can the cells in a single organism look and behave differently even though they all share the same genome? Challenged to compare the transcriptional profiles of genes from dividing cells to those from quiescent cells, students conduct a DNA microarray experiment. After setting up the experiment, they observe the construction of the DNA microarray, obtain samples from experimental and control cells, apply the samples to the microarray, and visualize the data obtained by a laser scanner. When presented with four previously uncharacterized genes, students apply their knowledge to classify each into a functional category. Through an integrated series of narrated audio, 3D graphics, FLASH movies, and interactives, students work at their own pace and check their understanding after each subsection. A final assessment allows instructors to measure student understanding. The summary reiterates the powerful role microarrays play in answering fundamental genomic questions. Delivered over the Internet and accessed using any standard browser equipped with the Macromedia Flash plug-in, the customized, centralized delivery enables easy management and allows real-time updates to the content. The system maintains a constant connection to a back-end server, facilitating the storage and retrieval of student data. Automated tracking allows instructors to identify students' interactions with each aspect of the content and provides Web access to student scores. The module has been evaluated at a math and science high school, a major research university, and two historically, underserved minority colleges (N=95). Results from student and teacher surveys showed high levels of satisfaction with both content and delivery at all sites. Pre-test performance indicated low levels of knowledge about microarrays in comparison to the post-test assessment. This module was supported by NHGRI grant #: 5R42HG002983-03.

Designing optimal two-stage genome-wide association studies. *A.D. Skol, L.J. Scott, G.R. Abecasis, M. Boehnke* Dept Biostatistics, Univ Michigan, Ann Arbor, MI.

Genome-wide association (GWA) is a promising approach to identify common genetic variants that predispose to human disease. Because of the high cost of genotyping hundreds of thousands of markers on thousands of subjects, GWA studies will often follow a staged design in which a proportion ($_{samples}$) of the available sample is genotyped on a large number of markers in stage 1, and a proportion ($_{markers}$) of these markers are followed up by genotyping them on the remaining samples in stage 2. We describe a strategy to identify cost-effective two-stage GWA designs that preserves the power of the one-stage design while minimizing the study cost. We explore how the optimal design and study cost is influenced by differences between stage 2 and stage 1 genotyping costs, the desired genome-wide false-positive rate, and power measured as a proportion the one-stage design's power.

We find that the ratio R of stage 2 to stage 1 per genotype cost dramatically influences both the optimal design and study cost. Increasing R shifts the genotyping burden, and much of the studys cost, to stage 1, both by increasing the proportion of the samples genotyped in stage 1, and by decreasing the proportion of markers to follow-up in stage 2. When stage 1 per genotype costs are constant, increasing R can increase study costs substantially. This cost increase can be partially mitigated by adopting a design with reduced power or by increasing the false-positive rate (while preserving power).

For example, when R is 5 an optimal two-stage GWA study which retains 95% of the one-stage design's power to detect a disease variant of modest effect will genotype 40% of the sample in stage 1 and follow-up 2% of the markers in stage 2 and costs 46% of the one-stage design. If R is 25, the optimal design genotypes 50% of the sample in stage 1, follows up 0.5% of the markers into stage 2, and costs 55% of the one-stage design. By allowing 5 rather than 1 false-positive genome-wide, this study's cost decreases to 50% of the the one-stage design's cost.

Array comparative genomic hybridization analysis of 20 first-trimester spontaneous abortions with normal karyotype. *O. Shimokawa[1,2,3], N. Harada[2,3], N. Miyake[1,4], K. Satoh[4], T. Mizuguchi[3,4], N. Niikawa[1,3], N. Matsumoto[3,4]*
1) Department of Human Genetics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; 2) Kyushu Medical Science, Nagasaki, Japan; 3) Solution-Oriented Research for Science and Technology (SORST), JST, Kawaguchi, Japan; 4) Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan.

We performed array comparative genomic hybridization (aCGH) analysis to detect genomic copy-number changes in 20 first-trimester spontaneous abortions with normal G-banding chromosomes. DNA was extracted directly from frozen villous samples of 20 first-trimester spontaneous abortions (46,XY karyotype: 10 cases; 46,XX karyotype: 10 case). A microarray, containing 2,173 BAC clone with a 1.5-Mb resolution, was constructed and used in the analysis. Two deletions were identified: a 1.4-Mb deletion at 3p26.2-p26.3 and a 13.7-Mb deletion at 13q32.2-qter. Re-examination of chromosome preparations from the sample with the 13q deletion showed a mixture of cells with the 13q- chromosome and those mostly with 46,XX chromosomes, the latter of which is likely to have been derived from maternal decidual cells. Detection rate of genomic imbalance in this series is thus 10% (2/20). The aCGH could successfully detect submicroscopic copy number changes and may overcome drawbacks of tissue culturing and chromosomal analysis, such as tissue culture failures or overgrowth of maternal cells.

Sequencing of *BBS2, BBS4* and *BBS5* genes in non-syndromic obesity patients reveals a small contribution of these genes that is likely to involve a multifactorial model. *A. Slavotinek[1], B. Bogert[2], A. Moshrefi[1], M. Skhiri[3], A. Landin Malt[1], C. Vaisse[3], K. Ashrafi[2]* 1) Dept Pediatrics, UCSF, San Francisco, CA; 2) Dept Physiology, UCSF, San Francisco, CA; 3) Diabetes Center, UCSF, San Francisco, CA.

Bardet-Biedl syndrome (BBS) is a genetically heterogeneous malformation syndrome comprising obesity, retinal dystrophy, polydactyly, learning disability, hypogonadism and renal anomalies. Mutations in eleven genes have been identified with the encoded proteins shown to be involved in ciliary formation; the role of these genes in non-syndromic obesity has not been established. We used RNAi to target the *BBS1-BBS8* gene orthologues in C.elegans and used Nile Red fluorescence to examine the fat content of mutant worms. Inactivation of the worm orthologues of the *BBS1*, *BBS2*, *BBS5* and *BBS8* genes resulted in enlarged fat droplets in the wild-type strain N2 Bristol compared to vector controls at 72 hours after plating. C. elegans mutants for the orthologues of *BBS1*, *BBS7* and *BBS8* also showed a moderate increase in fat. Based on these results and the literature, we screened *BBS2*, *BBS4* and *BBS5* for mutations in human subjects with non-syndromic obesity. We sequenced the coding regions of each gene in 188 severely obese patients with a median BMI of 49 kg/m^2. For *BBS2*, we identified one missense alteration predicting p.R539W that was absent in more than 600 control chromosomes. For *BBS4* and *BBS5*, we have identified six novel sequence alterations that we are examining in controls. The data on BBS gene variations and obesity is so far inconclusive; one study sequenced *BBS6* in 60 men with obesity and identified the rare p.A242S mutation in two families, showing partial segregation of this mutation with the obesity phenotype and concluding that a major role for *BBS6* in non-syndromic obesity was unlikely. However, it has been noted that p.A242S could increase susceptibility to obesity in combination with other predisposing gene variants and it is plausible that the alterations in *BBS2*, *BBS4* and *BBS5* could interact with other genetic or environmental factors to produce obesity according to a multifactorial model of disease. .

Transferability of Tag SNPs between CHB and JPT: A Warning of Performing tSNPs across Genetically Close Populations. *R. Yang*[1], *S. Xu*[1], *L. Jin*[1, 2] 1) MOE Key Laboratory of Contemporary Anthropology and Laboratory of Theoretical Systems Biology, Institute of Genetics, School of Life Science, Fudan University, Shanghai, China; 2) CAS-MPG Partner Institute of Computational Biology, SIBS, CAS, Shanghai, China.

International HapMap project made an effort to generate a whole genome single-nucleotide polymorphisms (SNPs) data base in the hope of providing researchers with tag SNPs (tSNPs) to be used in association studies. Many recent studies evaluated the performance of tSNPs between HapMap populations and those outside the project, all supporting the use of HapMap data in other populations. Here we present a result of evaluating the transferability of tSNPs between CHB and JPT, which are two samples used in HapMap project and put together in all the studies for their genetically close relationship. Our results are based on the data of ten 500-kb ENCODE regions and focused on SNPs with minor allele frequency of more than 15%. We found that the transferability of tSNPs between CHB and JPT is not as high as expected, only 84.9% (80.2-89.5%) from CHB to JPT and 86.8% (83.1-90.4%) from JPT to CHB, in contrast to the higher transferability across Caucasian populations (90-95%). Although the transferability of tSNPs between genetically close populations is higher than that of between those genetically distant (72.6% [60.4-84.8%] from CEU to CHB and 68.5% [55.5-81.6%] from CEU to JPT), our results indicate that people should keep in mind about the information loss when applying tSNPs from one population to others, even between those genetically close populations.

Development of objective tools to assess the number and volume of dermal neurofibromas in adults with Neurofibromatosis 1. *A. Theos[1], A.M. Shih[3], Y. Ito[3], B.S. Burns[2], B.R. Korf[2]* 1) Department of Dermatology; 2) Department of Genetics; 3) Department of Mechanical Engineering, University of Alabama at Birmingham, Birmingham, AL.

Purpose: To evaluate standardized digital photography and surface laser scanning as objective tools for quantifying the number and volume of dermal neurofibromas in adults with NF 1.

Methods:(1) Standardized digital photography was used to assess neurofibroma density. A 100 cm^2 frame was applied to predetermined regions on the back, abdomen, and thigh of adults with NF1. Discrete dermal neurofibromas that measured 2 mm in diameter were marked with a washable marker and counted. Standardized digital photographs were taken. Tumor density was calculated for each of the three body regions.(2) Surface laser scanning was used to calculate the volume of dermal neurofibromas. A defined 100 cm^2 area containing discrete dermal neurofibromas was scanned 4 separate times with a Minolta Vivid 9i laser scanner, which has an accuracy of 0.05 mm. The scanned data was converted to a 3-D triangulated mesh and the volume of three random tumors in each patient was calculated using Rapidform software. The reproducibility of this method was determined.

Results:(1) 31 patients with NF1 were evaluated with standardized digital photography. Tumor density ranged from 0-2.1/cm^2. Significantly higher tumor burden was observed on the back and abdomen.(2) 3 adults with NF1 were evaluated with surface laser scanning. This method proved to be rapid and easy to use. The reproducibility was assessed and the coefficient of variation ranged from 1 to 7.28.

Conclusion: The lack of objective methods for following the growth of dermal neurofibromas is a significant impediment to the study of new preventative and therapeutic measures in NF1. Based on our pilot studies, we conclude that standardized digital photography and surface laser scanning are useful methods for quantifying the number and volume of dermal neurofibromas.

Delineation of breakpoints in patients with 9q34.3 chromosomal rearrangements using fosmid array-CGH. *S. A. Yatsenko[1], S. W. Cheung[1], C. Shaw[1], F. Scaglia[1], A. Patel[1], T. Sahoo[1], G.M. Weissenberger[1], C. Chinault[1], J.R. Lupski[1,2,3]* 1) Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Pediatrics, Baylor College of Medicine; 3) Texas Children Hospital, Houston, TX.

Cryptic unbalanced subtelomeric rearrangements are emerging as an important cause of mental retardation and malformation syndromes. Constitutional disorders of 9q34.3 including terminal and interstitial microdeletions, duplications, derivative chromosomes and complex rearrangements are relatively frequent. Recently, we presented the FISH mapping of breakpoints in 11 patients with 9q34.3 microdeletion syndrome using BAC clones, and identified an ~700 kb critical region encompassing *EHMT1* (euchromatic histone methyltransferase) and *CACNA1B* associated with a minimal phenotype (Yatsenko et al, 2005). To further refine the location of the breakpoints and gain insight into the recombination mechanisms leading to 9q34.3 chromosomal instability we constructed a CGH-microarray using 239 fosmid clones spanning ~ 8 Mb of the most distal 9q region as well as subtelomeric regions of all chromosomes. Using this approach we studied a total of 14 rearrangements of 9q34.3 including 8 deletions, 2 duplications, 3 derivative chromosomes and an unbalanced translocation in a fetus. The majority of 9q34 rearrangements occur *de novo*, except in one patient who inherited a der(9)t(9;13)(q34.3;p12) from a balanced carrier mother. A CGH-based rapid identification of cytogenetically undetected 9q34 chromosomal imbalances demonstrates their complexity, and delineates the breakpoints to a 30-40 kb resolution in each case studied. Our investigation reveals that duplications of 9q34 vary considerably in size, in contrast with deletions that appear limited to the 9q34.3 region; for both types of rearrangement there is no single common breakpoint. Further cloning and sequencing of breakpoints is ongoing to gain insights concerning the mechanism of formation of terminal rearrangements and to unravel dosage sensitive genes potentially responsible for the observed phenotypes.

The role of inflammatory genes in a gastric precancerous lesion. *Y.Y. Tsai[1], S.W. Wang[2], I.C. Wu[2], R. Wu[1], D.C. Wu[2], S.H. Juo[1,3]* 1) Graduate Institute of Medical Genetics, Kaohsiung Medical University, Taiwan; 2) Division of Gastroenterology, Kaohsiung Medical University Hospital, Taiwan; 3) Department of Clinical Research, Kaohsiung Medical University Hospital, Taiwan.

INTRODUCTION: Intestinal metaplasia (IM) is a precancerous lesion of gastric cancer. Inflammation may play a role to initiate the gastric carcinogenesis, and therefore, inflammation may also be involved in the development of IM. The aim of the study is to evaluate the relationship between three inflammatory genes and IM.

METHODS: A case-control study with 190 IM and non-IM subjects was conducted. All controls did not have IM pathological findings for two or more consecutive biopsy examinations during a 3-year or longer follow-up. Single nucleotide polymorphisms (SNPs) were selected for genotyping using the Taqman technology. We selected one functional promoter SNP (i.e. SNP1) and one tSNP (i.e. SNP2) at the COX-2 gene, two tSNPs (SNP3 and SNP4) at the IL-6 gene and, two tSNPs (SNP5 and SNP6) at the IL-10 gene from the HapMap project. The genotype data were analyzed by using chi-square test. We also used the Hap-clustering program to perform the haplotype analysis.

RESULTS: The six SNPs in cases and controls were all in Hardy-Weinberg equilibrium. The statistical results showed significant association at IL-6 SNP3. Compared with the reference TT genotype, the G carriers had an OR of 1.62 (95%CI: 1.07~2.46, p=0.024). A borderline significance was found at IL-6 SNP4:the G carriers of SNP4 had an OR of 1.51(95%CI: 0.98~2.32, p=0.06) compared with the reference AA genotype. The haplotype analysis for IL-6 polymorphisms also reached a statistical significance (p=0.046). However, there was no statistical significance for either of the COX-2 or IL-10 polymorphisms.

CONCLUSIONS: The IL-6 gene may play a moderate role in the development of IM in the Taiwanese population. More subjects will be recruited in the near future to provide a more solid result.

Allele frequencies of CYP2C19 and CYP2D6 in a Japanese population. *T. Uemichi[1], T. Suzuki[1], T. Nobori[2], N. Yasui-Furukori[3], M. Mizuno[4], S. Shirahama[5], Y. Miyoshi[6]* 1) Kinki Central Hospital, Itami, Japan; 2) Department of Laboratory Medicine, Mie University School of Medicine, Tsu, Japan; 3) Department of Neuropsychiatry, Hirosaki University School of Medicine, Hirosaki, Japan; 4) Department of Neuropsychiatry, Toho University School of Medicine, Tokyo, Japan; 5) SRL, INC., Hachioji, Japan; 6) Roche Diagnostics K.K., Tokyo, Japan.

Cytochrome P450 (CYP) 2C19 and 2D6 play important roles in drug metabolism. Recently, polymorphisms in the genes coding these proteins have been shown to be associated with the decreased, or lack of, enzyme activity. To promote the individualized pharmacotherapy based on the genetic difference, the knowledge of the allele frequency of these genes in various ethnic groups is essential. In this study, we have examined the CYP2C19 and CYP2D6 allele frequency in a large-scale Japanese population. This study was conducted at four hospitals located on Honshu, the main island of Japan. One thousand and two subjects participated in the study. CYP2C19 and CYP2D6 genotypes were determined using DNA chip (AmpliChip, Roche Diagnostics) which was designed to detect 3 allelic variants of *CYP2C19* and 32 of *CYP2D6*. DNA sequencing analysis was carried out when the result of genotyping by DNA chip was not conclusive. *CYP2C19* analysis showed that allele frequencies of *1, *2, and *3 in this population were 0.57, 0.36, and 0.13, respectively. According to the result, extensive metabolizers (*1/*1, *1/*2, *1/*3) were 82% while poor metabolizers (*2/*2, *2/*3, *3/*3) were 18%. The *CYP2D6* allele frequencies of *1, *10, *2, *5, *41, and others were 0.40, 0.38, 0.13, 0.06, 0.01, and 0.02, respectively. For CYP2D6, 76.7% of the Japanese population were considered extensive metabolizers, 22.0% intermediate metabolizers, 0.2% poor metabolizers, and 1.1% undetermined phenotype. This is the first study to demonstrate the allele frequencies of the CYP2C19 and CYP2D6 genes in over a thousand Japanese subjects. The CYP2D6 gene shows a number of allelic variants including SNPs, deletions, and duplications in Japanese as previously described in other ethnic groups, and DNA chip is expected to be a powerful tool for genotyping CYP2C19 and CYP2D6 in individualized medicine.

The Effects of Recombination and Mutation on Patterns of Variation surrounding the -globin HbS allele. *E. Wood[1], D. Stover[2], M. Nachman[1], M. Hammer[1,3]* 1) EEB, Univ Arizona, Tucson, AZ; 2) Arizona State Univ, Tempe, AZ; 3) Division of Biotechnology, Univ Arizona, Tucson, AZ.

A longstanding question in population genetics is whether the malarial-resistance -globin HbS allele originated multiple times via recurrent mutation or if the HbS mutation arose once and moved to different haplotypic backgrounds through recombination. We PCR-cloned and sequenced a 5.2-kb region spanning the -globin recombinational hotspot and gene in a sample of 46 HbA and 31 HbS chromosomes. We estimate that recombination rates in the hotspot, which encompasses the HbS allele, are >50-fold higher than the genome average. We infer that a single mutation followed a crossing-over event is sufficient to explain the origin of Senegalese and Bantu HbS haplotypes, while Benin haplotype may have arisen via independent mutation or gene conversion. These results underscore the importance of recombination (crossing-over or gene conversion) in generating haplotypic diversity surrounding the HbS allele.

Audiological manifestations in mexican patients with Down syndrome. *L.Acosta Ramos[1], G. Garcia Sanchez[4], S.G. Juarez Garcia[2], L. Hernandez Gomez[3]* 1) Depto. de Comunicación Humana, CRIS Ameca Ameca, Edo. Mex,; 2) Servicio de Neuropsicologia Infantil. Instituto Nacional de Rehabilitación Mexico D.F; 3) Servicio de Audiologia. Instituto Nacional de Rehabilitación Mexico D.F; 4) Depto. de Investigación. Instituto Nacional de Rehabilitación. Mexico, D. F.

The syndrome of Down is a very frequent trisomía, with an incidence of 1 in 700 births, characterized by facies typical dismórfica with depressed nasal bridge, epicanto, anterior_posterior brachycephaly, variable orofaciales anomalies, fold simiano, mental weakness hipotonía generalized, mental weakness, hipotonía generalized, defects cardiac, from the 40 to 77% of the patients with this syndrome they present/display hearing problems that can be of conductivo type, sensorial or mixed due to congenital malformations and infections of superior respiratory routes due to its anatomical alterations. This study of made with 40 patients with syndrome of Down, to that interrogation was made to them, physical exploration, timpanometría and conventional audiometría, 25 patients were of masculine sex (62%) and 15 Feminine ones (38%), with an age average of 15 years and 5 months, of which 22 presented/displayed superficial normal hearing (55%) and 15 (36%) 12 of conductivo type and flat profile and 3 of sensorial type and slowly descendent pattern, 2 of moderate degree (5%) of conductivo type and flat pattern and 1 (2.5%) of sensorial type and descendent neurosensorial pattern. Most of our patients they presented/displayed hipoacusia of superficial degree of conductivo type and flat audiometrico pattern and a minority of deep degree of sensorial type and descendent superficial slowly descendent pattern as well as some cases of sensorial that they suggest data of presbiacusis.

Chromosome Microarray Analysis (CMA) Detects a Large X Chromosome Deletion Including *FMR1* and *IDS* in a Female Patient with Mental Retardation. *F.J. Probst[1], E.R. Roeder[2], V.B. Enciso[2], M.L. Cooper[1], P. Eng[1], J. Li[1], Y. Gu[1], A.C. Chinault[1], Z. Ou[1], C.A. Shaw[1], S.W. Cheung[1], D.L. Nelson[1]* 1) Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Division of Genetics and Metabolic Disorders, Department of Pediatrics, The University of Texas Health Science Center at San Antotonio, San Antonio, TX.

Chromosome Microarray Analysis (CMA) by array-based comparative genomic hybridization (CGH) is a new clinical test that can screen for hundreds of different submicroscopic chromosomal deletions and duplications. We report here a 6-year-old girl who presented for evaluation of mildly dysmorphic facies and marked developmental delay. She did not walk until 24 months of age and did not speak her first words until 5 years. Physical examination revealed overgrowth, a prominent forehead with nevus flammeus, mildly upslanting palpebral fissures, and a flat nasal bridge. Previous studies included a normal brain MRI; a normal 46, XX karyotype; and Fragile X studies yielding a single allele of 30 CGG repeats. CMA showed a loss in copy number of four clones from the genomic region cytogenetically defined as Xq27.3-Xq28. This loss was also seen by FISH analysis. Further studies have confirmed a complete loss of one copy each of the *FMR1* and *FMR2* genes (which are mutated in Fragile X Syndrome and FRAXE Syndrome, respectively) and at least a partial deletion of one copy of the *IDS* gene (which is mutated in Hunter Syndrome). Skewed X-inactivation has been previously reported in females with deletions in this region and can lead to a combined Fragile X/Hunter Syndrome phenotype in affected girls. A possible diagnosis of Hunter Syndrome is therefore being pursued, as enzyme replacement therapy is possible for this disease. Currently, efforts are underway to define precisely the childs deletion breakpoints, to determine this childs X-inactivation status (normal vs. skewed), and to assess her IDS activity. This clinical case demonstrates the utility of CMA for both making the diagnosis of a submicroscopic chromosomal deletion and for suggesting further testing that could possibly lead to therapeutic options for patients with developmental delay.

The quantification of the allelic variations of gene expression in peptidylarginine deiminase type 4 (PADI4). *A. Suzuki[1], Y. Kochi[1], K. Kobayashi[1], K. Hayashi[2], R. Yamada[1, 3], K. Yamamoto[1, 4]* 1) SNP Research Center, RIKEN, Yokohama City, Kanagawa, Japan; 2) Division of Genome Analysis, Research Center for Genetic Information, Kyushu University, Fukuoka, Japan; 3) Center for Genomic Medicine, Kyoto University, Kyoto, Japan; 4) Department of Allergy and Rheumatology, Graduate School of Medicine, the University of Tokyo, Tokyo, Japan.

Recently, multiple studies have shown that human single nucleotide polymorphisms (SNPs) affect gene regulation, resulting allelic imbalances of gene expression. The finding of these regulatory SNPs leads to understanding phenotypic diversity and the identification of alleles that modify disease risks. Therefore, it is important to quantify the allelic variations of gene expression in vivo for identification of regulatory SNPs. For measurement of these variations, we performed TaqMan real-time RT-PCR system and PLACE-SSCP system using cDNA from tissues of heterozygous individuals. In this study, we examined allele-specific gene expression in PADI4, which was reported to be associated with rheumatoid arthritis (RA), and of which allelic imbalance of the gene expression was observed. We used TaqMan probes, rs11203366 (G/A) as a marker located in exon and also used this SNP as a target for PLACE-SSCP analysis. Briefly, we measured the signal intensity using cDNA and DNA from heterozygous individuals, and calculated allele-specific gene expression ratio. We performed these analyses using cDNA from peripheral blood leukocyte of heterozygous individuals and showed difference of allele-specific PADI4 expression in vivo. This data supported the hypothesis that expression levels of PADI4 are associated with RA susceptibility. In this study, we also indicated that TaqMan assay was available for quantification of the allelic variation in gene expression with high accuracy. In addition, the method based on TaqMan assay can be used for the high-throughput assay of allelic gene expression in vivo. Further advances of analysis of allele-specific gene expression will contribute to understanding of transcriptional gene regulation and will provide strategies for studying complex traits, including common diseases.

A lethal autosomal dominant defect of mitochondrial and peroxisomal fission. *H.R. Waterham[1], J. Koster[1], C.W.T van Roermund[1], P.A.W. Mooijer[1], J.V. Leonard[2], R.J.A. Wanders[1]* 1) Laboratory Genetic Metabolic Diseases (FO-224), Academic Medical Center/Univiversity of Amsterdam, Netherlands; 2) Department of Pediatrics, Institute of Child Health, University College London, United Kingdom.

Mitochondria form a dynamic network which is subject to continuous fusion and fission processes for which several proteins involved have been identified. We report a deceased newborn female who had microcephaly, abnormal brain development, optic atrophy and hypoplasia, failure to thrive, persistent lactic academia, and raised plasma very long chain fatty acids. Extensive laboratory investigations revealed a defect in the fission of both mitochondria and peroxisomes due to a single heterozygous, but dominant negative c.1184C>A (p.A395D) mutation in the DLP1 gene. DLP1 codes for the dynamin-like protein DLP1, previously shown to be involved in the fission of these two organelles. Over-expression of mutant 395D-DLP1 in control fibroblasts resulted in the aberrant mitochondrial phenotype, whereas over-expression of normal 395A-DLP1 in fibroblasts of the patient reversed the aberrant mitochondrial phenotype to normal. The autosomal dominant inheritance of the observed defect was confirmed by the absence of the c.1184C>A mutation in the DLP1 gene of the patients parents. Our finding represents the first patient from a new class of disease with a combined defect in both mitochondria and peroxisomes.

Pseudoxanthoma Elasticum-like disorder with generalized cutis laxa and clotting deficiency represents a novel genetic entity. *O.M. Vanakker[1], L. Martin[2], D. Gheduzzi[3], B.P. Leroy[1], V. Guerci[4], P. Coucke[1], I. Pasquali-Ronchetti[3], A. De Paepe[1]* 1) Ctr. Medical Genetics, Ghent University Hospital, Belgium; 2) Dept. of Dermatology, Porte-Madeleine Hospital, France; 3) Biomedical Sciences Dept., University of Modena and Reggio Emilia, Italy; 4) IRCCS Burlo Garofalo, Metabolic Disorders, Italy.

OBJECTIVE: Pseudoxanthoma Elasticum (PXE) is an autosomal recessive disorder characterized by oculocutaneous and cardiovascular manifestations. The causal ABCC6 gene (chrom. 16p13.1), encodes an ATP-dependent transmembrane transporter. We performed mutation analysis in over 150 patients and identified mutations in all but five individuals. Although these patients showed fragmented and calcified elastic fibres typical for PXE, they revealed a severe, progressive and generalized skin laxity (resembling cutis laxa) but only mild retinopathy. Moreover, all five had a deficiency of the vitamin K-dependent clotting factors. We performed a detailed study to determine whether this condition is genetically distinct from PXE. MATERIAL AND METHODS: Direct sequencing was performed for ABCC6. In addition, , we also performed molecular analysis of VKORC1 and GGCX. Both candidate genes cause hereditary deficiency of the vitamin K-dependent clotting factors (VKCFD1&2). RESULTS: Although microscopical findings in the dermis were indistinguishable from PXE, at the ultrastructural level the organization of the mineralized elastin was found to be different. Direct sequencing revealed causal mutations neither in ABCC6, nor in VKORC1. In contrast, GGCX harboured missense mutations in four out of five patients. The GGCX enzyme, important for -carboxylation of gla-proteins such as the vitamin K-dependent clotting factors, is involved in bone mineralization and in preventing calcium precipitation in soft tissue. As such, it may play a role in the aberrant elastic fibre mineralization in this phenotype. CONCLUSION: In view of the differences in clinical and ultrastructural findings and the absence of ABCC6 mutations, we conclude that this disorder is genetically separate from PXE or cutis laxa. Our data suggest GGCX as a good candidate gene for this disorder.

Determination of parental origin of *de novo* mutations that were detected in the past protein studies of Atomic bomb survivors children. *Y. Satoh, E. Nishikori, N. Takahashi* Dept. Genetics, RERF, Hiroshima, Japan.

We have studied the effects of atomic bomb radiation on human germ cells. During 1975 to 1984, a large-scale survey was conducted at RERF to detect genetic effect of radiation by screening serum and erythrocyte proteins with altered electromobility on starch gel electrophoresis in the offspring (and the parents), which is most likely due to amino acid substitutions. Total five *de novo* mutations [phosphoglucomutase 2 (PGM2), nucleoside phosphorylase (NP), 6-phosphogluconate dehydrogenase (6PGD), adenosine deaminase (ADA), and glutamic-pyruvate transaminase (GPT) genes] were detected after testing more than 1 million loci from about 23,000 offspring consisted of over .005 Gy exposed group and under .005 Gy control group. Although it was not possible in those days to determine the parental origin of the mutations, it is now possible to do it in the post genomic era. For this purpose, lymphoblastoid cell lines consisting of offspring-mother-father trios were established from 4 families. The observed electrophoretic mutations were confirmed by base sequencing followed by determination of polymorphic sequences located near the mutation sites to determine the parental origin. About NP, offspring had a G747A (Arg > His) mutation which is consistent with the electrophoretic phenotype, and parents did not have. And then neighboring SNPs of NP mRNA were examined. G266 and C286 existed on the same chromatid which mutation existed. They were inherited only from father not mother to offspring. It suggests that this mutation occurred in a gamete of paternal origin. 6PGD and GPT had a T1049G (Leu > Arg) and G699A (Gly > Arg) mutation respectively, which are consistent with the electrophoretic phenotypes. But, there have not been distinguishable neighboring inherited SNP among the family, so far. In this study, these methods can detect the parental origin of *de novo* mutation. Further characterization experiments are underway to distinguish mutation rate of exposed and non-exposed gametes.

Chromatin immunoprecipitation identifies direct neural targets of a gene implicated in human speech and language disorder. *S.C. Vernes[1,2], E. Spiteri[3], J. Nicod[2], M. Groszer[2], K.E. Davies[1], D. H. Geschwind[3], S.E. Fisher[2]* 1) Physiology, Anatomy & Genetics, University of Oxford, UK; 2) Wellcome Trust Centre for Human Genetics, University of Oxford, UK; 3) UCLA Department of Neurology, UCLA, USA.

Heterozygous mutations in the human FOXP2 transcription factor cause problems with sequencing orofacial movements during speech (verbal dyspraxia) plus a wide-range of expressive and receptive language deficits. Mutations reported include a missense change yielding a substitution in the DNA-binding domain, and a nonsense mutation that dramatically truncates the protein. Identification of neural genes regulated by FOXP2 will shed light on molecular pathways that can go awry in verbal dyspraxia, suggest novel candidate genes for mutation testing in language-related disorders, and provide fundamental insights into the neurogenetic bases of speech and language. Here, we detail the isolation of candidate targets in neuronal-like cell culture using chromatin immunoprecipitation (ChIP), followed by validation of these candidates in these cells and in mice carrying Foxp2 point mutations mimicking those implicated in the human disorder (*Groszer et al, manuscript in preparation*). We adopted two FOXP2-ChIP strategies; i) immunoprecipitated chromatin was characterised via shotgun sequencing. *In silico* analysis was used to prioritise fragments near 5 ends of coding regions and containing predicted FOXP2 binding sites, ii) DNA from FOXP2-ChIP was applied to promoter arrays for high-throughput identification of target sites (ChIP-chip). This technique provided more rapid and comprehensive screening but promoter arrays usually cover only 1-5 kb spanning the start site of a subset of genes. Therefore shotgun screening allows for detection of genomic regions not covered by the arrays and the two techniques represent complementary approaches. Validation included the use of EMSA binding assays, and quantitative RT-PCR to demonstrate regulation of candidates by FOXP2. The biological significance of candidates was evaluated *in vivo* by studying CNS expression of Foxp2 mutant mice, providing the first *in vivo* confirmation of Foxp2 targets.

Balanced 1;21 translocation associated with Kleine Levin syndrome. *B. Tuysuz[1], L. Telvi[2], O. Kaya[1], N. Mütevelli[3], H. Kaynak[4]* 1) Istanbul University,Cerrahpasa Medical School, Department of Pediatric Genetics Istanbul, Turkey; 2) Hôpital St. Vincent de Paul, Laboratoire de Cytogénétique Constitutionnelle, Paris, France; 3) Istanbul University, Istanbul Medical School, Department of Child Psychiatry, Istanbul, Turkey; 4) Istanbul University, Cerrahpasa Medical School, Department of Neurology, Istanbul, Turkey.

The Kleine-Levin hibernation syndrome (MIM 148840) is a rare disorder that usually affects males and is characterized by episodic hypersomnia, increased feeding and aberrant behavioral / psychiatric disturbances. The case of an adolescent boy (13 year-old) was referred to our department with a diagnosis of Kleine-Levin syndrome. He was presenting with recurrent sleepiness and hyperphagia. He had a sleeping and eating period for 7-10 days followed by an awakeness period. Between episodes he was normal. He had fall down from 10 meter when he was 2 year-old. On physical examination, weight, height and head circumference were (>97p), (50-75p) and (97p), respectively. He had no major and minor abnormalities. At the end of two years, he had episodes with progressively milder manifestations under lithium and valproate therapy. Chromosome analysis from blood lymphocyte revealed de novo balanced translocation [46,XY, t(1;21)(p12;q22.2)]. FISH analysis was done using two whole chromosomes 1 and 21 painting probe and confirmed the balanced translocation. Etiology and pathogenesis of this syndrome are unknown. Precipitated factors such as infection, head trauma and serious medical and psychiatric diseases in 61% of the patients have been reported. Premature centromere division with chromatid puffing in area of constitutive heterochromatin only in a women with periodic hypersomnia had been described. She had also short stature and late menarche like Turner syndrome clinical findings and her chromosomes showed a mosaicism in 45,X/46,XX. Other chromosomal abnormalities had not been reported in Kleine-Levin syndrome up to date. The balanced translocation may be a coincidence in this patient. On the other hand, Kleine-Levin syndrome-associated genes can be localized to the breakpoints on the chromosome 1 and 21.

Estimation of allele frequencies of 100K SNPs in 500 individuals by combination of the pooled DNA method and the 100K SNP Array: Identification of a possible susceptibility gene for Parkinsons disease. *W. Satake[1], S. Ishikawa[2], M. Yoshida[1], I. Mizuta[1], M. Yamamoto[3], N. Hattori[4], M. Murata[5], H. Aburatani[2], T. Toda[1]* 1) Div Clinical Genetics, Osaka Univ Grad Sch Med; 2) Div Genome Sci, RCAST, Univ Tokyo; 3) Dept Neurol, Kagawa Pref Central Hosp; 4) Dept Neurol, Juntendo Univ Sch Med; 5) Dept Neurol, NCNP, Japan.

Genotyping large numbers of SNPs on large sample sizes is required to perform genome-wide association studies, but is very expensive and laborious. To solve this problem, we combined Affymetrix 100K SNP Array (100K Array) with the pooled DNA method, and made it possible to estimate allele frequencies of 100K SNPs in 500 individuals in a short period and by low cost. In order to assess the reliability of quantification of the 100K Array, we mixed 2 DNA samples at various proportions (1:4, 2:3, 3:2, 4:1), assayed these mixed samples on the 100K Array, and compared the relative allele signals (RAS) with the calculated allele frequencies. The RAS were highly correlated with the calculated allele frequencies (average of 0.989). We concluded that allele frequencies of the pooled DNA could be estimated using RAS. We therefore used this approach in order to identify susceptibility genes for idiopathic Parkinsons disease (PD). A PD pool and a control pool were prepared from the DNA of 500 PD patients and 500 controls respectively. Each pool was assayed on six separate 100K arrays to average experimental variances. We estimated allele frequencies with RAS, and selected SNPs which shows associations with PD. A SNP in -*synuclein*, which we recently reported to be a susceptibility gene for PD (HMG 15, 2006), was also included with the significant association (estimated $P10^{-8}$). Selected SNPs were genotyped individually for more than 800 PD patients and controls respectively using TaqMan assay. One SNP showed a significant association ($P=6.6x10^{-4}$). We performed association studies with the tagSNPs surrounding this SNP, and found one with a more significant association ($P=5.7x10^{-5}$). Our approach made it possible to perform effective genome-wide association studies in a short period and by low cost.

Identification of new and recurrent glucokinase mutations in Belgium and Luxembourg. *W. Wuyts[1], D. Beckers[2], M. Craen[3], C. de Beaufort[4], K. Dahan[5], M. Giri[6], A. Van den Bruel[7], C. Mathieu[8], L. Vits[1]* 1) Dept Medical Genetics, Univ Antwerp, Antwerp, Belgium; 2) Department of Paediatrics, University of Louvain (Yvoir) and University of Leuven (KUL), Belgium; 3) Department of Paediatrics, University Hospital Ghent, Belgium; 4) DECCP, Department of Paediatrics, CHL, Luxembourg; 5) Centre for Human Genetics, Université Catholique de Louvain, Brussels; 6) Department of Internal Medicine and Endocrinology, Algemeen Ziekenhuis Sint Jan, Brugge, Belgium; 7) Department of Endocrinology, University Hospital Gent, Belgium; 8) Department of Endocrinology, University of Leuven (KUL), Leuven, Belgium.

Glucokinase (GCK) is a key regulatory enzyme in the pancreatic beta cell acting as a glucose sensor which regulates insulin secretion. Mutations in the GCK gene encoding the glucokinase protein are responsible for the autosomal dominant Maturity Onset Diabetes of the Young type 2 (MODY2). After clinical examination, 161 Belgian and Luxembourg patients belonging to 124 families were selected for their clinical characteristics suggestive for MODY. Biochemical and clinical parameters including fasting glucose, HbA1c, BMI, age of diagnosis and treatment are presented. Molecular analysis of the GCK gene was performed by sequencing analysis of exon 1a and exons 2-9. In 33 probands, 19 different GCK mutations were identified, of which 11 have not been described previously. Among these, 6 were missense mutations (p.Phe152Leu, p.Ala188Val, p.Met202Arg, p.Asn231His, p.Leu315Phe and p.Cys434Phe), 3 were frameshift mutations resulting in premature termination of translation (c.171delG, c.1261delG and c.663-673dupGGTCGGCATGA) and the last two novel alterations were the splice site mutations IVS6-1 G/A and IVS6-6 C/A. Two recurrent mutations, p.Cys129Tyr and p.Ala378Thr, were identified in our patient population where they represent 42.4% of the GCK mutations identified. In our study 26,6 % of the 124 probands showed a GCK mutation, demonstrating that GCK mutations are a frequent cause of MODY presentation in the Belgian/Luxembourg diabetic patient population.

Severe myoclonic epilepsy of infancy: the impact of a mutation screening service on clinical management. *S. Stenhouse[1], R. Birch[1], L. Conlin[1], S. Zuberi[2]* 1) Inst Medical Genetics, Yorkhill Hosp, Glasgow, United Kingdom; 2) Fraser of Allander Neurosciences Unit, Royal Hospital for Sick Children, Glasgow, United Kingdom.

Severe Myoclonic Epilepsy In Infancy (SMEI) / Dravets Syndrome is an epilepsy syndrome characterised by early onset prolonged generalised, or unilateral clonic seizures usually induced by fever. During the first few years of life a number of seizure types arise, these include; generalised tonic-clonic seizures (GTCS), generalised clonic seizures (GCS), alternating unilateral clonic seizures, myoclonic seizures, atypical absences, obtundation status, and focal seizures. Developmental delay and/or regression occur concurrently with onset of first seizure. Mutations in the sodium channel gene SCN1A are commonly found in SMEI patients and the discovery of such mutations allows a definitive diagnosis to be made much earlier in the progress of the disorder. Having a diagnosis can in turn guide the clinical management of the patient allowing the clinician to focus on the few medications that have been shown to be helpful in this syndrome. These are rarely used in other epilepsies and include stiripentol and the bromides. Better seizure control may potentially prevent or ameliorate the cognitive decline associated with SMEI with obvious benefits to the patient. With the help of a grant from the Muir-Maxwell Fund, SCN1A mutation searching has recently been established in the West of Scotland Regional Molecular Genetics Department for patients suffering from SMEI. This service has been set up as a collaboration between the molecular genetics service in Glasgow and Dr Sameer Zuberi, Consultant Paediatric Neurologist and lead clinician for Scottish Paediatric Epilepsy Managed Clinical Network. SCN1A has 26 exons (7848bp) which are amplified in 40 fragments. A pre-screen is performed using Conformational Sensitive Capillary Electrophoresis (CSCE) and fragments containing apparent changes are sequenced. To date we have tested and reported on 51 individuals including 7 parents of children with SMEI. 7 mutations have been identified in affected patients but none in the parents confirming previous evidence that the majority of mutations are de novo. (Claes et al 2001) Unless their seizures were previously well controlled the finding of a mutation in a patient allows treatment to be altered. Three patients who are now on revised treatment as a result of finding a mutation already show clear improvement. Others are still being titrated up on dosage but early signs are hopeful. From this initial study it is clear that mutations are found in those patients who exhibit particular clinical features and in order to target testing most effectively Dr Zuberi has designed a questionnaire for referring clinicians which is now in use. We will report further on mutation detection rates before and after the use of the questionnaire. Claes et al, 2001. De novo mutations in the sodium channel gene SCN1A cause severe myoclonic epilepsy of infancy. Am J Hum Genet. 68:1327-1332.

Extensive in silico analysis of pathogenic *NF1* splicing defects identified by RNA-based mutation analysis uncovers predictors for splicing outcome of 5 splice-site disruption. *K. Wimmer[1], X. Roca[2], H. Beiglboeck[1], T. Callens[3], R.R. Atmakuri[2], J. Etzler[1], C. Fonatsch[1], L. Messiaen[3]* 1) Department of Human Genetics, KIMCL, Medical University of Vienna, Austria; 2) Cold Spring Harbor Laboratories, Cold Spring Harbor, NY; 3) Department of Genetics, UAB, Birmingham, AL.

Here we describe 94 pathogenic *NF1* gene alterations identified in a cohort of 97 Austrian neurofibromatosis type 1 patients meeting the NIH criteria. All mutations were fully characterized both at the genomic and mRNA transcript level. Thirty-six (38%) of the identified mutations altered pre-mRNA splicing and fall into five groups: exon skipping as a result of mutations at the authentic splice-sites (type I), cryptic exon inclusion caused by deep intronic sequence alterations (type II), creation of novel splice-sites causing loss of exonic sequences (type III), activation of cryptic exonic and intronic splice-sites due to the disruption of the natural splice-sites (IV) and exonic sequence alterations causing exon skipping (type V). The splice-site strengths of all wild-type, mutated and cryptic splice-sites were estimated using four different algorithms. With very few exceptions, they readily predict a dramatic decrease in splice-site strength upon mutation and an increase at sites of sequence alterations creating novel splice-sites. However, cryptic splice-sites used upon disruption of the natural splice-sites generally have a substantially lower calculated strength than natural splice-sites. Extensive *in silico* analyses of 37 *NF1* exons and their surrounding intronic sequences uncovered that the availability of a cryptic splice-site combined with a strong natural 3 splice-site are the main determinants for cryptic splice-site activation upon 5 splice-site disruption. Furthermore, the exonic sequences downstream of authentic exonic cryptic 5 splice-sites resemble more intronic than exonic sequences with respect to ESE/ESS density, providing an additional predictive parameter. In summary this study identifies valuable predictors for the outcome of 5 splice-site disruption, but also underscores the indispensable application of RNA-based techniques for most effective and reliable *NF1* mutation analysis.

A R184Q mutation in the GJB2 gene causes autosomal dominant nonsyndromic hearing impairment in a large Chinese Family. *R. Yang[1], G.R. Zhang[2], X. Ren[1], C. Zhou[2], J.Y. Liu[1], Q.K. Wang[1,3], M.G. Liu[1]* 1) Center for Human Genome Research and College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, Hubei, 430074, P.R. China; 2) The first affiliated hospital of jilin university, Jilin, P.R. China; 3) Department of Molecular Cardiology, The Cleveland Clinic Foundation, Cleveland, OH 44195, USA.

Approximately one in 1000 children is affected by severe or profound hearing loss at birth or during early childhood. GJB2 on chromosome 13q11, which encodes the gap-junction protein Connexin 26 (Cx26), is the most important deafness causative gene. Mutations in the GJB2 gene not only responsible for as much as 50% cases with autosomal recessive Hearing loss, but also cause autosomal-dominant hearing loss in a small number of families. In this study, we identified a 8 generation Chinese family with congenital autosomal dominant nonsyndromic hearing impairment, 74 individuals in the family are affected with bilateral, moderate to severe hearing impairment, exhibiting intrafamilial variability for the onset and severity of hearing loss. Linkage analysis revealed that the causative mutation in the family is linked to the DFNA3 locus. Direct DNA sequence of whole coding region and exon -intron boundary of GJB2 showed a heterozygous G to A transition at nucleotide 551, resulting in an arginine to glutamine substitution at codon 184 (R184Q). PCR-RFLP analysis showed that the R184Q mutation co-segregated with all affected individuals, and was not present in unaffected members in the family and 200 controls. Our data first provide strong evidence suggested that R184Q mutation of GJB2 gene can cause autosomal dominant nonsyndromic hearing impairment.

A computerized interactive database for diagnosis and study of Atypical Spinal Muscular Atrophies. *L. Viollet[1], I. Maystadt[2], N. Brahimi[1], S. Quijano-Roy[3], A. Munnich[1]* 1) INSERM U781, Hopital Necker, Paris, France; 2) Universite Catholique de Louvain, Bruxelles, Belgique; 3) Hopital Raymond Poincaré, Garches, France.

Atypical Spinal Muscular Atrophies (SMA), also named non-5q-linked-SMAs, form a very heterogeneous group of rare disorders characterized by progressive anterior horn cell degeneration, paralysis, denervation, and amyotrophy. Diagnosis of Atypical SMA is assessed upon clinical and electrophysiological critera and classification is based on the distribution of paralysis, age at onset and mode of inheritance. To date, many phenotypes have been described in case reports or series but the diagnosis of these rare disorders is usually hardly made. The main reasons are the lack of information due to the rarety of the phenotypes and the lack of an easily accessible and well documented database for clinicians. Only few genes have been identified and genetic research is hampered by the small number of informative families. To proceed in Atypical SMAs, we intend to set up an international interactive computerized database, allowing the collection of clinical and biological data from patients of both sexes and of all ages, though they may be few and dispersed geographically and in time, facilitating the emergence of research projects. These informations will allow to study the natural history of the diseases and to construct correlations genotype/phenotype, improving the quality of diagnostic approach. The database will be accessible to neurologists, neuropaediatricians and geneticists of the Neuromuscular Centers in France and Europe and could be extended to the worldwide community. The project vill be granted by the french Agence Nationale Pour la Recherche and the Association Française contre les Myopathies. The goal of this project is to improve the knowledge of the mecanisms leading to motor neuron degeneration by the identification of novel proteins involved in motor neuron disorders. Characterization of novel mutations in Atypical SMAs should allow a better understanding of the motoneuronal physiopathology, an important step towards the developement of specific treatments. Email: viollet@necker.fr.

Expression variation of HSA21 genes in Down syndrome and control individuals: understanding phenotypic variability in DS patients. *P. Prandini[1], R. Lyle[1,2], M. Gagnebin[1], C. Gehrig[1], S. Deutsch[1], S.E. Antonarakis[1]* 1) Dept Genetics & Development, Univ Geneva, Geneva 4, Switzerland; 2) Norwegian Institute of Public Health, Oslo, Norway.

Down Syndrome (DS) individuals display considerable phenotypic variability. Major goals in DS research are the identification of the underlying causes of phenotypic variation and of genes involved in specific DS phenotypes. Since DS is a disease caused by alterations of gene dosage, gene expression variation of human chromosome 21 (HSA21) genes in DS and normal individuals is likely to have a substantial impact on pathogenesis and phenotypic penetrance. We studied gene expression variation on 14 lymphoblastoid (LCLs) and 17 fibroblast cell lines from DS individuals and an equal number of age, sex and ethnic matched controls. Gene expression was assayed using Taqman qRT-PCR on a total of 105 HSA21 expressed genes and 20 non-HSA21 genes. Analysis of expression levels in LCLs, revealed a general over-expression of HSA21 genes in DS patients compared to normal. Around 43% of genes showed a statistically significant difference between DS and normals ($p<0.005$), with an average upregulation of 1.66 fold. According to the expression levels, we broadly classified genes in three categories: (a) 60 genes with no significant differences in gene expression between DS and controls, (b) 40 genes with significant over-expression and minor overlapping distributions and (c) 5 genes with significant over-expression in DS individuals but with largely overlapping distributions. We hypothesize that genes in category (b) could be involved in the constant features of trisomy 21, while genes in category (c) are likely to be involved in variable DS traits. We are currently performing a similar analysis in the fibroblasts cell lines. This study provides the first extensive data set on HSA21 gene expression variation in DS and addresses its potential role in underlying phenotypic variability observed in this disorder.

Screening for mutations in NEMO in a large cohort of Incontinentia Pigmenti patients. *M.V. Ursini, F. Fusco, G. Fimiani, A. Pescatore, G. Napolitano, M.G. Miano, M. D'Urso* "Institute of Genetic and Biophysics A.B. Traverso" CNR, Naples, Italy.

Incontinentia Pigmenti (IP) is an X-linked genodermatosis, lethal for males and presents in females with abnormal skin pigmentation and high variable clinical signs. IP results from mutations in NEMO. This gene encodes for the NF-kB essential modulator/IKKgamma (IkB kinase-gamma) required for the activation of the transcription factor NF-kB, which is central for many immune and apoptotic pathways. The genomic deletion of exons 4-10 of NEMO accounts for 80% of molecular IP phenotype. Loss-of-function mutations are lethal in males while the females survive for an extremely skewed X-inactivation pattern. Less deleterious mutations that preserve the NEMO activity can result in male affected by EDA-ID. A non-functional copy of the gene, pseudoNEMO, identified in opposite direction to NEMO, maps 22kb distal to NEMO and only contains exons 3-10. We report on 150 patients diagnosed with IP for which we revealed the presence of the recurrent NEMO 4-10 deletion in 81 females. By using a PCR diagnostic methods to discriminate between NEMO gene and pseudo-gene, we found that 4 patients carried also a pseudoNEMO 4-10 deletion. In addition 4 IP females had only pseudoNEMO deletion. Whether or not this deletion has a pathological effect will be discussed. Mutational analysis of all NEMO coding exons in the remaining 65 patients revealed the presence of 13 new mutations. We will report on the role of those protein-affecting-mutation on the function of NEMO protein and therefore, on the activity of IKK complex. Finally we identified a new class of IP males with clinical characteristic typical of IP in females, and exclusion of EDA-ID diagnosis. This cohort is composed of 17 males, 2 of them were post-mytotic mosaics for a loss-of-function deletion of NEMO, while all the others present no NEMO protein-affecting mutations in their DNA. We will present data supporting the hypothesis that other gene/pathway may be involved in this IP-like pathology in males.

RECQL4: One gene behind three syndromes with an increased risk for malignancies. *H.A. Siitonen[1], H. Kääriäinen[2], M. Kestilä[1]* 1) Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland; 2) Department of Medical Genetics, University of Turku, Turku, Finland.

RAPADILINO, Rothmund-Thomson (RTS) and Baller-Gerold (BGS) syndromes are caused by mutations in the RECQL4 gene. These syndromes are chracterised by growth retardation and bone malformations, mainly hypo- or aplasia of thums, radius and patellae. Differential diagnosis in these three syndromes relies on two cardinal features. The first is poikilodermatous rash seen in RTS and BGS but never in RAPADILINO. The second feature craniosynostosis is unique to BGS. RTS patients are also known for their elevated osteosarcoma incidence. We have recently found out that RAPADILINO patients may have increased susceptibility to develop lymphoma. We have mainly investigated RAPADILINO syndrome which is overrepresented in Finnish population where all patients are either homo- or heterozygous for the IVS7+2delT mutation. This mutation leads to inframe skipping of exon seven and causes a 44 amino acid deletion out of 1208 amino acid protein. The mutations in RTS and BGS are mainly truncating mutations and the outcome of these mutations could be unfunctional protein which could explain the elevated osteosarcoma risk in RTS. In contrast the IVS7+2delT mutation might preserve some of the proteins function. Hence we are studying the genotype-phenotype correlation in order to further understand the role of RECQL4 defects. RECQL4 is one of the five human RecQ homologs and mutations in three of them, RECQL4, BLM and WRN, are known to be associated with cancer predisposition. RECQ proteins are helicases with a role in maintaining genomic stability. RECQL4 is the only human RECQ protein that does not possess helical activity. Yet it has been suggested to have role as a genome caretaker and thus the defect in maintenance of genomic stability which could explain the elevated cancer incidence. When transfecting HeLa and COS-1 cells with wt and mutant (lacking exon seven) constructs we observed mislocalisation of the mutant constructs. Other studies at the protein level are in progress. We are currently analysing genomewide expression arrays to furter understand the role of RECQL4 defect in RAPADILINO.

Silencing of the oncosuppressor gene FHIT in normal cells leads to dysregulation of mitosis related pathways. *L. Roz, F. Andriani, G. Sozzi* Dept Experimental Oncology, Ist Nazionale Tumori, Milano, Italy.

The inactivation of the tumor suppressor gene Fhit in many epithelial cancers has been reported in several studies. Restoration of Fhit expression in Fhit-negative cancer cells results in reduction of tumorigenicity and induction of apoptosis, however the precise mechanism of action remains to be elucidated. In this study we used small interfering RNAs technology to transiently inhibit the endogenous Fhit expression in 293-HEK cells and in normal bronchial epithelial cells (NHBE) and thereby investigate the effect of its inactivation in the regulation of various critical cellular processes that lead to tumorigenesis. After reduction of the FHIT transcript, we tested by real-time PCR the expression of some of the target genes already identified in a microarray experiment which demonstrated the involvement of Fhit in the regulation of processes related to DNA replication and synthesis, cell cycle control and mitosis. The decrease of Fhit transcript was associated with a regulation of more than 50% of the selected genes, including TOP2A, HEC, CCNB1, BUB1, KNSL1 and CENPF, suggesting a putative role of the Fhit protein in mitosis control also in non-transformed human epithelial cells. The effect of Fhit repression on regulation of these genes was also investigated using retroviral-mediated long term silencing in human bronchial epithelial cells-KT (HBEC-KT), an immortal cell line generated from normal cells by stable expression of cyclin dependent kinase-4 (CdK4) and hTERT. Real-time PCR showed a marked reduction of the level of FHIT gene expression and regulation of 8/12 (67%) proliferation-related genes (CCNA2, CCNB1, CCNB2, KNSL1, CENPF, STK12, BUB1, PKMYT). These results demonstrate a good reproducibility of gene regulation obtained with different approaches and provide further support for the relevance of the Fhit gene in proliferation control. Interestingly HBEC-KT cells carrying both Fhit knockdown and p53 knockdown cassettes showed a more evident regulation of the same genes compared to cells with wild type p53, suggesting a putative cross-talk of these oncosuppressor genes in the control of different pathways related to the tumorigenesis process.

Microdeletion encompassing the MAPT gene at chromosome 17q21.3 is associated with developmental delay and learning disability. *C. Shaw-Smith[1], A. Pittman[2], L. Willatt[3], H. Martin[4], L. Rickman[5], S. Gribble[5], R. Curley[5], S. Cumming[4], C. Dunn[3], D. Kalaitzopoulos[5], K. Porter[5], E. Prigmore[5], A. Krepischi-Santos[6], M. Varela[7], C. Koiffman[7], A. Lees[2], C. Rosenberg[6], H.V. Firth[1], R. de Silva[2], N.P. Carter[5]* 1) Medical Genetics, University of Cambridge, Cambridge, United Kingdom; 2) Reta Leila Weston Institute of Neurological Studies, University College, London UK; 3) Regional Cytogenetics Laboratory, Addenbrooke's Hospital, Cambridge, UK; 4) Regional Molecular Genetics Laboratory, Addenbrooke's Hospital, UK; 5) Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK; 6) Department of Genetics and Evolutionary Biology, Institute of Biosciences, Sao Paulo, Brazil; 7) Human Genome Study Centre, Department of Genetics and Evolutionary Biology, University of Sao Paulo, Brazil.

Recently, the application of array-based comparative genomic hybridization (array-CGH) has improved rates of detection of chromosomal imbalances in patients with mental retardation. Here, we describe three individuals with learning disability and a heterozygous deletion at chromosome 17q21.3, detected in each case by array-CGH. There are some clinical similarities between the three patients: low birth-weight, neonatal hypotonia and feeding difficulties were present in each case, as well as moderate to severe learning difficulties. FISH analysis demonstrated that the deletions occurred as de novo events in each patient and were between 500 kb and 650 kb in size. A recently described 900 kb inversion which suppresses recombination between ancestral H1 and H2 haplotypes encompasses the deletion. We show that, in each trio, the parent of origin of the deleted chromosome 17 carries at least one H2 chromosome. This region of 17q21.3 exhibits complexity of genomic architecture with well-described low copy repeats (LCRs). We show that the orientation of LCRs flanking the deleted segment in inversion heterozygotes is likely to facilitate the generation of this microdeletion by the mechanism of non-allelic homologous recombination. We believe this to be the first microdeletion syndrome to be described in which all the patients have been ascertained by array-CGH.

Landmark study of LD in a genetic Sardinian isolate using 500k SNP: prerequisite for GW association approach.

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The knowledge of the genetic features such as linkage disequilibrium (LD) patterns in isolated populations has acquired a great importance to determine the feasibility of genome wide association studies to map genes that underlie complex traits. We analyzed 500K SNPs (Affymetrix) in 50 unrelated individuals (kin<0.0625) from the village of Urzulei (URZ) in Ogliastra region of eastern Sardinia. This isolate (~1000 inhabitants) is characterized by a low number of founders, high endogamy (95%;) and slow demographic growth with very little immigration. 83.5%; of the SNPs were informative with a mean MAF of 0.18 and heterozigosity of 0.3. For comparison we selected a subset of 300,000 SNPs typed both in URZ and in 60 unrelated Caucasian individuals obtained from the HapMap project. Genomewide LD patterns were calculated using pairwise D' and r2 in both populations. The average distribution of LD was very similar (=0.9), although in the URZ it was uniformly higher. We constructed metric LD maps for both populations for the whole genome and found similar patterns of LD blocks and recombination hot spots. Differences in length of the LDU maps imply that LD extends twice as far in the URZ population than in the CEU; on average 129 kb vs 62.75 kb, respectively. Although LD Blocks shared by both populations show similar haplotypes with highly correlated frequencies (=0.9), their distribution within each block shows that more than 50%; differ significantly in the 2 samples independently from LD block sizes, possibly due to genetic drift. Using Gene Ontology, we compared the distribution of genes involved in 40 biological processes (BP) within high and low LD regions vs the whole genome gene distribution. 18 BPs in URZ, mostly shared with CEU, showed a significant difference between the observed and the expected distribution suggesting a correlation between gene function, LD distribution and evolution of the village. These results suggest that this isolate, while resembling the general population, posses an enhanced LD power to detect the genetic make up of the biological processes.

Cost-optimal two-stage designs for genome-wide association studies. *H. Schaefer, H.H. Mueller* Inst Medical Biometry, Philipps Univ, Marburg, Marburg, Germany.

Genome-wide association studies for complex diseases have become practical since genome-wide SNP chips are available. To reduce the cost of genome-wide association studies, two-stage designs with intermediate substantial reduction of the marker set after the first stage have been proposed (Satagopan et al.,2002,2003,2004; Thomas et al.,2004). Efficient statistical analysis strategies for two-stage studies have recently been proposed (Skol et al.,2006). We present cost-optimal two-stage designs for case-control association studies. These designs are based on the methodology of group sequential tests as known from clinical trials. For case-control studies using 500k SNP markers and achieving 80% power at genome-wide type I-error rate of 0.05, overall study costs can be reduced by more than 60% as compared to one-stage designs and by about 25% as compared to two-stage designs proposed in the literature (Satagopan,2003). Only the most promising 5% of the total marker set must be genotyped in the whole sample in the second stage of the study. As in the work by Satagopan et al.,2003, the cost function used for design optimisation includes both sampling and genotyping costs, with user specified cost ratio. In addition it was extended to fit different costs in the two stages, e.g., lower genotyping costs per marker in the first stage due to high throughput chip technology. References Satagopan JM, et al (2002).Two-stage designs for gene-disease association studies. Biometrics 58(1):163-170. Satagopan JM, Elston RC (2003). Optimal two-stage genotyping in population-based association studies. Genetic Epidemiology 25:149-157 Satagopan JM, Venkatraman ES, Begg CB (2004).Two-stage designs for gene-disease association studies with sample size constraints. Biometrics 60(3):589-597 Skol AD, Scott LJ, Abecasis GR, Boehnke M (2006).Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. Nature Genetics 38(2):209-213 (Corrigendum: Nature Genetics 38(3):390) Thomas D, Xie R, Gebregziabher M (2004).Two-Stage sampling designs for gene association studies. Genetic Epidemiology 27:401-414.

Patterns of admixture in Latino populations. *A. Ruiz-Linares[1], S. Wang[1], G. Bedoya[1], C. Bortolini[2], H. Nicolini[3], W. Klitz[4], J. Molina[5], N. Freimer[5], R. Barrantes[6], G. Mazzotti[7], C. Gallo[7], J. Dipierri[8], E. Alfaro[8], N. Bianchi[8], G. Bailliet[8], F. Rothhammer[9], F. Salzano[2], M. Feldman[10], N. Rosenberg[10]* 1) Dept Biol, Wolfson House, Univ Col London, London, United Kingdom; 2) Departamento de Genetica, Univ Federal do Rio Grande do Sul, Porto Alegre, Brazil; 3) Department of Neuropsychiatry, Neurological Emergencies of the National Institute of Neurology and Neurosurgery, Mexico City, Mexico; 4) School of Public Health, Univ California, Berkeley, CA; 5) Univ California, Los Angeles, Los Angeles, CA; 6) Dept Genetics, Escuela de Biol, Univ de Costa Rica, San Jose, Costa Rica; 7) Univ Peruana Cayetano Heredia, Lima, Peru; 8) IMBICE, La Plata, Argentina; 9) Faculty Medicine, Univ Chile, Santiago, Chile; 10) Univ Michigan, Ann Arbor, MI.

We examined the diversity of 13 Latino populations from seven countries (Mexico, Guatemala, Costa Rica, Colombia, Chile, Argentina and Brazil) typing 745 autosomal microsatellite markers in 250 individuals. Estimates of genetic ancestry for these populations varied substantially. Native American ancestry varied between 19.6% and 70.3%, European ancestry between 26.9% and 70.6%, and African ancestry between 1.1% and 9.8%. Genetic structure analysis provides evidence of a genetic continuity between pre- and post-Columbian populations for specific geographic regions. For instance, a Chibchan-Paezan ancestry is detectable in Latinos from lower Central America and northwest South America. Individual admixture estimates vary considerably between populations. Some Latinos (e.g. Mexico City) show marked variation in individual admixture, whereas others (e.g. Antioquia and Costa Rica) show little variation. This variation is likely to reflect the history of admixture of each geographic region examined: some Latino populations are still undergoing substantial admixture whereas others underwent admixture mostly in early colonial times. These results have important implications for admixture mapping and association mapping studies in Latino populations.

The human X chromosome in the etiology of Premature Ovarian Failure (POF): epigenetic control of the critical region on autosomal genes translocated to the X chromosome. *F. Rizzolio[1], C. Sala[1], S. Bione[1,2], V. Causarano[1], S. Alboresi[1], M. Goegan[1], O. Zuffardi[3], D. Toniolo[1]* 1) Dept of Molecular Biology and Functional Genomics, DIBIT-HSR, Milano, Italy; 2) Institute of Molecular Genetics, CNR, Pavia, Italy; 3) Dept. of Pathology and Medical Genetics, University of Pavia, Pavia, Italy.

Premature Ovarian Failure (POF) is a disorder characterized by lack of ovulation and elevated gonadotropin level before 40 years of age. POF has a frequency of about 1% among females and has become a relevant cause of female infertility. A genetic component of the disorder was demonstrated by numerous familial cases and by the frequent observation of X chromosome rearrangements. Mapping of chromosomal rearrangements associated with POF led to the definition of a POF critical region in Xq. Characterization of the POF critical region by FISH mapping of a large panel of X chromosome rearrangements showed that most of the breakpoints mapped to a 16 Mb gene poor region corresponding to Xq21, where only 3 genes were interrupted by POF associated breakpoints. Expression analysis of X-linked genes surrounding some of the X;autosome balanced translocation breakpoints did not reveal ovary-specific genes. Surprisingly, analysis of the corresponding autosomal regions showed in all instances genes with a specific expression in mouse ovaries, in granulosa cells and/or in oocytes. Investigation of the position effect of the breakpoints on chromatin organization of X chromosome and autosomal regions, showed chromatin alterations occurring exclusively at the promoters of the autosomal genes translocated to the X. The data demonstrates a role of the X chromosome critical region on ovarian gene expression, during oogenesis and follicular maturation, when the global active X chromosome gene expression is down regulated .

Exact type I error rate for association study using SNPs. *A. Takahashi[1], T. Nakamura[1], N. Kamatani[1,2]* 1) Laboratory for Statistical Analysis, SNP Research Center, RIKEN, Japan; 2) Institute of Rheumatology, Tokyo Women's Medical University, Japan.

Association study using single nucleotide polymorphisms(SNPs) is one of the methods to discover genes related with diseases. Large number of marker SNPs over the genomes have become available by the HapMap project. Large number of tests are performed in association study generally. Therefore problem of multiple testing has to be considered. Bonferroni's correction is one of the corrections for multiple testing. However it is well known that Bonferroni's correction is too conservative. We derived the equation of exact type I error rate for association study using SNPs. We calculated the probability of distributing all samples to case and control groups on the condition that the size of both groups had fixed. Exact type I error rate is depend on sample size, the number of SNPs and significance level of test. Exact type I error rate can be calculated computationally in principle. However if sample size is too large, it is difficult to calculate this rate because calculation time becomes very long. Markov chain Monte Carlo methods was developed to calculate type I error rate by the approximation. The results of simulated data will be reported. We will discuss the relation between our results and other correction methods.

Development of a new assay for monitoring the *in vivo* effects of drugs on *SMN* mRNA in clinical trials of spinal muscular atrophy. *M. Vezain[1], P. Saugier-Veber[1,2], J. Melki[3], A. Toutain[4], E. Bieth[5], M. Husson[6], J.M. Pedespan[6], L. Viollet[7], I. Pénisson[8], S. Férenbach[2], J. Bou[1,2], T. Frebourg[1,2], M. Tosi[1]* 1) Inserm U614, Faculty of Medicine, Rouen, France; 2) Department of Genetics, University Hospital, 76031 Rouen, France; 3) Laboratory of Molecular Neurogenetics, Inserm E.0223 and University, 91000 Evry, France; 4) Department of Medical Genetics, University Hospital, 37044 Tours, France; 5) Department of Medical Genetics, University Hospital, 31079 Toulouse, France; 6) Unit of Child and Teenager Neurology, University Hospital, 33076 Bordeaux, France; 7) Department of Medical Genetics, Necker-Enfants Malades Hospital, 75743 Paris, France; 8) Department of Neurology, University Hospital, 49033 Angers, France.

Spinal muscular atrophy (SMA) mainly results from homozygous deletion or gene conversion of the survival motor neuron (*SMN1*) gene. All patients have at least one copy of the highly homologous *SMN2* gene. Two main mRNA isoforms are produced from the *SMN2* gene: a full length (FL) mRNA, and mRNA lacking the exon 7 sequence (7mRNA). An inverse correlation between *SMN2* copy number and disease severity has been reported, suggesting the possibility of treating SMA patients by increasing FL *SMN* mRNA levels produced from the *SMN2* gene. We have developed a sensitive assay based on multiplex fluorescent RT-PCR which allows to determine in the same reaction the levels of FL mRNA, 7 mRNA and total *SMN* mRNA, and to calculate directly the FL/7 mRNA ratio. This assay, applied to blood samples of 75 unrelated control subjects and 48 SMA patients, allowed us to show that, in both SMA patients and controls, the levels of 7 mRNA are linearly dependent on the number of *SMN2* copies. In addition, we show that FL mRNA levels are linearly dependent on *SMN2* copy number in SMA patients. We also show that, in patients, the FL/7 ratio is approximately 1 in blood cells and 0.5 in muscle. These data indicate that the FL/7 mRNA ratio is regulated according to tissue. This new assay should be valuable as a biomarker for monitoring the *in vivo* effects of various drugs on *SMN2* in forthcoming clinical trials of SMA.

Detection by functional analysis of splicing mutations in hereditary nonpolyposis colorectal cancer. *I. Tournier[1], M. Vezain[1], F. Charbonnier[1], A. Martins[1], M-P. Buisine[2], S. Olschwang[3], Q. Wang[4], T. Frebourg[1,5], M. Tosi[1]* 1) Inserm U614, Faculty of Medicine, Rouen, France; 2) Laboratory of Biochemistry and Molecular Biology, University Hospital, Lille, France; 3) Inserm UMR 599, Institut Paoli-Calmettes, Marseille, France; 4) Molecular Oncology Unit, Centre Léon Bérard, Lyon, France; 5) Department of Genetics, University Hospital, Rouen, France.

Hereditary nonpolyposis colorectal cancer (HNPCC) results from germline mutations of the *MMR* genes, especially *MLH1*, *MSH2* and *MSH6*. Numerous variants of unknown biological significance such as missense, silent, or intronic mutations have been found in these genes, which limits the molecular diagnosis and genetic couselling of HNPCC. A fraction of these variants may have an effect on RNA splicing by disturbing splicing regulatory sequences like exonic or intronic splicing enhancers and silencers (ESE, ESS, ISE, ISS). In order to determine the consequences of these variants on splicing, we have undertaken a systematic screening of these variants using a functional assay of exon inclusion or exclusion, performed on genomic DNA. This assay is based on an expression vector, containing a CMV promoter and exons 2 and 3 of the *SERPIN/G1* gene, separated by their natural intron, into which we have inserted appropriate cloning sites. For each family, mutant and wild-type exons to be tested are PCR amplified from genomic DNA, including about 150 bp of flanking sequences on both sides, and are inserted into the expression vector and transfected transiently into HeLa cells. The effects of mutations on splicing are evaluated by RT-PCR on total RNA and by direct sequencing of the relevant RT-PCR products. We have presently examined 53 different *MLH1* or *MSH2* mutations (32 missense, 9 silent, 2 deletions of a single codon and 10 intronic mutations). We found that 17 of these mutations affect splicing, including 5 exonic mutations that indicate the presence of ESE (exonic splicing enhancer) or ESS (exonic splicing silencer) elements. This assay should contribute to the molecular diagnosis and genetic counselling of HNPCC and can be easily applied to other genetic diseases.

Genomic diversity and population structure of Native Americans. *S. Wang[1], M. Jakobsson[2], S. Ramachandran[3], G. Bedoya[1], W. Rojas[1], M. Parra[1], J. Molina[4], G. Mazzotti[5], C. Gallo[5], D. Labuda[6], W. Klitz[7], R. Barrantes[8], C. Bortolini[9], F. Salzano[9], F. Rothhammer[10], M. Feldman[3], N. Rosenberg[2], A. Ruiz-Linares[1]* 1) Dept Biol, Univ Col London, London, United Kingdom; 2) Univ Michigan, Ann Arbor, MI; 3) Stanford Univ, Stanford, CA; 4) Univ California, Los Angeles, Los Angeles, CA; 5) Univ Peruana Cayetano Heredia, Lima, Peru; 6) Univ Montreal, Montreal, Canada; 7) School of Public Health, Univ California, Berkeley, CA; 8) Dept Genetics, Escuela de Biol, Univ de Costa Rica, San Jose, Costa Rica; 9) Univ Federal do Rio Grande do Sul, Porto Alegre, Brazil; 10) Faculty Medicine, Univ Chile, Santiago, Chile.

We examined 745 autosomal microsatellite markers in 432 individuals sampled from 24 indigenous populations in the Americas. These data were analyzed jointly with similar data available in 54 other indigenous populations from across the world (including an additional 5 Native American groups). The populations from the Americas show lower diversity and more differentiation than populations from other continental regions (global Fst=0.08). Signals of long-range linkage disequilibrium are detectable to a greater extent in Native Americans than in other populations, as are signals of recent bottlenecks followed by population growth. A negative correlation is observed between population diversity and geographic distance from the Bering Strait, an observation consistent with the north-to-south dispersal of humans upon initial entry into the continent. A higher diversity is observed in western vs. eastern South American populations, potentially reflecting differences in long-term effective population size or in colonization routes within South America. Phylogenetic trees relating Native American populations show a marked differentiation between Canadian and other Native populations. Canadian natives also show a detectable shared ancestry with contemporary Siberian populations, which is less visible for more southerly Americans. A substantial agreement is observed between phylogenetic relatedness and population affiliation according to the linguistic classification of Greenberg.

Prevention of oculopharyngeal muscular dystrophy-associated nuclear protein aggregation with intracellularly expressed camelid-derived antibody domains. *S. van der Maarel[1], P. Verheesen[2], A. de Kluijver[2], S. van Koningsbruggen[1], H. de Haard[2], G. van Ommen[1], T. Verrips[2]* 1) Dept Human Genetics, Leiden Univ Medical Ctr, Leiden, Netherlands; 2) Dept Mol Cell Biology, Utrecht University, Utrecht, Netherlands.

Oculopharyngeal muscular dystrophy (OPMD) is considered a paradigm for neurodegenerative protein aggregation disorders. OPMD is caused by extensions of the N-terminal polyalanine stretch in the nuclear polyA-binding protein 1 (PABPN1) leading to the presence of intranuclear aggregates in skeletal muscle. Intranuclear aggregation of mutant PABPN1 is also observed in animal and cell models for OPMD, and these models support a direct role for protein aggregation in OPMD pathogenesis. We have isolated camelid-derived single domain antibody reagents (VHH) against different epitopes in PABPN1 from a non-immune phage display library. These VHH specifically detect and label normal and mutant endogenous PABPN1. When expressed intracellularly as intrabodies, autofluorescent VHH specifically recognize endogenous PABPN1. In a cell model for OPMD, aggregation was prevented in a dose-dependent manner by some of these intrabodies. These intrabodies were also able to reduce already existing aggregates. Currently, the intrabodies are being used in living cells to study the spatiotemporal behaviour of normal and mutant PABPN1. Given the domain specificity of VHH-mediated aggregation interference, this approach facilitates definition of the nucleation kernel in aggregation-prone proteins, thus providing etiological insight into this and other protein aggregation disorders. It may also provide useful therapeutic agents.

Male-to-female sex reversal due to an ~250 Kb deletion upstream of *NR0B1 (DAX1)*. *M. Smyk[1,2,*], J.S. Berg[1,*], A. Pursley[1], F. Curtis[3], B. Fernandez[3], G.A. Bien-Willner[1], J.R. Lupski[1], S.W. Cheung[1], P. Stankiewicz[1,2]* 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) Disciplines of Medicine and Genetics, Memorial University of Newfoundland. *Equally contributing authors.

Sex determination and differentiation is a complex cascade involving a number of dosage sensitive genes. Although mutations in many of these genes were identified, the cause for sex reversal remains unknown in the majority of patients. *NR0B1 (DAX1)* is a dosage sensitive gene on Xp21.2 that plays a critical role in the development of the reproductive and adrenal systems. Deletion of *NR0B1* results in congenital adrenal hypoplasia, whereas *NR0B1* duplication in 46,XY individuals leads to gonadal dysgenesis and a female phenotype. We describe a 20 year old 46,XY female manifesting primary amenorrhea, a small immature uterus, and ovotestis, in whom analysis by array-CGH showed a loss in copy number detected by one BAC interrogating clone RP11-662D2 that harbors *NR0B1*. No signs of adrenal insufficiency were present in the patient. Surprisingly, FISH with this clone showed the fluorescence signal on Xp21. Using PCR, we found that *NR0B1* was present in the patient; however, we identified a 257,782 bp deletion located 11,320 bp upstream (proximal) to *NR0B1* and 75 kb distal to *GK1*. The deletion truncated 53% of the BAC RP11-662D2, which explains the discrepancy between array-CGH and FISH results. No additional nucleotides were found at the junction. The deletion was also found in the patients mother. Using bioinformatics and comparative genomics, we identified within the deletion several potential *cis*-acting regulatory elements upstream of *NR0B1* as well as 17 potential consensus-binding sites for SF1; the latter is thought to be a negative regulator of *DAX1*. Loss of regulatory sequences apparently resulted in a position effect up-regulation of *NR0B1* expression. We propose that this genomic region and by extension those surrounding the dosage sensitive *SRY, SOX9*, and *SF1* genes, should be examined for copy number variation in patients with sex reversal.

Apolipoprotein E polymorphism in patients with amyotrophic lateral sclerosis. *H. A. Idrisoglu[1], A. Sazci[2], G. Akpinar[2], E. Ergul[2], O. Ozsarac[1]* 1) Department of Neurology, University of Istanbul, Faculty of Medicine, Istanbul, Turkey; 2) Department of Medical Biology and Genetics, Faculty of Medicine, University of Kocaeli, Umuttepe, 41380, Kocaeli,Turkey.

Amyotrophic lateral sclerosis(ALS) is the most common adult onset neurodegenerative disorder affecting the motor neurons. ALS is considered a multifactorial disease, though its etiopathogenesis is still unknown. We were interested to know whether apolipoprotein E polymorphism play a role in the susceptibility to ALS in a turkish population.We used a PCR-RFLP method to determine the genotypes of 113 ALS patients and 406 healthy controls. The frequency of the 2,3, and 4 alleles of ApoE gene was 8.37%, 84.61% and 7.02% in the controls and 7.52%, 83.18% and 9.29% in ALS cases respectively. There was no allele association between ALS cases and controls (Chi2= 6.333; df=5; P=0.275). The ApoE3/4 genotype had a 1.666 -fold increased risk for ALS when compared to the controls (OR=1.666; 95%CI= 0.903-3.074; Chi2=2.716;df=1;P=0.99), though it was statistically insignificant. And also, the ApoE4/4 genotype had a 1.811-fold increased risk for ALS as compared to the controls. However it was statistically insignificant (OR=1.811;95%CI=0.327-10.015; X2=0.476; df=1; P=0.490). The individuals with the APOE2/3 genotype had a 1.107-fold increased risk for ALS as compared to the controls (OR=1.107; 95%CI=0.615-1.992; X2=0.114;df=1;P=0.735), though the results were statistically insignificant. In conclusion, individuals with the ApoE3/4,4/4 and 2/3 genotypes showed increased risk for ALS in the turkish population. However the results did not reach the statistical significant level. The study should be carried out on a large sample size in the future.

Recurrent campomelic dysplasia due to a mosaic *SOX9* deletion in a father. *E. Obersztyn[1], M. Smyk[1], B. Nowakowska[1,2], E. Bocian[1], S.W. Cheung[2], T. Mazurczak[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.

Campomelic dysplasia (CD) is a fully penetrant autosomal dominant semilethal skeletal malformation syndrome with or without XY sex reversal caused by mutations in *SOX9*, a dosage-sensitive gene that plays a critical role in the development of the skeletal and reproductive systems. To date, multiple intragenic *SOX9* mutations and 19 chromosomal rearrangements have been identified in patients with CD: two entire gene deletions, one deletion upstream of *SOX9*, 14 translocations and one inversion with breakpoints scattered up to 1 Mb 5' to *SOX9*, and one translocation with breakpoint mapping 1.3 Mb downstream of *SOX9*. We present a family with two children diagnosed with CD. From the first pregnancy a child with CD was born in the 31st week of gestation and died after one hour of life. During the second pregnancy, an ultrasound examination showed an increased nuchal translucency (NT) and amniocentesis revealed a normal fetal karyotype. A child with CD was born in the 35th gestational week and died on day of life 3 due to upper respiratory insufficiency. Postmortem array CGH study revealed a >600 kb deletion involving the entire *SOX9* gene. During the third pregnancy, a slightly increased NT was observed; however, no evidence of the deletion was found by FISH in amniocytes. The second ultrasound at 24th weeks of gestation was normal. FISH analysis of the parental samples revealed that the father was a carrier of a *SOX9* deletion present in 66% of lymphocytes. He had short stature (<3rd centile). We are currently studying the grandparental samples and determining the size and tissue distribution of the deletion. This is the first report of mosaic deletion of *SOX9*; few familial CD cases with germline and somatic mutation mosaicism have been described. Our findings reveal the utility of aCGH in the evaluation of SAB and indicate that for a more accurate estimate of the recurrence risk for a completely penetrant autosomal dominant disorder, parental somatic mosaicism should be considered in healthy parents.

Locus specific mutation databases for human genetics: catalogue and survey of curators. *C. Talbot[1], R. G. H. Cotton AM[2], O. Horaitis[2], M. Phommarinh[3], K. Phillips[2], Consortium to collect mutations* 1) Inst Genetic Medicine, Johns Hopkins Sch Medicine, Baltimore, MD; 2) Genomic Disorders Research Centre, St. Vincent's Hospital, Australia; 3) McGill University Health Centre (MUHC), Montreal Children's Hospital.

Complete and accurate information on mutations in disease genes is required for efficient delivery of genetic health care. Whilst possible to obtain this information by surveying scientific journals and/or data mining, it is well known that many variations are unpublished for numerous reasons. Information in scientific journals does not have all the information that those dealing with patients or research need. Curators of locus specific databases (LSDBs) collect mutations and their effect in an organised manner usually collecting unpublished mutations.

The HGVS (www.hgvs.org) has been collecting LSDB lists with the first list available in 1998. We recently updated this and found 571 genes have an LSDB (www.hgvs.org/dblist/glsdb.html).

We surveyed the curators of these databases. Whilst their existence is essential to those dealing with research and patients, funding and content is variable. 125 curators were sent a questionnaire and we received 47 responses. Some individuals performed curation on up to 69 genes. Time spent on curation was variable, varying from no time to up to five curators spending over four hours per week. Funding estimated for proper curation ranged from USD 600-4,500 per year. A majority of databases were stimulated by the HUGO-Mutation Database Initiative (now-HGVS) using their guidelines. Many reported unpublished and all but one reported errors in the literature. Of the 13 who reported hits, nine reported over 52,000 per year. The need for expert curation is underlined by the fact that curators found an enormous error rate in the literature and many contained unpublished mutations.

Detection of Duplication/Deletion of the PMP22 Gene in Patients with Charcot-Marie-Tooth Disease Type 1A and Hereditary Neuropathy with Liability to Pressure Palsy. *M. Rostami, M. Dehghanmanshadi, S.M. Seyedhasani, A. Ebrahimi, M. Tonekaboni, M. Houshmand* medical genetic, NIGEB, Tehran, Tehran, Iran.

Charcot-Marie-Tooth (CMT) disease is a clinically and genetically heterogeneous group of hereditary peripheral neuropathies. The clinical characteristics of the disease include distal symmetric muscle weakness, atrophy, bilateral pes cavus, and diminished or absent deep tendon reflexes. Peripheral nerve conduction velocities are often severely reduced. In hereditary neuropathy with liability to pressure palsy (HNPP), recurrent peripheral nerve palsies (e.g., ulnar nerve, median nerve, and peroneal nerve palsies) occur because of minor compression trauma, whereas foot deformity is less frequently observed than it is in CMT. Nerve conduction velocities are significantly reduced at compression sites of peripheral nerves. Charcot-Marie-Tooth disease type 1A (CMT1A) is the most common form of CMT (1, 2). A 1.5-Mb duplication on chromosome 17p11.2-p12 (CMT1A duplication) caused by a misalignment of the CMT1A repeat sequences (CMT1A-REPs) is associated with Charcot-Marie-Tooth disease type 1A (CMT1A). A hotspot of crossover breakpoints located in a 3.2-kb region of the CMT1A-REPs accounts for three-quarters of the rearrangements in CMT1A patients. In contrast, deletion of the PMP22 characteristically results in HNPP. Point mutation in the PMP22 may result in CMT1A or HNPP. Thus heterozygous carriers of the deletion (HNPP) or duplication (CMT1A) have one or three copies of the PMP22, respectively.We used some methods for study of this gene: PCR-based diagnostic method to detect a recombination Hotspot associated with the CMT1A duplication Methods for molecular diagnosis of CMT1A use Southern blot and/or amplification by PCR of polymorphic poly (AC) repeats(microsatellites) located within the duplicated region, or the detection of junction fragments specific for the duplication. Real-time quantitative PCR using SYBR Green I to detect the PMP22 duplication and deletion.

Alleles of reelin gene show association with working memory in Finnish schizophrenia families. *J.O. Peltonen[1], A. Tuulio-Henriksson[2], A. Loukola[1], J. Ekelund[1,2], T. Paunio[1,2,4], T. Varilo[1,3,5], J. Suvisaari[2], T. Partonen[2], J. Lönnqvist[2,3,4], L. Peltonen[1,3,5]* 1) Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland; 2) Department of Mental Health and Alcohol Research, National Public Health Institute, Helsinki, Finland; 3) Department of Medical Genetics, University of Helsinki, Finland; 4) Department of Psychiatry, University of Helsinki, Finland; 5) Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, MA, USA.

Using a large, well-characterized nationwide collection of schizophrenia families we have obtained evidence for multiple genetic loci, including 7q22. The initial linkage evidence emerged from 52 families (n=236). We later replicated this finding in an independent sample of 352 families (n=1626).

RELN gene on 7q22 encodes the reelin protein critical for neuronal development. It regulates neuronal migration by guiding neurons and glia to their positions during brain development, and is expressed in brain regions with synapse remodelling. No reports exist for genetic association of *RELN* with the clinical diagnosis of schizophrenia. However, specific alleles of *RELN* have been suggested to contribute to response to antipsychotics.

We hypothesized that since *RELN* takes part in essential brain functions it might associate with cognitive traits affected in schizophrenia. We collected detailed neuropsychological data from 186 schizophrenia families (n=618) and tested association with five microsatellites in *RELN* using sex, age, and affection status as covariates. This revealed associations (p=0.002 to 0.000001) with traits related to working memory, controlled by the frontal brain areas and known to be impaired in schizophrenia. Carriers of the most associated allele scored lower in the visual working memory (p=0.0005) and verbal working memory tests (p=0.000008), as well as in learning (p=0.001) and memory tests (p=0.0002). Our findings suggest a role of *RELN* in frontal brain functions and raise the possibility that some allelic variants of this gene might contribute to cognitive disturbances related to schizophrenia.

A study of common SNP variation around 10 genes in the SREBP and HMG CoA reductase pathway and a testing of their association with BMI in a Caucasian case control panel. *E.K. Speliotes[1,2], H. Lyon[2,3], J.L. Butler[3], J.A. Drake[2,3], J. Nemesh[2,3], M.J. Daly[1,4], D. Altshuler[1,2,4], S. Purcell[1], J.N. Hirschhorn[2,3,4]* 1) Massachusets General Hospital, Boston MA; 2) Broad Institute, M.I.T., Cambridge MA; 3) Children's Hospital, Boston MA; 4) Harvard Medical School, Boston MA.

Obesity is a heritable risk factor for significant illness and death in the U.S. and worldwide. The genetic variants that predispose some but not other individuals to becoming obese are largely unknown. We with others recently showed that the SNP rs7566605, located 10 kb upstream of INSIG2 (insulin induced gene 2), is associated with BMI in multiple human populations (1). Data from animal studies suggest that INSIG2 regulates the localization of the SREBP transcription factor and the stability of HMG CoA reductase, which in turn control lipid and cholesterol biosynthesis pathways. Using HapMap data, we selected a set of tagged SNPs that capture most of the common variation in individuals of European ancestry for 10 genes in these pathways: INSIG1, SCAP (SREBP cleavage activating protein), SREBF1(SREBP1), SREBF2(SREBP2), LXRA (LXR alpha), LXRB (LXR beta), HMGCR (HMG CoA reductase), MBTPS2(site 2 protease), FASN (fatty acid synthase) and ACAS2 (acetyl coA reductase). We genotyped 94 SNPs in over 2800 individuals from the United States and Poland drawn from the near-extremes of the population distribution of body mass index (BMI). One SNP upstream of the LXRB showed a nominally significant association with obesity in both samples (Mantel-Hanzel p-value 0.006 and OR 0.83). We are genotyping this SNPs in additional samples to determine whether this nominal association with BMI is reproducible. We will also be testing for gene-gene interactions between SNPs in this pathway, with a particular focus on rs7566605. (1) Science. 2006 312(5771):279-83.

Genetic Heterogeneity and Genetic Interaction study of Parkinsons Disease in Seventy-Six Multiplex Tunisian Families. *L. Warren[1], R. Gibson[1], L. Ishihara[2], S. Thomas[1], R. Amouri[3], N. Gouider-Khouja[3], M. Kefi[3], M. Zouari[3], S. Sassi[3], S. Yahmed[3], E. Euch-Fayeche[3], A. Roses[1], L. Middleton[1], F. Hentat[3]* 1) GlaxoSmithKline, Res Triangle Park, NC; 2) Department of Public Health and Primary Care, University of Cambridge, Cambridge CB2 2SR, United Kingdom; 3) Service de Neurologie, Institut National de Neurologie, La Rabta, Tunis 1007, Tunisia.

Parkinsons disease (PD) is the second most common neurodegenerative disease after Alzheimers disease. To identify genetic causes of the disease, GSK has established a unique PD family-based collection from Tunisia. This collection is characterized by having large consanguineous pedigrees, excellent clinical assessment and a relatively homogenous population background. All of these factors have provided a great opportunity for genetic research to identify regions harboring PD susceptibility genes. Whole genome linkage scan was conducted in 76 consented PD families with 455 individuals genotyped for 1000 4cM-spaced microsatellite markers. Five major linkage regions have been identified with highly significant statistical support (LOD > 3). Of particular interest is the region on Chromosome 12 (LOD = 3.64), where a single mutation (G2019S) within the LRRK2 gene has been confirmed. The objective of the current study is to identify genetic interactions among these major linkage loci and examine genetic heterogeneity in the data set, with the potential to identify additional novel linkage regions. Linkage analyses stratified by LRRK2 G2019S mutation were performed and a significant linkage peak on Chromosome 13 (LOD = 3.05) was identified in families without the LRRK2 G2019S mutation. A series of Ordered Subset Analyses (OSA) were performed by treating the per-family linkage scores as covariates in non-parametric linkage analyses. A possible genetic interaction between genes under chromosome 1 and chromosome 13 was identified (permutation-based P-value = 0.017). Detailed results will be presented at the meeting. These results warrant further molecular follow-up work for confirmation and validation.

Animal Model of Fabry disease: new findings. *L.G. Rodrigues[1], M. Pais-Vieira[2], M.J. Ferraz[1], M.C. Sá-Miranda[1]* 1) UniLiPe, Institute for Molecular and Cellular Biology, Porto, Portugal; 2) Morphophysiology Unit, Institute for Molecular and Cellular Biology, Porto, Portugal.

Purpose: Fabry (Fb) disease is an X-linked inherited disorder of glycolipid metabolism resulting from deficient activity of the lysosomal enzyme, a-galactosidase A. Neutral glycosphingolipids, predominantly globotriaosylceramide, accumulate several tissues. Pain is one of the most distressing and less understood symptoms. As far as we know to date the Fb knockout mouse model was not used to study the pain mechanism.

Methods: All animals analysed were genotyped and two different ages, 24 and 48 weeks (wks), were analysed and only males were used. We characterised the behaviour of Fb knockout mice and their respective background (C57BL/6) by subjecting them to a primary behavioural screen (SHIRPA protocol). Sensitivity to noxious heat stimuli was measured with a hot plate (HP) test. Morphological and ultrastructural studies in myelin of sciatic nerves were carried out examining cross sections between Fb mice and controls with the light and electron microscope.

Results: When screening for the SHIRPA protocol it was found: a higher body weight for the Fb mice at 24 wks but these effects were not found at 48 wks; it was also found for both ages studied a decreased in locomotor activity and an increased in righting reflex for the Fb mice when compared with their respective aged matched controls. When compared Fb mice to their respective controls for the HP test it was found for both ages studied that Fb mice respond to a higher temperature. In the 24 wks Fb mice only responded approximately 4ºC above their respective controls and for the 48 wks group the difference was even higher, 5ºC. Its seems also to be a decreased in the ratio of myelin/unmyelin number of fibres in the sciatic nerve of the 48 wks Fb mice when compared with their respective aged matched controls.

Conclusions: An important conclusion from this study is that the nociception phenotype of the Fb mice is mainly one of hypoalgesia. The significance of this finding remains to be established.

Prevalent and uncommon filaggrin mutations cause ichthyosis vulgaris. *A. Terron-Kwiatkowski[1], A. Sandilands[1], F.J.D. Smith[1], M. van Geel[2], M.A.M. van Steensel[2], W.H.I. McLean[1]* 1) Human Genetics Unit, University of Dundee, Scotland, UK; 2) Department of Dermatology, University Hospital Maastricht.

The aim of this study was to identify filaggrin (FLG) mutations that cause ichthyosis vulgaris (IV) and that predispose individuals to atopic dermatitis (AD). The 400 kDa polyprotein profilaggrin is the major protein of keratohyalin granules and its proteolytically processed end-product, filaggrin, is essential for skin barrier function. Prevalent loss-of-expression mutations in FLG were recently shown to cause IV and predispose carriers to AD and other atopic phenotypes in European populations. Exon 3 of FLG contains multiple filaggrin repeat units arranged in tandem. These highly repetitive DNA sequences make PCR amplification and diagnostic re-sequencing of this gene problematic. Furthermore, the number of repeats has been shown to vary from 10-12 in the population. By designing repeat-specific primers we were able to develop a range of overlapping PCR fragments each of which spans a few filaggrin repeats. Here we analysed the FLG gene in several families with IV and found that mildly affected patients were heterozygous for the previously reported mutations R501X or 2282del4, whereas severely affected individuals were homozygous for either mutation, consistent with the suggested semidominant inheritance model. Interestingly, three individuals with the marked IV presentation were found to be heterozygous for R501X or 2282del4. Further sequencing revealed three novel compound heterozygous mutations: E2422X and 7267delCA, both at the end of filaggrin repeat 6; and 11033del4 in filaggrin repeat 10. All of the novel mutations lead to premature termination codons within exon 3 of the FLG gene. Immunohistochemical and biochemical analysis suggested that although these more distal mutations allow synthesis of several filaggrin repeats, they disrupt profilaggrin processing and therefore act as null-alleles. This strongly suggests that sequences near the C-terminus of profilaggrin are required for correct processing to occur. These data confirm the role of the FLG gene in IV and suggest that other family-specific or population-specific mutations should be sought in IV and AD.

Copy number variation of immune genes. *S.J. White[1,2], E. van den Akker[1], E. van de Vosse[3], A.W. de Visser[3], J.T. den Dunnen[1], M.H. Breuning[1]* 1) Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands; 2) Immunohematology and Blood transfusion, Leiden University Medical Center, Leiden, Netherlands; 3) Department of Infectious Diseases, Leiden University Medical Center, Leiden, Netherlands.

Recent studies have revealed a new type of genetic variation encompassing relatively large genomic segments, referred to as copy number variation (CNV). CNV is significantly associated with segmental duplicons, defined as stretches of DNA (>1 kb) with a high degree of homology (>90%) present in at least two copies in the genome. These duplicons are enriched for genes, particularly those involved in the immune system. We have searched for CNV in immune-related genes present in segmental duplicons using Multiplex Ligation-dependent Probe Amplification (MLPA), which is currently the best method available to analyze duplicons in a high-throughput, high-resolution manner. A large proportion (1142 of 1562) of the genes in the Immunogenetic Related Information Source (IRIS) was screened for the presence of exon containing segmental duplications. Analysis showed that 137 (12.0%) of the immune related genes tested were completely (n=73, 6.4%) or partially (n=64, 5.6%) located within one or more segmental duplicons. At least two MLPA probes were designed for 41 target genes, preferentially within two outmost exons, each on one end of the particular gene. These probes were used to screen genomic DNA from healthy individuals. CNV was detected for SIGLEC5 and C4A/C4B, and suspected for several other loci. We are currently confirming the potential CNV detected, as well as expanding the number of genes to be tested. Our results demonstrate that CNV occurs in at least some immune genes with segmental duplicons, which may be associated with disease susceptibility.

Mapping recombination hotspots in the three Indian consanguineous communities. *M.S. Song[1], H.S. Savithri[2], X.M. Lu[1], X. Li[3, 4], B.S. Gong[4], H.S. Venkatesha Murthy[5], A.H. Bittles[6], N. Appaji Rao[2], W. Wang[1,3]* 1) Department of Biology, Graduate School of the Chinese Academy of Sciences, Beijing, PR China; 2) Indian Institute of Science, Bangalore; 3) Capital University of Medical Sciences, Beijing, PR China; 4) Bioinformatics Department, Harbin Medical University, Harbin, PR China; 5) Annapurna Clinic, Bhadravati, India; 6) Edith Cowan University, Perth, Australia.

Mapping recombination hotspots should ultimately illuminate the as yet mysterious factors that direct the location and frequency of recombination and aid in understanding the genomic landscape of human evolution. Previous studies have shown that uniform genetic background and controlled breeding schemes can avoid the variability which often confounds genetic effects such as environmental effects, therefore the sample size required to identify the specific gene in question will be decreased and fewer biomarkers needed owing to increased linkage disequilibrium (LD) and decreased allelic diversity. Here we studied recombination rates of chromosomes 13, 15, 16, 17 and 18 by genotyping of 67 STR loci in the three 4-generation Indian consanguineous communities (Sankethi kindreds A, B and C) with a total of 128 individuals (69 males and 59 females). The results evidenced the extreme local recombination rate variations on individual chromosomes among the isolated Sankethi kindreds, indicating that it is essential to look at patterns of recombination rates in the consanguineous populations.

LADD syndrome is caused by mutations that reduce the tyrosine kinase activity of FGFR2. *E. Rohmann[1, 2], I. Lax[3], E.D. Lew[3], V.P. Eswarakumar[3], H.G. Brunner[4], Y. Li[1, 2], H. Kayserili[5], J. Schlessinger[3], B. Wollnik[1, 2]* 1) Center for Molecular Medicine Cologne (CMMC), Cologne, Germany; 2) Institute of Human Genetics, University of Cologne, Cologne, Germany; 3) Department of Pharmacology, Yale University School of Medicine, New Haven, CT, USA; 4) Department of Human Genetics, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands; 5) Medical Genetics Department, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey.

Lacrimo-auriculo-dento-digital (LADD) syndrome is an autosomal dominant disorder mainly affecting the lacrimal system, the ear-shape and hearing, teeth development, and digit patterning. We recently identified the molecular basis of LADD syndrome and found heterozygous missense mutations in LADD families in FGFR2, and FGFR3, and FGF10, one of FGFR2 ligands. Notably, the mutations in the FGF-receptor molecules were located in regulatory regions of the tyrosine kinase domain of the receptors. Previous studies have shown that different craniosynostosis syndromes are caused by gain-of-function mutations in FGFR2 which result in elevated tyrosine kinase activity of FGFR2. We have compared the tyrosine kinase activity of three LADD FGFR2 mutations and demonstrated that their intrinsic tyrosine kinase activity is reduced as compared to the tyrosine kinase activity of wild type FGFR2 or to the constitutively activated tyrosine kinase activity of FGFR2 mutant responsible for Pfeiffer syndrome. Furthermore, stimulation with a LADD FGF10 mutant protein led to a decreased FGFR2 activation and to compromised MAPK stimulation as compared to stimulation of these responses induced by wild type FGF10. We conclude that FGFR2- and FGF10-LADD mutations lead to reduced FGFR2 autophosphorylation and diminished cell signaling via FGFR2 and that the reduced tyrosine kinase activity of FGFR2 is a hallmark underlying the molecular basis of LADD syndrome.

Phenotypic Variability of Vascular Ehlers-Danlos Syndrome in a Pedigree with a COL3A1 Mutation. *J. Yang*[1], *W. Chen*[2], *J. Tran*[1], *N.B. McDonnell*[2], *C.A. Francomano*[2,3] 1) Laboratory of Genetics, National Institute on Aging/NIA, Baltimore, MD; 2) National Institute on Aging, NIH, Baltimore, MD; 3) Harvey Inst Hum Genetics, GBMC, Baltimore, MD.

Vascular Ehlers-Danlos Syndrome (VEDS) is a rare genetic disorder affecting approximately 1 in 100,000 individuals, resulting from mutations in the gene for type III procollagen (COL3A1). Affected patients are at risk for arterial, bowel, and uterine rupture and have a significantly reduced life expectancy. We describe a pedigree in which a single base pair substitution leading to a G786R mutation in Col3A1 was detected in a 55 year old proband and her 30 year old son. Previously performed protein gel electrophoresis analysis on a clinical basis found abnormal type III procollagen motility. A review of the family history revealed that at least 10 additional members of the family are affected with VEDS. A total of 6 premature deaths (age range 14-55) were reported due to aneurysms and bleeding complications. However, there was no history of uterine rupture or pregnancy- related death in five affected females with a total of ten pregnancies. A vascular work-up including MRI/MRA imaging and echocardiography in the 30 year old son who carries the G786R mutation was completed and did not reveal any abnormalities. A review of the clinical history in affected family members revealed variability in the facial characteristics as well as musculoskeletal features of the disorder, in addition to the vascular presentation. The phenotypic variability speaks to the role of environmental interactions as well as possible influence of modifier loci. The study of modifying influences may lead to the identification of therapeutic approaches to reduce the complications of VEDS.

Aortic smooth muscle reactivity in -galactosidase A knockout mice. *P. Rozenfeld[1], M. Frtiz[1], A. Kulkarni[2], R. Brady[2], C. Fossati[1], G. Rinaldi[1]* 1) Univ Nacional de La Plata, Argentina; 2) National Institutes of Health, USA.

Introduction: Fabry disease results in deposition of glycosphingolipids, particularly in vascular endothelium and smooth muscle cells, leading to vessel occlusion and ischemia. Previous reports demonstrated increased endothelium-mediated vascular reactivity in Fabry disease. We used the -galactosidase A knockout (AGA null) mice as a murine model to study the pathophysiology of the vasculopathy in Fabry disease. The aim of this work was to evaluate the aortic reactivity in AGA null mice compared with wild type littermates (WT). Methods: Contraction forces (in milligrams of force per milligram of tissue, mgF/mgT) of aortic rings from AGA null and WT mice were measured after exposure to either 80 mM KCl or 1 M norepinephrine (NE). The relaxant agents acethylcholine (Ach) and sodium nitroprusside (SNP) were added to the bath when the maximum force of the agonists was attained, and the relaxation was expressed as a percent of that contraction. The effect of L-NAME in Ach-induced relaxation was also studied. Results: Aortic rings from AGA null mice developed a higher contractile force than WT mice in 80 mM KCl (1526 169 vs. 1083 117 mgF/mgT, $P<0.05$); and with NE (1412 119 vs. 1174 141 mgF/mgT,). After the addition of Ach, the relaxation was significantly greater in AGA null mice. L-NAME inhibited Ach-induced relaxation to a similar extent in both groups. The percentage of relaxation with SNP was lower in AGA null mice than in WT mice ($p<0.03$). Conclusions: 1) Aortic smooth muscle of AGA null mice exhibited augmented contractile response upon activation of either voltage-operated or receptor-operated channels. 2) An exaggerated response to Ach was observed, demonstrating an increased endothelium-dependent vasodilation. 3) SNP-induced relaxation was significantly decreased in AGA nulls with respect to WT. 4) A similar blunting of Ach response after L-NAME was observed in both groups. These findings indicate an augmented contractile response and a higher vasodilation response in AGA null mice mediated mainly by non-NO endothelial mediators.

DNA microarray-based resequencing analysis of LRRK2 in familial Parkinsons disease. *H. Tomiyama[1,2], N. Seki[2], Y. Takahashi[2], J. Goto[2], E. Rogaeva[3], A. Lang[3], M. Murata[4,5], T. Toda[5,6], M. Yamamoto[5,7], M. Funayama[1], Y. Mizuno[1], N. Hattori[1,5], P.S. George-Hyslop[3], S. Tsuji[2]* 1) Neurology, Juntendo University School of Medicine, Japan; 2) Neurology, University of Tokyo, Japan; 3) Centre for Research in Neurodegenerative Diseases, Dept of Medicine, University of Toronto, Canada; 4) Neurology, Musashi Hospital, National Center of Neurology and Psychiatry, Japan; 5) CREST, Japan; 6) Functional Genomics, Osaka University Graduate School of Medicine, Japan; 7) Neurology, Kagawa Prefectural Central Hospital, Japan.

Objectives: LRRK2, the causative gene for PARK8, is a large gene with 51 exons containing 7584bp. Recently, we have established a custom-made DNA microarray-based high throughput resequencing system for the comprehensive mutational analysis of genes of familial Parkinsons disease (PD). To clarify the molecular epidemiology of mutations in the large LRRK2 gene, we applied the microarray-based resequencing system to extensive mutation analysis of LRRK2. Methods: We determined the nucleotide sequences of all the 51 coding exons and the splice junctions of LRRK2 of 42 autosomal dominant PD (adPD) probands from Japan (23 families) and Canada (19 families) using the microarray-based resequencing system. Results: We found one patient with R1514Q from Canada. The R1514Q has been described in adPD patients of Norway, thus confirming as the causative mutation. The G2385R was found in 21.7% (=5/23) of Japanese adPD probands, while it was not identified in adPD patients from Canada. The G2385R was also present in 4.8% (=22/457) of Japanese normal controls. Discussions: High throughput DNA microarray-based resequencing system is highly useful for comprehensive mutational analysis of causative genes for PD, especially for genes containing numerous exons like LRRK2. Very recently, the frequency of heterozygotes G2385R has been reported to be higher in sporadic PD patients (10.0%) than in controls (4.8%) in Taiwan. Given the much higher overrepresentation of the G2385R in Japanese adPD probands (21.7%), the results strongly support that LRRK2 G2385R is a common genetic risk factor for PD in Asian but not in Caucasian.

Dilated Cardiomyopathy In Propionic Acidemia Patients. *S. R. Romano[1], V. V. Valayannopoulos[1], F. L. Lacaille[1], D. B. Bonnet[1], D. R. Rabier[3], A. R. Rotig[2], A. M. Munnich[2], G. T. Touati[1], P.dL de Lonlay[1,2]* 1) Department of Pediatrics Hôpital Necker-Enfants Malades, Paris, France; 2) Department of Medical Genetics and INSERM U-393Hôpital, Necker-Enfants Malades, Paris,France; 3) Department of Biochemistry Necker-Enfants Malades, Paris,France.

Objective. Dilated cardiomyopathy is now a well known complication of propionic acidemia (PA) even if it has been reported in only a small number of PA patients. We reviewed PA patients for evidence of cardiomyopathy and we described follow up and management of these patients. Methods. Five patients with PA had the diagnostic criteria of cardiomyopathy and were treated with standard therapy. For each patient we detailed clinical follow up, age at the onset of cardiomyopathy and medical management. Endomyocardiac biopsy (EMB) was performed for mitochondrial respiratory chain investigation (two patients). Results. Dilated cardiomyopathy occurred at age height (n=1), six (n=1), nine (n=1), and five (n=2). All patients had neonatal onset of PA (neonatal coma) except one (10 months). Complex III deficiency was identified for one patient and complex II deficiency for the other. Liver transplantation (LT) was performed for two patients. The first was transplanted thirteen years ago. Cardiac echography was normal one year after the transplantation. The second child has been transplanted one month ago and shortening fraction was 28 % before LT. Cardiac echography is now normal. Conclusion. Dilated cardiomyopathy in propionic acidemia is a severe complication due to secondary Oxphos deficiency. A toxic accumulation of metabolites excreted in PA probably is implicated as the cardiac defect was reversible after liver transplantation for two patients.

Are periventricular intracranial cysts on prenatal MRI an early diagnositic feature for mitochondrial depletion syndrome? *M. Rohrbach[1], D. Chitayat[1, 2], G. Maegawa[1], G. Davidzon[3], S. Shanske[3], K. Chong[1, 2], S. Blaser[4]* 1) The Hospital for Sick Children, Department of Pediatrics, Division of Clinical and Metabolic Genetics, University of Toronto, Toronto, ON, Canada; 2) Mount Sinai Hospital, The Prenatal Diagnosis and Medical Genetics Program, University of Toronto, Toronto, Ontario, Canada; 3) Department of Neurology, Columbia University College of Physicians and Surgeons, New York, USA; 4) The Hospital for Sick Children, Department of Pediatrics, Divisions of Neuroradiology, University of Toronto, Toronto, ON, Canada.

Mitochondrial DNA (mt DNA) depletion syndrome (MDS) refers to a quantitative defect in mtDNA in which affected tissues have markedly reduced mtDNA copy number. MDS is inherited as an autosomal recessive trait and mutations in a number of nuclear genes involved in mtDNA replication and nucleotide metabolism have been reported in patients. We describe a patient who first presented at 27 weeks gestation with isolated prenatal MR findings of bilateral periventricular/subependymal cysts adjacent to the frontal horns, and fetal diffusion-weighted-images which showed restriction. The pregnancy was continued and resulted in a boy, who presented with severe lactic acidosis, elevated lactate/pyruvate ratio (56.6) and methylmalonic aciduria. Postnatal MRS revealed high lactate peak and MRI confirmed prominent periventricular cysts with no other brain abnormalities. Muscle biopsy showed low activities for multiple respiratory chain complexes, suggesting MDS. Quantitative PCR analysis in muscle showed a severe reduction of mtDNA, a depletion of approximately 90%. However, sequencing of two of the genes affected in some patients, thymidine kinase (TK2) and polymerase gamma (POLG) did not reveal any mutations. The karyotype was 46,XY. To our knowledge this is the first report correlating fetal MRI abnormalities and mitochondrial depletion syndrome. Due to unfavorable prognosis, prenatal recognition of this disorder would be very useful for counseling.

Chromosome 16p duplications: phenotype and microarray analysis. *J. Puechberty[1], M. Noruzinia[3], A. Schneider[1], G. Lefort[1], J. De Vos[2], C. Coubes[1], P. Blanchet[1], V. Cacheux[1], P. Sarda[1]* 1) Genetique Medicale, CHU Arnaud de Villeneuve, MONTPELLIER, Languedoc, France; 2) Institut de Recherche en Biotherapie, CHU Hopital Saint-Eloi, MONTPELLIER, Languedoc, France; 3) School of Medical Sciences, Tarbiat Modarres University, Teheran, Iran.

Partial trisomy 16p is a rare chromosomal anomaly. Duplications of chromosome 16p are often the products of unbalanced maternal reciprocal translocations and consequently the phenotype of patients is not typical of pure partial trisomy 16p. The cytogenetic difficulty is to determine the precise limits of duplicated regions, which is important for predicting the phenotype. We report an analysis of eleven cases of pure partial duplications of chromosome 16p, six from the literature and five new cases from our laboratory. This study determines three groups of duplications. The first group includes patients with short proximal 16p11-p12.1 euchromatic duplication considered as a silent duplication without phenotypical expression. Two of our patients fall into group 1. Two other patients belong to group 2 which corresponds to patients with larger 16p11-p12/p13 duplications. Phenotype is characterized by severe mental retardation, dysmorphism, variable malformations and recurrent infections. The third group includes patients with terminal 16p13-pter duplication and is not well defined to date due to the difficulty of comparing published reports. Our last patient falls into this category. The correlation phenotype-karyotype is difficult to define according to the data presently reported in the literature. In our patients, studies using SNP microarrays (Affymetrix) have permitted to define the borders of the duplications and have led to the discovery of an additional unexpected micro-rearrangement in one case.

Carnitine palmitoyltransferase-1b (CPT-1 muscle isoform) deficiency in the mouse. *P.A. Wood[1], S. Ji[1], D.A. Hamm[1], J. Kerner[2], C.L. Hoppel[2], T.R. Schoeb[1], W.S.H. Chick[3], Y. You[3]* 1) Dept Genetics, Univ Alabama at Birmingham, Birmingham, AL; 2) Depts Pharmacology & Nutrition, Case Western Reserve Univ, Cleveland, OH; 3) Oak Ridge Nat Lab, Oak Ridge, TN.

Carnitine palmitoyltransferase-1 (CPT-1) catalyzes the rate-limiting step of mitochondrial beta-oxidation of long chain fatty acids. CPT-1b, also known as the muscle isoform, is expressed in skeletal and cardiac muscle, as well as brown fat, and to a lesser extent in other tissues. Patients with CPT-1b deficiency have been extremely rare. In order to better understand the function of this gene, we have developed a mouse model with CPT-1b deficiency by gene targeting a deletion of exons 1-3 in the mouse CPT-1b gene (*Cpt-1b*). To date we have not detected any homozygous CPT-1b deficient pups, suggesting that homozygous mutants do not survive to birth. There were no CPT-1b homozygous mutant embryos detected from heterozygous matings at 9.5 dpc (n=10) and 11.5 dpc (n=8). In both male and female CPT-1b heterozygous mice, the total CPT-1 enzyme activity in skeletal muscle was reduced to ~60 percent of that in wild-type littermates, whereas no change in total CPT-1 activity was seen in liver. This is consistent with the predominate tissue-specific expression of CPT-1b in skeletal muscle but not liver. Following an 18 hour fast, adult CPT-1b heterozygous mice showed significant fatty change in brown adipose tissue only, whereas skeletal muscle and heart appeared unaffected as compared to CPT-1b wild-type controls. Furthermore, there were no significant differences in fasting blood glucose and triglyceride concentrations. The development of this model will provide an important tool to investigate the role of CPT-1b in many disease conditions including type 2 diabetes.

Histological Findings in GUCY2D-/- x PDErd10/rd10 Double Mutant Mice Suggest a Protective Effect of cGMP Depletion in Photoreceptors Lacking Rod-Specific PDE Function. *J.M. Rozet[1], I. Perrault[1], K. Bigot[2], A. Provot[2], L. Vede[2], G. Pivert[3], A. Munnich[1], M. Abitbol[2], J. Kaplan[1]* 1) INSERM U781, Hopital des Enfants Malades, Paris, France; 2) CERTO, Faculte de Medecine Necker, Paris, France; 3) IRNEM, Faculte de Medecine Necker, Paris, France.

This purpose of this study was to characterize the phenotype of a mouse lacking both GUCY2D and rod-specific PDE functions. GUCY2D knock-out mice were crossed with homozygous PDErd10/rd10 mice to generate double mutant GUCY2D-/- x PDErd10/rd10 mice. Comparative histological studies of the retinas of rd10 and double mutant mice were performed at ages 2, 3, 4, 5, 6, 7 weeks. For that purpose, the animals eyes were fixed in paraformaldehyde, embedded in wax, sectioned at 4m and coloured in HES. As soon as 4 weeks of age, the retinas of homozygous PDErd10/rd10 mice showed a massive reduction of the outer segment, inner segments layer and outer nuclear layers. Conversely, the retina of double mutant mice of the same age demonstrated slower photoreceptor degeneration. In PDErd10/rd10 mice a dramatic degeneration was noted in both central and peripheral retina (one or two rows of photoreceptors). In double mutant mice the degeneration followed a concentric gradient from the central retina to the peripheral retina where a significant number of photoreceptors was still present up to 7 weeks of age, especially in the superior retina. In conclusion, it has been shown that the invalidation of GUCY2D in mice resulted in severe cone degeneration. Rods do not degenerate but are functionnaly impaired. On the other hand, the missense mutation in the rod-specific beta-subunit of the PDErd10/rd10 mice resulted in a fast degeneration of both rods and cones. Interestingly, in mice lacking both the GUCY2D and PDE functions, the degeneration of photoreceptors was less severe than in rd10 mouse. Although preliminary, these data suggest that the retinal-specific guanylate cyclase gene might play a role in rod photoreceptors of mouse and that the lowering of cGMP synthesis might partially protect these cells from degeneration.

Genome wide linkage analysis of two Norwegian families with epilepsy. *K.K. Selmer[1], T. Egeland[1], M.H. Solaas[1], K. Brandal[1], K.O. Nakken[2], L.A. Corey[3], D.E. Undlien[1]* 1) Inst. of Medical Genetics, University of Oslo, Norway; 2) National Center for Epilepsy, Sandvika, Norway; 3) Dept. of Human Genetics, Virginia Commonwealth University, Richmond, VA, USA.

Epilepsy is one of the most common brain disorders with a prevalence of ~1%. Most forms of epilepsy are considered to be of multifactorial origin and the clinical expression of the different subtypes is very heterogeneous. During the past decade mutations in several different genes have been detected as a cause of some monogenic forms of epilepsy. The aim of our study is to identify mutations leading to the development of epilepsy. More than 40 families with assumed monogenic epilepsy were ascertained from the population based Norwegian Twin Panel. Epilepsy classifications were done by experienced neurologists according to the International League Against Epilepsy classification, based on clinical and family interviews and medical records. Further selection of 12 families for genome wide linkage analysis was based on power analyses and clinical diagnoses. 3862 SNP markers were genotyped in 285 persons using the ABI Linkage Mapping Set 4K. Analyses of two of the families were performed in Merlin, which takes linkage disequilibrium (LD) between markers in clusters into account to reduce possibly inflated LOD scores due to LD between markers. The remaining 10 families will be analyzed in Simwalk2 due to pedigree size. Altogether, the two families consist of 46 members, in which 11 are affected by either epilepsy or febrile seizures. Estimated maximum LOD scores (based on power estimations) were 1.8 and 1.5 respectively and parametric analyses assuming autosomal dominant mode of inheritance with penetrance of 0.80 indicated linkage to chromosomal region 14q31.1 with LOD scores in the same range. Suggestive linkage to other loci was also found in the two families, but the linkage peak on chromosome 14 was the only one the two families had in common. Our study provides evidence for linkage to chromosome 14 in two Norwegian families with epilepsy and further analyses of the remaining 10 families with a similar form of epilepsy are planned to see if we can confirm the linkage to this locus.

A method for finding risk haplotypes using cases and their parents. *M. Shi, D.M. Umbach, C.R. Weinberg*
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We propose a method for association studies based on dense SNP markers using cases and their parents. Our approach uses the genotypes of affected individuals and their parents directly, without requiring the user either to know or to estimate haplotypes and their phases. Our testing procedure works by comparing the affected offspring with their corresponding complement through permutations, where the complement is the equally likely hypothetical offspring of the same parents that would have received the sequences of DNA un-transmitted to the affected offspring. We describe a method, the max_Z2 test, for testing the hypothesis that the inherited diplotype is not associated with the disease phenotype under study. The max_Z2 is based on the difference vector between the childs and complements genotypes, and P-values are obtained by referring to a permutation distribution, derived by randomly assigning disease status to either the offspring or their complement. This method can be used when there are missing SNPs or individuals with entirely missing genotypes. To take advantage of different testing procedures we propose an approach to effectively combine different tests. Our approach can be easily extended to test for maternal effects. When the null hypothesis is rejected, a subsequent procedure permits identification of the informative risk-haplotype tagging SNPs, to enable discovery of the haplotype that is associated with the phenotype under study. We characterize the performance of the proposed approach and compare it with several competing procedures through realistic simulation studies of case-parent triads based on the haplotype frequencies and structures for four genes, derived from the HAPMAP project. We apply this method to data from an orofacial cleft study.

Molecular Studies on *DYX1C1* a candidate gene for dyslexia. *I. Tapia-Paez*[1], *S. Massinen*[2], *J. Kere*[1,2] 1) Biosciences and Nutrition, Stockholm, Stockholm, Sweden; 2) Dept. of Medical Genetics, Biomedicum, University of Helsinki, Finland.

Dyslexia is a complex disorder characterized by reading disability despite normal intelligence, senses and education; it affects 5-10% of the population. Genetic studies have repeatedly pointed out loci linked to dyslexia on at least chromosomes 2, 3, 6, 11, 15 and 18. Until now, four genes have been associated with dyslexia: *DYX1C1*, *DCDC2*, *KIAA0319*, and *ROBO1*. These genes are involved in neuronal migration or brain development and their functional role in dyslexia remains to be elucidated. The *DYX1C1* gene on chromosome 15 (Taipale et al. PNAS 2003) was the first gene implicated in dyslexia, cloned based on a translocation t(2;15)(q11;q21) that cosegregated with dyslexia in a Finnish family. Two sequence changes in *DYX1C1* showed association with dyslexia in unrelated families, one that introduced a stop codon truncating the protein by four amino acids, and the second in 5UTR, close to the translation initiation site. The association of *DYX1C1* with dyslexia has been ambiguous in replication studies, prompting us to look for new SNPs in the promoter of the *DYX1C1* gene. Two new SNPs were found, and when combined with additional results from German samples, they showed supportive evidence for *DYX1C1* as a candidate dyslexia gene (Dahdouh F et al. manuscript). To understand possible functional consequences of these changes, we made constructs to study the binding of proteins in electrophoretic mobility shift assays. Allele-specific differential retardation of mobility was observed with the promoter and 5UTR SNPs, suggesting a functional effect of these variations. We also prepared constructs for reporter assays in pGL3 basic and promoter vectors using the -3G/A polymorphism. We could detect differences in luciferase expression, suggesting that a repressor molecule binds to the -3 position and that this effect is stronger when the -3G variation is present. In another approach, to study protein-protein interactions we have created a stable cell line overexpressing constitutively native DYX1C1. Elucidation and understanding of the biology behind *DYX1C1* and the other genes related to dyslexia may open new pathways in the study of brain function.

The polymorphism of *INSIG2* is associated with fatty liver, hypertriglyceridemia and insulin resistance in type 2 diabetes. *M. Zenibayashi, Y. Hirota, K. Miyake, T. Teranishi, K. Kouyama, K. Sakaguchi, T. Ohara, M. Kasuga*
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Introduction Insulin-induced gene (Insig) 1 and 2 are closely related proteins that block proteolytic activation of sterol regulatory binding proteins (SREBPs). We studied the association of polymorphisms in Insig genes with type 2 diabetes and its relation to lipid parameters in type 2 diabetic patients. **Method** We screened 5' flanking region (1000bp) and the coding region of the human *INSIG1* and *INSIG2* in 16 diabetic subjects by direct-sequencing. 5 SNPs in *INSIG1* and 2 SNPs in *INSIG2* were identified. We also extracted 4 SNPs around *INSIG1* and 9 SNPs around *INSIG2* from the public databases for analyzing linkage disequilibrium (LD) in 87 non-diabetic subjects. We selected 3 SNPs of *INSIG1* and 7 SNPs of *INSIG2* based on allele frequencies and LD patterns. We genotyped these SNPs in 340 Japanese patients with type 2 diabetes and 180 non-diabetic subjects. We evaluated hepatic lipid content by ^{1}H-Magnetic Resonance Spectroscopy. **Results** None of SNPs or haplotypes in both genes was associated with type 2 diabetes. We also tested their association to the lipid parameters of 100 patients with type 2 diabetes. We found one haplotype of *INSIG2* was significantly associated with hepatic lipid content (p=0.015) and serum triglyceride level (p=0.016). This haplotype was determined by -231G>A polymorphism (nucleotide number of the SNP is counted from the transcriptional start site), so we examined whether this SNP affects serum triglyceride level and HOMA-IR in 308 patients with type 2 diabetes. We found A allele carriers had significantly higher levels of serum triglyceride (p=0.031) and HOMA-IR (p=0.008) than non-carriers. These differences remained after adjusting for sex, age and BMI using multiple regression model. We found no association of this polymorphism with BMI in these 308 patients. **Conclusion** These results suggest that the -231G>A polymorphism of *INSIG2* is associated with fatty liver, hypertriglyceridemia and insulin resistance in type 2 diabetes.

Therapeutic perspectives for the treatment of breathing dysfunctions in Rett syndrome. *J. C. Roux, E. Dura, A. Moncla, J. Mancini, L. Villard* Génétique Médicale, INSERM U491, Faculté de Médecine de la Timone 13385 Marseille, France.

Rett syndrome is a severe X-linked neurological disorder, in which most patients carry a mutation in the gene encoding methyl-CpG binding protein 2 (MECP2). The clinical course of the disease consists of normal in utero and neonatal development followed by a period of regression showing signs of neurodevelopment defects (arrest of brain development, loss of acquisitions such as speech and walk, apparition of behavioural troubles). Twenty six percent of deaths in Rett girls occur with sudden respiratory arrhythmia. We investigated breathing dysfunction in Rett syndrome using an animal model deficient for the Mecp2 gene. We performed experiments on wild-type and Mecp2-deficient mice to understand the role of the Mecp2 gene in respiration and bioaminergic systems. We have previously shown that adult mice deficient for the Mecp2 gene have erratic breathing with highly variable respiratory rhythm and frequent apneas likely due to reduced norepinephrine content and a drastic decrease of tyrosine-hydroxylase (rate limiting enzyme in the catecholaminergic synthesis) expressing neurons in the medulla. Recently, using TUNEL method, no apoptotic cells have been identified at the brainstem level indicating that the neurons did not die and probably lost their ability to synthesize tyrosine-hydroxylase protein. We are currently investigating the stimulation of noradrenergic metabolism in the same animal model using specific norepinephrine reuptake inhibitors. Our results show that we can improve both, the respiratory rhythm of the mutant animals and increase significantly their lifespan. Moreover, we determined that our treatment significantly increased the number of tyrosine-hydroxylase expressing neurons which could explain at least in part the breathing improvement. In conclusion, these results open new perspectives for the treatment of the respiratory deficits of Rett syndrome children.

Evidence of a single founding event responsible for the infertility phenotype macrocephalic spermatozoa. *P.F. Ray[1], K. Dieterich[1], J. Perrin[1], S. Hennebicq[1], B. Ben Amar[2], M. Zahi[2], S. Rousseaux[3], B. Sèle[1], J. Lunardi[1]* 1) Dpt Génétique et Procréation, CHU de Grenoble, France; 2) Rabat, Morocco; 3) INSERM U309,Grenoble.

Rare cases of infertile men have been reported presenting with 2-3 fold enlarged spermatozoa with an aneuploidic content observed by FISH. The anomalies suggested a segregation defect during meiosis I and II and a failure of nuclear division. The remarkable likeness of i) the spermatograms, ii) the morphological aspect of the spermatozoa and iii) the FISH results strongly suggested a common genetic etiology for this rare phenotype. We investigated four unrelated French patients (3 from Algier and one from Tunisia), all born to first cousins, and ten patients from the Rabat region in Morocco. All showed oligoasthenoteratozoospermia with 100% abnormal spermatozoa with macrocephalia and a variable number of flagella. An autosomic recessive transmission was suspected, potentially due to a founder effect. A microsatellite whole genome scan was realized to identify homozygous regions common to the patients. DNA was extracted from blood and a genome-wide scan was realized for the 22 autosomes with the 10cM Linkage Mapping v2.5 Applied Biosystems. Homozygosity ranging from 1 to 6 adjacent kit markers (<10 - 58 Mb) was observed on chromosome 19 in 9/14 patients. Detailed polymorphisms analysis confirmed the region homozygosity for the nine patients and allowed to identify a smaller region in 3 other patients that had passed unoticed with the first low coverage screen. A 1Mb critical interval with a common haplotype was identified in all 12 patients. In conclusion our data indicate that 12/14 patients have inherited two copies of a common genetic event localized on chromosome 19. All four French patients have inherited the "Rabats haplotype"; indicating that the genetic event is probably at least 10 generations old. We are currently sequencing a candidate gene expressed in the testis. Preliminary results indicate that this gene is likely to be involved.

This work was supported in part by the Fond d'Aide à la recherche Organon (FARO) and the DRC of Grenoble CHU.

GOLDsurfer2: A comprehensive tool for the analysis and visualization of whole genome association studies. *F. Pettersson[1], A.P. Morris[1], P.S. Derwent[2], M.R. Barnes[2], L.R. Cardon[1]* 1) Dept Bioinformatics, Wellcome Trust Centre, Oxford, United Kingdom; 2) GlaxoSmithKline Pharmaceuticals, Genetic Bioinformatics, Harlow, Essex, UK.

With recent advances in the efficiency of high-throughput single nucleotide polymorphism (SNP) genotyping technology, genome-wide association studies are now routinely undertaken with the sample sizes necessary to detect the modest genetic effects we expect for complex diseases. There is now a clear demand for efficient analysis tools that allow data pre-treatment, together with evaluation, visualization and interpretation of results. To meet these demands, we have further developed the GOLDsurfer software [1]. GOLDsurfer2 can be used for pre-filtering of genotype data, using user-defined quality control thresholds based on Hardy-Weinberg disequilibrium, missing genotype rates and minor allele frequencies, and for statistical association analysis. Interactivity in terms of both visualization and data management are key concepts in the user-friendly GUI. Basic statistical calculations, including single-locus and pairwise models of SNP association with disease, are built in. The architecture is written to accommodate methods for more complex analyses as external modules. Functional annotation is of great importance for design of studies and evaluation of statistical results. GOLDsurfer2 can link to extract annotation from public databases such as the UCSC genome database and it also supports browsing of gene ontologies. A core feature is the 3D interactive visualisation of linkage disequilibrium (LD) which has been further developed to function on a genome wide level. Extended regions of disease association within LD blocks can suggest regions for further investigation in order to find putative causal alleles. To this end, we include a capability to functionally weight SNPs characterized by the International HapMap project. GOLDsurfer2 is implemented in Java and distributions are available for Mac OS X, Linux and Windows. References. [1] Pettersson, F., Jonsson, O. and Cardon, L.R., GOLDsurfer: three dimensional display of linkage disequilibrium. Bioinformatics. 2004;20(17):3241-3.

A polyalanine contraction in HOXD13 is associated with complex brachydactyly. *X. Zhang[1], X. Zhao[1,2], M. Sun[1,3], W. Yang[1], X. Zeng[1], W.H.Y. Lo[1], E.W. Jabs[3], X. Shan[2]* 1) Department of Medical Genetics, Peking Union Medical College, Beijing, China; 2) Southeast University, Nanjing, China; 3) The Johns Hopkins University, USA.

HOXD13, the homeobox-containing gene located at the most 5 end of the HOXD cluster, plays a critical role in limb patterning and growth. Mutations in human HOXD13 may give rise to different limb malformations. Polyalanine expansions in HOXD13 cause synpolydactyly (SPD [MIM 186000]) while missense mutations in the homeodomain are associated with brachydactyly types D and E. SPD was the first disease in which a polyalanine expansion was identified as the disease-causing mechanism. This novel type of mutation has been found in nine different human genetic diseases. Unequal crossing-over has been suggested as the mechanism for polyalanine expansion. Polyalanine contraction, the alternative allele of unequal crossing-over, has not yet been confirmed to cause a human genetic disease. We identified a large seven-generation Chinese family with an autosomal dominant complex brachydactyly phenotype to discover the disease gene. Fifty-seven family members, including 27 affected individuals (10 males and 17 females), were clinically investigated. The constant phenotypic feature in the family is bilateral generalized brachydactyly with limb features overlapping brachydactyly types A, D and E. Forty-five members were recruited for linkage and mutation analysis. Two-point linkage analysis was performed using 31 microsatellite genetic markers at chromosome 2q. A maximal LOD score of 6.46 (=0) was obtained at D2S2314. Haplotype analysis defined a region between D2S1379-D2S324 where HOXD13 was located. A deletion of 21 bp in the polyalanine encoded by exon 1 of HOXD13 was detected. This polyalanine contraction of 7 residues was shown to completely cosegregate with the disease phenotype and was not detectable in all unaffected individuals in the family and controls. Our data establish the association of a polyalanine contraction with a genetic disease.[This work has been supported in part by the International Collaborative Genetics Research Training Program (NIH D43 TW 06176)].

Antidepressant treatment response in mood disorders is associated with a brain-derived neurotrophic factor haplotype. *V. Soria[1], M. Gratacos[2,3], J.R. Gonzalez[2,3], M. Bayes[2,3], R. de Cid[2,3], J.M. Crespo[1], M. Urretavizcaya[1], X. Estivill[2,3]* 1) Mood Disorders Clinical and Research Unit, Psychiatry Dept, Hospital Universitari de Bellvitge. Catalonia. Spain; 2) Genes and Disease Program, and National Genotyping Center (CeGen), Center for Genomic Regulation, Catalonia, Spain; 3) Department of Health and Life Sciences, Pompeu Fabra University, Barcelona, Catalonia, Spain.

To test whether BDNF is a susceptibility locus for mood disorders phenotypes and antidepressant treatment response, we performed a case-control study with eight TagSNPs (including the Val66Met functional variant), covering the entire BDNF region. We have genotyped 342 control subjects and a clinical sample of 374 patients with mood disorders diagnosed according to DSMIV criteria. Single SNP case-control association analysis considering the mood disorder phenotype identified a significant association to a SNP located in the 5 upstream region of the gene ($p = 0.029$), which did not remain significant after Bonferroni correction. We further investigated the association of this nominal positive SNP with the antidepressant treatment response phenotype. Patients were allocated to one of two groups according to Thase and Rush staging scheme: those with clinical remission after major depressive episode treated with adequate antidepressant monotherapy were denoted responders. After testing for five genetic models of inheritance, the additive model was the one that best fit the data. Thus, patients heterozygous for variant allele have almost 3-fold probabilities of being responders and almost 6-fold if they are homozygous carriers (OR = 2.95; CI95% = 1.48-5.88; $p = 0.0025$). We have further identified a haplotype conferring risk to this particular phenotype through a 3 SNPs sliding window approach (OR = 2.71; CI95% = 1.30-5.63; $p = 0.0078$). While our results failed to detect a positive association of BDNF with mood disorders diagnosis, they support the implication of BDNF in the therapeutic response to antidepressants.

Clinical evaluation of Prader-Willi syndrome treated with growth hormone in early infancy. *H. Yoshihashi[1], D. Ariyasu[1], T. Hasegawa[1], K. Samejima[2], K. Kurosawa[2]* 1) Pediatrics, Tokyo Metropolitan Kiyose Children's Hospital, Tokyo, Japan; 2) Division of Medical Genetics, Kanagawa Children's, Medical Center, Yokohama, Japan.

Prader-Willi syndrome (PWS) is characterized by infantile hypotonia, obesity in childhood, small hands and feet, hypogonadism, mental retardation, and occurs at a frequency of 15,000 births. The major pathogenic mechanisms are a deletion of a paternal chromosome 15(q11-q13)(70%) or maternal uniparental disomy(UPD) of chromosome 15(25%). The majority of patients with PWS can be diagnosed by using methylation analysis, even if in the neonatal period. Growth hormone replacement therapy is known as one of the treatments, after assurance that sleep apnea and respiratory compromise are not present. The age for starting growth hormone replacement is still controversial. We evaluated for the effects of growth hormone replacement therapy to three PWS female babies, including 2 cases with mUPD15 and 1 case with deletion, diagnosed in early infancy (13-62day). Hypotonia, feeble fetal activity, poor suck, labial hypoplasia, hip-joint abduction are common to these cases. Data are presented on perinatal events, dysmorphic features, body composition, development, and cytogenetics. Growth pattern is estimated in comparison with growth charts representing Japanese patients with PWS (Nagai T et al. Am J Med Genet. 2000) . All cases, who started on growth hormone replacement therapy (0.25mg/kg/week) at 7-18month, grow well on treatment and especially improve in development. In one case, facial and body phenotype are almost normal. There are no adverse effects. It is appropriate to start growth hormone replacement therapy in early infancy . Further study may provide insight into the consideration of the indication of replacement therapy.

Unexpected results of molecular diagnostics in a patient with CDG Ia. *K. Vesela[1], T. Honzik[1], H. Hansikova[1], E. Schollen[2], G. Matthijs[2], J. Zeman[1]* 1) Pediatric Department, 1st Medical Faculty, Charles University, Prague, Czech Republic; 2) Center for Human genetics and Center for Metabolic Diseases, University of Leuwen, Belgium.

Congenital disorders of glycosylation represent a heterogeneous group of disorders affecting assembly or processing of the glycoproteins. Up to now, 24 distinct subgroups of CDG syndrome have been identified. We present unexpected results of molecular analyses of PMM2 gene in 16-year old boy with CDG Ia syndrome and persisting thrombocytopenia and leucopenia. Methods: We analyzed (PCR, RFLP and sequencing analysis) coding sequence and 59 SNPs around in gDNA from whole blood, cultivated fibroblasts, buccal cells, urinary sediment, isolated platelets and CD3+, CD19+, CD15+ and CD14+ cells separated from fresh blood sample and cDNA from whole blood. Results: Molecular analyses confirmed the diagnosis CDG-Ia, but according to found mutation layout, the exact hereditary transfer is unclear. First analysis of gDNA from whole blood revealed novel homozygous mutation IVS2+3A>T. Analysis of parental DNA showed this mutation only on one fathers' allele, mother carried heterozygous form of common mutation 422G>A. Therefore we continued in analyses of other tissues. In cultivated fibroblasts, buccal cells, urinary sediment we confirmed the presence of both mutations in heterozygous forms, but in separated blood cells we found lower proportion of 422G>A only in platelets, the other cells carried homozygous IVS2+3A>T. Discussion: We assume that there is a mosaic in the stem cells. DNA differences between platelets and other cells might correlate with patients' symptoms. Mechanisms of forming cells with homozygous IVS2+3A>T is unclear. Existence two different genotypes in one blood cell lineage might be explained by unknown selection benefit of blood cells carrying homozygous mutation IVS2+3A>T compare to the cells with IVS2+3A>T/422G>A, or by alternative model of haematopoieses. Supported by GAUK 33/2005/c, GAUK 18/2004/c and LSHM-CT-2005-512131.

Genetic association of LDLR SNP with plasma low density lipoprotein cholesterol level. *H. Zhu[1], H.M. Tucker[1], R.K. Gopalraj[1], J. Simpson[1], L.A. Cupples[2], A.K. Manning[2], S. Estus[1]* 1) Department of Physiology and Sanders-Brown Center on Aging, University of Kentucky, Lexinton, KY; 2) Department of Mathematics and Statistics, Boston University, Boston, MA.

Alternative splicing increases transcript diversity and maximally utilizes genome sequence. Single nucleotide polymorphisms (SNPs) may locate in exonic splicing enhancers (ESEs) and act as cis genetic components to modulate mRNA splicing efficiency. Recently, our lab identified a low density lipoprotein receptor (LDLR) SNP that was associated with a lower proportion of full length LDLR transcript in both human brain and minigene transfection experiments. In this study we further evaluated the association of this SNP with plasma LDL cholesterol level in a Framingham Offspring Study (FOS) series of 1716 individuals. Genomic DNA was extracted from peripheral blood leukocytes. ApoE genotypes were determined by a restriction fragment length polymorphism approach. SNP genotyping used a TaqMan approach designed by Applied Biosystems. Briefly, genomic DNA was denatured at 95º for 10min, and then amplified for 40 cycles by using 92ºC for 15sec and 60ºC for 1min. Genotypes were determined using an ABI PRISM 7000 sequence detection system (Applied Biosystems). The SAS General Linear Model (GLM) procedure was used to evaluate genotypic association of LDL cholesterol level with this LDLR SNP. The resulting p values were considered significant if less than 0.05. Our results suggested that LDLR SNP rs688 was significantly associated with a higher plasma LDL cholesterol level in pre-menopausal women and that this effect was dominant (p=0.0129) when adjusting for age, BMI, apoE, estrogen level, menopause. Hence, this LDLR SNP needs further study for its functional mechanism and may represent a good genetic biomarker for cardiovascular disease.

Are we providing excellent care? Development of novel quality measures for clinical genetics. *A. Shealy, C. Scacheri, M. Mettler, R. Rerko, D. Clements, C. Clough, C. Eng, M.R. Natowicz* Center for Personalized Genetic Healthcare, Cleveland Clinic Genomic Medicine Institute, Cleveland, OH.

Many medical specialties have already developed outcomes measures, parameters to evaluate operational aspects of clinical practice. These provide a structured, often quantitative, means to determine the quality of care provided by clinicians, facilitate standardization of healthcare delivery, and offer a way to monitor improvement over time. They also allow one to assess patient-oriented parameters including patient satisfaction and education. Finally, they may facilitate reimbursement when the clinical value of a program can be documented. To our knowledge, quality measures specific to clinical genetics have not been published. However, standardization of the delivery of genomic medicine is critical. We are using two broad categories of quality measures: structure and outcome. Structural measures evaluate an institutions infrastructure to assess whether resources and logistics are sufficient to provide high-quality care. Examples of our structural measures are: (1) parameters relating to the consultations physical setting, including space accessibility and (2) the number of relevant personnel (eg, schedulers, board eligible/certified genetic counselors, physician geneticists) in total and per 100 patients seen. Outcome measures evaluate the quality and effectiveness of patient care. Examples of our outcome measures include: (1) the amount of time before consultation letters are sent to the physician and patient; (2) several standard measures of patient satisfaction; and (3) patient education as measured by evaluating pre- and post-counseling knowledge. We are accessing patient-related outcome data through questionnaires submitted either electronically or by mail after the appointment. Quality control is imperative in the practice of medicine, especially in evolving fields like clinical genetics. We believe outcomes measures such as these will enable clinical genetics programs to objectively evaluate their performance, provide a tool with which to longitudinally measure improvement of clinical services, and facilitate intra-and inter-institutional standardization.

Genetic Analysis of Benign Positional Paraoxysmal Vertigo. *L.R. Peddareddygari[1], M. Gizzi[1], D. Gordon[2], A. Dutra[1], M. Hirano[3], A. Abubakr[1], R.P. Grewal[1]* 1) New Jersey Neuroscience Institute, Edison, NJ; 2) Department of Genetics, Rutgers, The State University of New Jersey, Piscataway, NJ; 3) Columbia University College of Physians & Surgeons, New York, NY.

Vertigo is a common neurological symptom, occurring in up to 42% of the general population. One form of vertigo, benign positional paroxysmal vertigo (BPPV), is characterized by recurrent episodes of a violent whirling sensation lasting less than 30 seconds. These episodes are typically triggered by a change in body position. It is estimated that BPPV is present in up to 17% of patients reporting dizziness.

The risk factors for developing this disorder are not known and may include environmental and/or genetic factors operating alone, or gene/environmental interactions. Family histories of patients with BPPV indicate that multiple family members are often affected, suggesting a genetic contribution to the pathophysiology of this disorder.

We ascertained a large family three-generation Caucasian-American family in which BPPV segregates as an autosomal dominant trait with incomplete penetrance. Nine individuals are definitely affected. We performed a genome-wide scan with a set of 400 microsatellite markers spaced at 10cM (Weber lab screening set 13, Prevention Genetics Inc., Marshfield, WI). Linkage analysis revealed a maximum 2-point LOD score of 2.47 at theta=0. Recombinations identified through haplotype reconstruction indicates that the BPPV gene in this family maps to a critical chromosomal interval of 25 MB.

A Genomic Approach to Neurofibromin Function. *A. Pemov*[1], *L. Messiaen*[2], *D. Stewart*[1] 1) Dept GDRB, NHGRI/NIH, Bethesda, MD; 2) UAB, Birmingham, AL.

Background: Neurofibromatosis type 1 (NF1) is a monogenic disorder of dysregulated tissue growth. The causative gene, NF1, encodes a 220 kD protein called neurofibromin. Neurofibromin is a tumor suppressor but its other putative functions are not well understood. We hypothesized that a genomic approach characterizing gene expression differences between NF1-affected and NF1-unaffected individuals could offer insight into the function of neurofibromin. We reasoned that gene expression profiling in a single large NF1 pedigree could benefit from limited heterogeneity of NF1 mutations. Methods: 1) We obtained genome-wide expression profiles of 33 lymphoblastoid cell lines (LCLs) from an NF1 pedigree from the Coriell Cell Repository. Twelve LCLs were from NF1-affected individuals and 21 LCLs were from NF1-unaffected family members. Total RNA was isolated from the LCLs, reverse-transcribed to cDNA and labeled with Cy3. Universal human RNA (Stratagene) was labeled with Cy5. Both were then hybridized to in-house printed microarrays containing 34,580 oligos representing known and putative human genes. 2) We analyzed the coding region of the NF1 gene for mutations in the proband. Results: 1) We compared genome-wide expression profiles from NF1-affected to NF1-unaffected family members. Of all probes on the microarrays, three corresponded to three different exons of the NF1 gene. We found that two of the three NF1 probes had the two highest t-scores. The third one had a t-score in top 30 probes. Accordingly, quantitative RT-PCR data showed that the level of NF1 mRNA was consistently lower in NF1-affected individuals. Although there were no other statistically significant genes in the list, some of them are potentially biologically relevant in NF1. 2) We detected a 4 bp deletion in exon 28 in one of the NF1 alleles; the mutation co-segregated with affected individuals. Conclusions: 1) The modest changes in the level of NF1 gene expression in heterozygous NF1 LCLs were detected by microarray technology and validated by qRT-PCR; 2) genes whose expression is affected by NF1 haploinsufficiency likely require a larger sample to detect; 3) LCLs may prove to be convenient tool for studying cellular and molecular mechanisms in NF1.

Genome scan follow up studies provide further evidence for a primary open angle glaucoma loci on chromosome 14q11-q22 and 15q11-q15. *J.L. Wiggs[1], R.R. Allingham[2], M.A. Hauser[3], L. Olson[4], C. Santiago-Turla[2], E.A. DelBono[1], K. Abramson[3], F. Lennon[3], M.A. Pericak-Vance[3], J.L. Haines[4]* 1) Dept Ophthalmology, Harvard Medical Sch, MEEI, Boston, MA; 2) Dept Ophthalmology, Duke University School of Med, Durham, NC; 3) Center for Human Genetics, Duke University School of Med, Durham, NC; 4) Center for Human Genetics Research, Vanderbilt University School of Med, Nashville, TN.

Primary open angle glaucoma (POAG) is a genetically and phenotypically heterogeneous disorder that causes irreversible damage to the optic nerve and is a leading cause of blindness worldwide. Three genes have been identified as factors that contribute to POAG and 7 additional loci have been localized using mendelian approaches. Using a collection of affected sibling pairs, we have previously completed a genome scan that provided evidence for POAG loci on chromosomes 2, 4, 14, 15, 17 and 19. Using additional sibling pairs (total of 195) and additional markers in the regions of interest, we have performed follow-up studies to further define these loci. Model dependent and model free linkage analysis gave highest values for markers D14S264 (LOD = 3.71) and D15S165 (LOD = 2.43). Haplotype sharing using additional markers in the areas of peak linkage identified a 50 cM interval on chromosome 14q11-q22 extending from marker D14S72 to D14S274 shared by affected individuals in 53% of the pedigrees. An 18 cM region extending from D15S122 to D15S219 was identified on chromosome 15q11-15, and coincides with the chromosome 15 region previously identified using age of onset ordered subset analysis. 38% of the pedigrees were found to share both the chromosome 14 and chromosome 15 affected haplotypes which suggests that the genes located in these regions may have an additive effect, and that together these two loci represent a significant portion of the genetic contribution to POAG in this population.

Paucity of mutations in *GJB2* and *GJB6* in African American and Caribbean Hispanic populations. *J.M. Samanich[1,2], R. Burk[3], C. Lowes[2], S. Shanske[4], A. Shanske[1], B.E. Morrow[2]* 1) Center for Craniofacial Disorders, Childrens Hospital at Montefiore, Bronx, NY; 2) Department of Molecular Genetics, Albert Einstein College of Medicine, Bronx, NY; 3) Department of Pediatrics, Albert Einstein College of Medicine, Bronx, NY; 4) Laboratory of Molecular Neurogenetics, Columbia University, New York, NY.

Approximately 1/1,000 children are born with hearing impairment, half due to genetic and half to environmental causes. Autosomal recessive non-syndromic sensorineural hearing impairment (ARNSHI) comprises 80% of familial cases. Approximately half of familial cases result from coding mutations in the Connexin 26 gene, *GJB2*, in Caucasian populations. Heterozygous mutations in the single coding exon of *GJB2* occasionally co-occur with a deletion in the Connexin 30 gene, *GJB6*. Few studies have focused on the mutation frequency of these in African American (AA) and Caribbean Hispanic (CH) admixture populations. In this study, we performed bidirectional sequencing of the *GJB2* gene and PCR screening for the common *GJB6* deletion in 109 predominantly simplex individuals of mostly minority ethnic backgrounds. The hearing impairment ranged from unilateral mild to bilateral profound. None of the AA patients and only one CH patient had a bi-allelic mutation in *GJB2*. Variations found were T101C (M34T; 1/109), G109A (V37I; 1/109), 35delG (mutation; 6/109, 2/6 CH), 167delT (mutation; 1/109), G139T (mutation; E47V; 1/109 homozygous, CH), C-15T (1/109, AA), G79A (V27I; 10/109, 3/10 CH, 1/10 AA, 1/10 CH/AA), G380A (R127H; 4/109), A670C (Indeterminate; K224Q; 1/109 CH), A503G (novel; K168R; 3/109, 2/3 CH) and C684A (novel; 1/109 CH). None had a *GJB6* deletion. Bi-directional sequencing of *GJB2* was performed in 187 AA and Hispanic healthy individuals. Our results reveal that *GJB2* mutations and *GJB6* deletions may not be a significant cause of HI in these minority admixture populations.

Resequencing of the 3UTR of SLITRKs and potential targets of miRNAs in patients with obsessive-compulsive disorder. *E. Saus[1], M. Gratacos[1,2], M.P. Alonso[3], J.R. Gonzalez[1,2], J.M. Menchon[3], C. Segalas[3], M. Bayes[1,2], J. Labad[3], J. Vallejo[3], X. Estivill[1,2,4]* 1) Genes and Disease Program, Center for Genomic Regulation, Barcelona, Barcelona, Spain; 2) Center for Genomic Regulation, Barcelona, Barcelona, Spain; 3) OCD Clinic, Department of Psychiatry, Hospital Universitari de Bellvitge, LHospitalet de Llobregat, Barcelona, Spain; 4) Department of Health and Life Sciences, Pompeu Fabra University, Barcelona, Catalonia, Spain.

Obsessive-compulsive disorder (OCD) is characterized by recurrent unwanted thoughts and/or repetitive behaviors, with prevalence estimate of 2.5% in the Spanish population. Recently, Abelson et al. (2005) studied SLITRK1 as a candidate gene for Gilles de la Tourette Syndrome (GTS) on chromosome 13q31.1, and identified two mutations in SLITRK1 in three out of 174 GTS probands. One of these mutations was a base change in the 3UTR of the gene, and corresponds to a highly conserved nucleotide within the predicted binding site for a miRNA, hsa-miR-189. Due to the overlap between GTS and OCD, it has been postulated that, in some cases, OCD and GTS may be alternative manifestations of the same underlying illness. We hypothesized that OCD patients may carry sequence variants in the 3UTR of the family of SLITRK genes, especially in the regions predicted to be targets for miRNAs. We have performed re-sequencing of the six genes of the SLITRK family (Slit and Trk-like family, members 1-6) in 86 OCD patients. We have identified four previously described SNPs and eight sequence variants not found in public available databases. We performed a case-control analysis between known and new SNPs in 95 control samples to test if the presence of these variants confers susceptibility to the development of OCD. Finally, conservation and miRNA target prediction programs have been used to define the potential disruption of the complementarities between known miRNAs expressed in the central nervous system and nuclotide changes affecting the 3UTR of SLITRK genes.

Unusual presentation of partial hypoxanthine phosphoribosyltransferase deficiency in a female patient. *I. Sebesta[1,2], O. Martincova[1], B. Stiburkova[1], L. Dvorakova[1], M. Hrebicek[1], J. Minks[1], Z. Vernerova[3], I. Rychlik[4]* 1) Institute of Inherited Metabolic Disorders; 2) Institute of Clinical Biochem. and Lab. Diagnostics,1st Faculty of Medicine, Charles University; 3) Depatment of Pathology; 4) 1st Department of Medicine,3rd Faculty of Medicine,Prague,Czech Republic.

Partial deficiency of hypoxanthine-guanine phosphoribosyltransferase (HPRT), described as Kelley-Seegmiller syndrome is an X-linked inborn error of purine metabolism. Marked uric acid overproduction resulting in hyperuricemia, nephrolithiasis and gout are common features. Female carriers have somatic cell mosaicism of HPRT activity, and are healthy with enzyme activity in erythrocytes within normal limits. Only one female with partial HPRT deficiency was described recently. We report 50-years old women, the sister of the proband with Kelly - Seegmiller syndrome, who did not experience neither gout, nephrolithiasis nor hyperuricemia. She was never on allopurinol. Uric acid was quantified by specific enzymic method and red cell enzyme with plasma purine metabolites were measured by HPLC methods. Results: Purine biochemical investigations few months ago revealed in repeat serum uric acid concentrations within normal limits (31412 mol/); increased plasma levels of hypoxanthine:19.8 mol/l (control values 2.51.0 mol/l) and xanthine: 7.5 mol/l (control values 2.40.7 mol/l); HPRT activity in erythrocyte lysate was surprisingly very low: 8.6 nmol/h/mg Hb (control values :113 11 nmol/h/mg Hb). Mutation analysis using direct sequencing revealed heterozygous form of previously described mutation in the 3rd exon of HPRT gene, c.215AG (Y72C). Our preliminary results showed skewed X-inactivation ratio in favour of mutant allele (25:75), which could explain enzyme defect. Although enzyme deficiency with urate overproduction (presenting as high plasma oxypurines) is evident, the reason for normal serum urate concentrations remains uncertain. Such results have not been reported in a female with HPRT deficiency. In conclusion our finding shows the need of detailed purine metabolic investigation in asymptomatic female members of family with partial HPRT deficiency. (Supported by grants - MSM 0021620806, VZ 64165 CZ).

A family longevity selection score. *P. Sebastiani[1], E. Hadley[2], M. Province[3], A. Yashin[4], K. Christensen[4], J. Vaupel[4], C. Kammerer[5], W. Rossi[2], T. Perls[6], A. Ash[1]* 1) Biostatistics, Boston Univ Sch Pub Health, Boston, MA; 2) Geriatrics, Boston Univ Med Cntr, Boston, MA; 3) Biostatistics, Washington Univ, St Louis, MO; 4) Public Policies Stud, Duke Univ, Durham, NC and Univ S. Denmark; 5) Epidemiology, Univ Pittsburgh, Pittsburgh, PA; 6) Geriatrics and Clin Gerontology, Natl Inst Aging, Bethesda, MD.

The Long Life Family Study requires a score to rank sibships as to their desirability for recruitment due to being unusually long-lived and having good numbers of old living members. We propose a family longevity selection score denoted by S, as a measure with several desirable properties: all family members, both living and dead, contribute; a sib's contribution to S increases sharply with very old age; a deceased sib adds less than a living sib of the same age, sex and birth cohort; sibs dying young decrease S. S has two parts: total sibship exceptionality(TSE) which depends on all members of the sibship, and living sibship exceptionality (LSE), which depends only on living members. Let A denote the age and C the sex and birth-year cohort of a sib; then LSE is defined as the sum over all living sibs of: max(-ln(Probability of survival past age A for a sib in birth cohort C) - Threshold, 0) Threshold is a tuning parameter that adjusts the tradeoff between the desire for more subjects and for older subjects in defining LSE by giving positive credit only for sibs with ages exceeding a percentile of the relevant survival distribution. If, say, Threshold = -ln(0.25), then s is only positive when a sib's age exceeds the 75th percentile of survival for a person of the same sex and decade of birth. TSE is calculated similarly, but by summing over all sibs and using the age at death for A for deceased sibs while replacing actual ages of living sibs by their expected ages A* at death, given their birth and sex cohorts. Finally, S is defined as a weighted average of LSE and TSE, the relative weight being a second tuning parameter. We used Danish family data to choose tuning-parameter values so that families with above-median values of S are slightly larger than average, but with high mean ages for both total and living sibships.

Robotic Microscopy of Amniotic Fluid Cells for the Fully-Automated Quantitation of FISH Signals. *T. Tafas*[1], *M.W. Kilpatrick*[1], *J. Tepperberg*[2], *S.M. Sharp*[1], *P. Tsipouras*[1] 1) Ikonisys Inc, New Haven, CT; 2) LabCorp of America, Research Triangle Park, NC.

FISH analysis is a valuable adjunct to cytogenetics that provides a rapid screen for common abnormalities. However, FISH is expensive, labor intensive, and requires a high skill level and subjective signal interpretation. A fully automated system for FISH analysis could improve laboratory efficiency and potentially reduce errors and costs. Ikonisys fastFISH is a fully robotic fluorescence microscopy platform requiring only that slide-containing cassettes are loaded into the instrument. Each slide is scanned and returned to its cassette with no requirement for user input. Images of cells are digitally captured and automatically analyzed for the presence of specific FISH signals, that are enumerated and reported, along with the Test Outcome from analysis of 50 selected nuclei. A gallery displaying the image of each cell and its FISH signals is provided, so that the operator can confirm the diagnostic relevance of the data reported. Slides were prepared and probed for chromosomes 13, 18, 21, X, and Y. The accuracy of the automated FISH dot counting was first evaluated by blindly comparing images of a total of 1129 chromosomes obtained using the fastFISH Auto System with the automated dot count of the same images. The agreement between the manual and automated FISH dot count ranged from an average of 76.9% for chromosome 18 to one of 95.3% for the Y chromosome. Subsequently, 161 amniocentesis samples were split and two slide sets were prepared per sample. One set was evaluated using standard manual microscopy and the other using the fastFISH Auto application. An automated diagnostic outcome was produced for 155 of the 161 amniocentesis samples, including a trisomy 21 and an X0. 100% concordance was observed between the results obtained using manual microscopy and the Test Outcome generated from automated FISH dot-count analysis. This data suggests that the automated system is capable of providing accurate detection and quantitation of FISH signals and has potential where the reliability and speed offered by an automated system would be of benefit.

Fetal stability of the 8893T>G mtDNA mutation load has implications for the feasibility of prenatal diagnosis in NARP syndrome. *J. Steffann[1], N. Gigarel[1], J. Corcos[1], M. Bonnière[1], F. Encha-Razavi[1], Y. Dumez[2], A. Yamgnane[2], R. Frydman[3], S. Prevot[3], J.P. Bonnefont[1], A. Munnich[1]* 1) Department of Genetics, Inserm U781, Hopital Necker, Paris, France; 2) Obstetrics, Hopital Necker, Paris, France; 3) Obstetrics and foetopathology, Hopital Béclère, Clamart, France.

Mitochondrial DNA (mtDNA) mutations cause a wide range of serious genetic diseases with maternal inheritance. Due to a high transmission risk and the absence of efficient therapy in these disorders, at risk couples often ask for prenatal diagnosis (PND). However, because loads of heteroplasmy (coexistence of mutant and wild type mtDNA) may vary among tissues and with time, the possibility that a single fetal sample may not reflect the whole organism impedes prenatal diagnosis of mtDNA disease. We carried out 13 prenatal diagnoses for the NARP (Neurogenic weakness, Ataxia, Retinitis Pigmentosa) m.8993T>G mtDNA mutation. Analyses were carried out from chorionic villous (CVS) and/or amniocytes (AS) samples, using a method enabling quantification of low DNA amounts. Maternal mutant loads ranged from 0 to 65% in blood and had no predictive value for the fetus status except for women with no detectable mutant DNA whose fetuses were constantly mutation free. In 8/13 PND, mutant load was below 30%. These children are healthy at 1 to 6 years of age. In 5/13 PND, mutant load ranged from 65 to 95% and parents elected to terminate the pregnancies (15-17 weeks of gestation). Single-cell analysis of 7 trophoblastic cells and 21 amniocytes from one fetus found a mutant load of 725%, and 767%, very close to the overall CVS and AS mutant loads (70% and 70%, respectively). m.8993T>G mutant load, assessed in 5, 7, and 11 different tissues from 3 termination products, was identical in all tissues from a given fetus (mean 631%, 752.8%, and 910.4%for the 3 fetuses, respectively). Our results suggest that the placental/amniotic mutant loads do reflect the NARP mutant mtDNA load in the whole fetus even when the sample amount is small. While these data establish the feasibility of PND for this mutation, assessing more precisely the correlation between mutant load and disease severity should further help interpreting PND results.

Uncommon Rearrangements and De Novo Mutations Associated With Leber Congenital Amaurosis (LCA). *I. Perrault[1], S. Hanein[1], N. Delphin[1], S. Gerber[1], J.L. Dufier[2], J. Kaplan[1], J.M. Rozet[1]* 1) INSERM U781, Hopital Necker-Enfants Malades, Paris, France; 2) Service d'Ophtalmologie, Hopital Necker-Enfants Malades, Paris, France.

The purpose of this study was to understand the uncommon segregation of RPE65 and RDH12 mutations in four unrelated LCA patients. Two unrelated patients whom clinical history strongly suggested the involvement of the RPE65 gene and two unrelated patients suggesting the involvement of the RDH12 gene, respectively, were screened for mutation using DHPLC and direct sequencing. Subsequently, haplotype analyses at the RPE65 or RDH12 loci on chromosome 1p31 and 14q23, respectively, were carried out using polymorphic markers flanking both genes. The paternity was checked for all four patients using markers of chromosomes 1 and 14 as well as 8 highly polymorphic markers localized on 4 distinct chromosomes. This study alowed to identify i) a partial maternal isodisomy of chromosome 1p31 carrying a RPE65 splice-site mutation (c.11+5G>A) responsible for the disease in the affected child of this woman, ii) a paternal de novo RPE65 mutation in an affected child carrying a maternal mutation in this gene, iii) two RDH12 deletions resulting in apparent homozygosity in two patients born to non-consanguineous parents; one of them was inherited from the father while the second consisted in a large intragenic paternal deletion. In conclusion, we report four families with unconventional mutations in two of the nine hitherto identified LCA genes. It is worth noting that a paternal isodisomy of the whole chromosome 1 responsible for LCA has been already reported. This study emphasizes the existence of not uncommon pitfals in the transmission of disease alleles in autosomal recessive LCA.

Characterization of a 1q21:6q25 Translocation Associated With Congenital Glaucoma. *K.V. Ramchand[1], M.D. Tocyap[1], J.L. Haines[2], J.L. Wiggs[1]* 1) Dept Ophthalmology, Harvard Medical School/MEEI, Boston, MA; 2) Center for Human Genetics Research, Vanderbilt University School of Medicine, Nashville, TN.

Congenital glaucoma can be inherited as an autosomal recessive or autosomal dominant trait. One gene for the autosomal recessive form of the disease, CYP1B1, has been identified. The purpose of this study is to identify genes that are disrupted by a 1q21:6q25 translocation in a patient with congenital glaucoma and evaluate those genes as candidates for autosomal dominant forms of the disease. To facilitate the identification and sequencing across the translocation breakpoints, somatic cell hybrids were constructed from a lymphoblastoid cell line derived from a patient with congenital glaucoma and a cytogenetically detectable translocation involving chromosomes 1q21 and 6q25. Using a PCR approach, the breakpoints were initially found between microsatellite repeat markers D1S2612 and D1S2347, and D6S1655 and D6S1581. Using the human genome sequence for design of PCR assays, the locations of the breakpoints were further refined to intron 1 of the KIAA0460 gene located at 1q21 and in intron 5 of the VILLIN2 gene located on chromosome 6q25. The breakpoint sequence of both derivative chromosomes was obtained by direct genomic sequencing after PCR amplification across the breakpoints. Secondary chromosomal abnormalities were not identifed. These data suggest that the form of congenital glaucoma affecting this patient may be the result of disruption of genes located at the translocation breakpoints. Mutation screening of these genes in a population of early onset glaucoma patients is currently underway.

Hyaluronan synthetase 2 is a target gene of human SIM2 transcription factor. *Y. Shimizu[1], A. Yamaki[1], S. Asai[1], A. Ueno[1], J. Kudoh[2], N. Shimizu[2]* 1) Department of Medical Genetics, Kyorin University School of Health Sciences, Tokyo Hachioji, Tokyo, Japan; 2) Department of Molecular Biology, Keio University School of Medicine, Tokyo, Japan.

The study using Sim2 knockout mouse has indicated that Sim2 contributes to neuroendocrine hormone gene expression in the anterior hypothalamus and craniofacial development. Human SIM2 gene locates on chromosome 21q22.2, Down syndrome chromosomal region. It belongs to the family of bHLH (basic helix-loop-helix)/PAS (Per-Arnt-Sim) transcription factor and interacts with ARNT or ARNT2 to repress the expression of unknown target genes. To find the target genes of SIM2, we established stable cell lines which overexpress SIM2 or SIM2/ARNT2. Then, we performed a differential display analysis using RNA from these cell lines (HeLa, HeLa-mock, HeLa-SIM2 and HeLa-SIM2/ARNT2). We identified 26 potential target genes of SIM2 and SIM2/ARNT2 and confirmed the expression by real-time PCR analysis. Transcription of Hyaluronan synthase 2 (HAS2) was inhibited drastically by overexpression of SIM2 and SIM2/ARNT2. Furthermore, we performed the promoter analysis of HAS2 using dual Luciferase assay and 11 deletion constructs which contain various parts of promoter regions, nt-4401~nt-480. We found that the inhibitory activity locates in the regions, nt-3114 ~ nt-1974 and nt-1767~nt-1751. In these regions, three binding sites for SIM2/ARNT2 heterodimer were estimated. To examine the cis-elements in HAS2 promoter, we made constructs that have two base substitutions in the consensus sequence. The mutations of nt-2421G>A, nt-2420T>A and nt-2309A>T, nt-2308C>T, made to increase the promoter activity. These data suggest that two cis-elements exist in 5 franking region of HAS2 gene and that HAS2 gene is a target of SIM2 transcription factor.

Integrated Analysis of Genetic and Proteomic Data Improves the Prediction of Adverse Drug Reactions. *D.M. Reif[1,2], A.A. Motsinger[1], B.A. McKinney[1,2], J.E. Crowe Jr.[1], J.H. Moore[2]* 1) Vanderbilt University, Nashville, TN; 2) Dartmouth Medical School, Lebanon, NH.

Complex clinical phenotypes such as adverse drug reactions (ADR) arise from the coordinate interaction among a myriad of genetic, genomic, proteomic, metabolic, and environmental factors. We propose as a working hypothesis that our ability to predict ADRs and other pharmacodynamic endpoints will be improved by jointly considering candidate biomarkers from multiple different levels of the biological hierarchy that maps the genome to a given phenotype. The goal of this study was to develop and evaluate an analytical strategy using random forests (RF) for modeling multiple different types (e.g. discrete and categorical) and sources (e.g. genetic and proteomic) of data. The RF approach generates thousands of decision trees on bootstrapped samples of the data for the purpose of selecting important variables. Selected variables can then be modeled using a decision tree or any other classification method on the entire dataset. In this study, we characterized the performance of RF on a range of simulated genetic and/or proteomic datasets. The simulated datasets are based upon real genetic data (genotypes at 1442 SNPs across 500 genes) and real proteomic data (circulating levels of 108 cytokines and chemokines) collected to identify biomarkers associated with ADR following smallpox vaccination of n=108 human subjects. This simulation strategy allowed us to evaluate RF with a known genetic/proteomic model while at the same time preserving the complex correlation structure of the real data. We compared the performance of RF for identifying relevant biomarkers when given genetic data alone, proteomic data alone, or a combined dataset of genetic plus proteomic data. Across all simulated datasets, the integrated RF analysis of both the genetic and the proteomic data yielded the most power for identifying functional biomarkers. Our results support the hypothesis that the integrated analysis of genetic, genomic, proteomic, metabolic, and environmental data will improve our ability to predict ADR. (Supported by NIH R01 AI59694, PI-Moore).

Molecular analysis of *SPG7* in patients with Hereditary Spastic Paraplegia. *H.G. Yntema, M. Ruiterkamp-Versteeg, J. Bokhorst, G. Schobers, H. Scheffer, W.M. Nillesen, E.A. Sistermans* Dept of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.

Hereditary Spastic Paraplegia (HSP) comprises a genetically heterogeneous group of inherited diseases, characterized by weakness and spasticity of the lower limbs. HSP types have been divided in pure HSP and complicated HSP, which is accompanied by additional neurologic symptoms. Genetic studies have revealed more than 30 different chromosomal HSP loci. Six loci for autosomal recessive complicated HSP have been identified, and four of these genes have been cloned: *SPG7*, *SPG20*, *SPG21*, and *SACS*. SPG7 (MIM 607259) is caused by mutations in the *SPG7* gene, which encodes paraplegin. *SPG7* belongs to the same gene family as *SPAST* (encoding spastin), which is mutated in SPG4, the most common form of autosomal dominant HSP. Both paraplegin and spastin are highly homologous to yeast mitochondrial ATPases and in muscle tissue of some patients morphological and functional abnormalities typical of mitochondrial OXPHOS defects have been described. As in other mitochondrial diseases, the clinical spectrum of paraplegin defects is variable. In this study, 170 patients with pure or complicated recessive HSP have been tested for mutations in the *SPG7* gene by a combination of DGGE, direct sequencing and MLPA. In 12 of these patients two mutations in *SPG7* have been detected. In total 14 different mutations were identified, 12 of which have not been reported in literature before. These novel mutations contained mostly missense mutations (9), but also splice site mutations (2), and one frameshift mutation were detected. One of the reported mutations, c.1454_1462del, was detected in 7 alleles, making it the most common mutation in our population. Furthermore, based on our results, c.1529C>T (p.Ala510Val), which was detected 4 times, is indeed a pathogenic mutation, as has recently been suggested by Elleuch et al (Neurology 2006:654). In conclusion, in this population, SPG7 mutations account for 7% of HSP families compatible with recessive inheritance. This percentage is slightly higher than the 5% reported by Elleuch et al (2006).

Prenatal diagnosis of a rare European Cystic Fibrosis (CF) mutation in a fetus of Jewish descent. *R. Shtoyerman, A. Kaftori, Z. Appelman* The Genetic Institute, Kaplan Medical Center, Rehovot and the Hebrew University, Jerusalem, Israel.

A 25 weeks pregnant woman (P1G0) of a Jewish-Turkish ethnic background, was referred to our clinic due to echogenic bowel in a level II ultrasound performed in the community. Repeated ultrasound in our facility confirmed intestinal obstruction due to meconium ileus. The father, of a combined Jewish Ashkenazi and Jewish Bulgarian background, was a known CF mutation carrier, as detected in routine carrier testing performed elsewhere. DNA fetal testing following amniocentesis confirmed the fetus carried the paternal W1282X mutation. Despite negative carrier status for the mother, as based on the routine ten mutation panel offered to non-Ashkenazi Jews (Orgad et al., 2001; Gazit et al., 2001), the couple opted to terminate the pregnancy with CF being the most probable diagnosis. Pathological examination following termination confirmed meconium ileus. In order to provide genetic counseling for future pregnancies, the mothers DNA was further analyzed at Genzyme Genetics, for an additional panel of 87 mutations (http://www.genzymegenetics.com/pdf/cf_physician_brochure.pdf). This revealed she carries the S1251N mutation. The same mutation was also detected in the fetal sample. S1251N was previously described only in European patients (Kalin et al., 1992; Mercier et al., 1993), thus this is the first report of this mutation in a Jewish subject. Carrier testing for CF is offered in Israel for Jews of most ethnic backgrounds, with an estimated detection rate of 85% for Jews originating from the Balkan countries. We suggest that when meconium ileus is diagnosed prenatally, and the fetus carries a known CF mutation, it is highly suggestive of CF, even when one of the parents is considered a non-carrier. The assumption in these cases should consider the fact that CFTR is a large gene with over a thousand mutations described to date (http://www.genet.sickkids.on.ca/cftr), and the other parent may be a carrier of an unknown/undetected mutation. Future analysis will determine if S1251N is a common mutation in Jews of this ethnic background, or a case of a private mutation.

Genomic Study of Human Complex Disorders Using Twins: Design Options and Sample Size. *F. Zhang[1], N. Whitehead[1], P. Levy[1], L. Corey[2], V. Vannappagari[3], P. Chulada[4], P. Blackshear[4]* 1) RTI International,Resarch Triangle Park,NC; 2) Virginia Commonwealth University, Richmond,VA; 3) GlaxoSmithKline,Research Triangle Park,NC; 4) National Institute of Environmental Health Sciences, Research Triangle Park,NC.

Twin studies have been a powerful tool for investigating the relative importance of genetic susceptibility and environment in the development of complex disorders or traits. As molecular technology advances, molecular geneticists are increasingly interested in determining specific genes that are responsible for the development of a certain disorder. Twin studies have recently received increased attention as a tool in the genomic study of human disorders and have increased focus upon the availability of established twin registries worldwide. In this study we evaluated several study designs and carried out the explicit calculation of sample size required:(1)Dizygotic(DZ)twin pairs are siblings of the same age. Statistical power will be increased in a linkage study where the trait varies by age. Although monozygotic(MZ) twin pairs, who are genetically identical, do not contribute to the detection of linkage in conjunction with DZ pairs they can improve power in linkage analysis.(2) DZ discordant twin pairs can serve as an ideal case-control study, which is believed to be more powerful in detecting susceptibility genes than is a population-based case-control study.(3)MZ discordant twin pairs are particularly powerful for detecting epigenetic effect in that the relative importance of genetic, environment, and epigenetic effects can be estimated.(4)Population-based twin cohort can be used to investigate the association of human disorders or traits with genetic variants. This design can reduce selection bias that is often concerned in case-control design. We have evaluated the sample size and power for an association study using discordant twin pairs. In population-based twin cohort design, twin pairs can be considered as a repeated measurement. Sample size can be calculated using longitudinal design while taking into account the correlation between twin pairs. Sample size increases remarkably with the intra-pair correlation.

Mutational model leading to Polyalanine expansions and contractions revisited. *D. Trochet, L. De-Pontual, B. Keren, A. Munnich, S. Lyonnet, J. Amiel* Dept Genetics, Hosp Necker-Enfants Malades, Paris, France.

Alanine stretches (AS) are common in all proteomes studied with the longer stretches being found in mammals. AS are coded by degenerated codons (GCN) and characterized by rapidly evolving nucleotidic sequences with high rates of expansions and contractions: the longer and more pure the sequence, the higher the length polymorphism of the AS in the population. These genic sequences are frequent in transcription factors and have recently been proposed as facilitators of evolution. Hitherto, polyalanine expansions and contractions have been ascribed to 9 human diseases, either autosomal dominant or X-linked. Considering both that : i) AS are coded by mixed (GCN)n codons and, ii) that transmission over generations is stable, a polymerase slippage mechanism is unlikely. A mechanism of unequal allelic homologous recombination has thus been proposed. Worth noting, expansions have been identified in asymptomatic parents for 3/9 disease causing genes namely HOXD13, ZIC2 and PHOX2B for which mutations account for synpolydactyly, holoprosencephaly and congenital central hypoventilation syndromes respectively. In the latest case, 5% of the asymptomatic parents from our series harbor an alanine expansion in their leucocytes. We tested carrier parents either by cloning the PCR fragment when a heterozygous SNP was present or by QMPSF. We show that, as speculated, carrier parents are somatic mosaics for the mutation. However, instead of the 3 alleles (wild-type, expansion and contraction) expected according to the mutational model, only 2 are observed in mosaic parents (wild-type and expansion). Therefore, an alternative mutational model to generate alanine expansions and contractions will be proposed.

Potential role of UBE3A and ATP10A, positional and functional candidate genes, in the aetiopathogenesis of the autistic disorder. *S. Russo[1], MT. Bonati[1], A. Gessi[1], G. Guffanti Masetti[2], M. Marchi[1], F. Cavalleri[1], F. Cogliati[1], M. Elia[3], M. Estienne[4], L. Larizza[1,5], F. Macciardi[2]* 1) Ist.Auxologico Italiano,Milano, Italy; 2) Polo LITA,University of Milan,Italy; 3) LOasi-Troina (EN),Italy; 4) Ist.C.Besta,Milano,Italy; 5) Hosp.S.Paolo,University of Milan,Italy.

Autism is a common neurodevelopmental disorder characterized by severe impairments in reciprocal social interactions and communication and restricted/stereotypical patterns of interests and behaviour. The complex genetic aetiology of autism has not been elucidated; however, the most common chromosomal abnormality seen in 1% of children with autism is gain of chromosome 15q11-q13 caused by interstitial duplication of this region or supernumerary marker chromosomes deriving from proximal 15q. Moreover genetic and epigenetic alterations of 15q11-q13 region, causing the lack of expression of the maternally inherited copy of the imprinted UBE3A gene, are responsible for Angelman syndrome, which features, such as severe mental retardation, absence of speech, abnormal EEG/epilepsy, inappropriate social behavior, overlap with those often seen in AD (Clayton-Smith and Laan, 2003). In 2001 a study of Nurmi et al. reported a significant evidence for marker D15S122, located at 5 end of UBE3A and autism, which was never replicated. Also SNPs in the nearby imprinted gene, ATP10A, were investigated to disclose association with autism without coming to relevant evidence.We here refer on the analysis of D15S122, and ATP10A,SNP3(intron12)-D15S1534 (intron 8)markers and D15S135 (intron 18) in a sample including 54 trios from idiopatic autism and 13 among non deleted Angelman patients,RTT and fragile X syndrome with autistic traits selected for displaying autistic traits.TDT test was used for statistical evaluation.Our results are consistent with a potential role of UBE3A and ATP10A in showing a significant association for D15S122 and for the haplotype including D15S122. Other tagged 18 SNPs in the region are under analysis to streghten these preliminary results.

Results of a whole genome association study for Major Depressive Disorder (MDD). *H. Sakul*[1], *D.A. Hinds*[2], *K.A. Frazer*[2], *D.R. Cox*[2], *C. Hyde*[1] 1) Pfizer Global R&D, CT; 2) Perlegen Sciences, CA.

Many genetic and environmental factors affect response to treatment with an SSRI or placebo in MDD clinical trials. While many candidate genes have been studied, results are often discordant and difficult to interpret due to small sample size, multiple testing, etc. The primary objective of this study was to identify genes affecting SSRI or placebo response, as well as time-to-response, to improve the design of MDD trials. DNA from 1,024 Caucasian subjects from 8 MDD trials were genotyped using 250,000 SNPs and analyzed using various statistical models. Primary endpoints were Total HAMD, and three subscales: Core Depression, Anxiety and Insomnia. Statistical significance was based on overall false discovery rate (q-value). The following numbers of SNPs were significant across binary, quantitative or time to response variables and annotated as CNS-relevant or novel based on the literature: For Placebo, 16 CNS and 466 Novel; for SSRI, 33 CNS and 440 Novel. False discovery rates were also computed for 11 CNS candidate genes and SNP rs10891539, located in the TTC12 gene (11q23.1) within 50 kb of the D2 dopamine receptor (DRD2), was the most significant marker. This SNP is a part of a 3 SNP haplo-block spanning both genes (D>0.9). Most literature reports for DRD2 highlight associations with the bipolar disorder while our data is from unipolar depression. As such, our finding may have a significant impact on MDD trials. Further, the effects of each allele of this SNP suggested that with all studies combined, screening out subjects homozygous for the reference allele results in SSRI treatment significance that is double the observed value without screening. For 2 of the 5 trials where SSRI treatment response was not significant, such screening would result in significant SSRI response. In conclusion, we have identified several genes with significant effects on either SSRI or placebo response in MDD trials. Our analyses also demonstrate that even the large sample size of this study has limited statistical power for replication, and we anticipate that our results will facilitate external replication studies and possible collaborations to join datasets.

Haplotype Trend Regression (HTR) Analysis of *OPRD1*, *OPRK1* and *GPR7* in Alcohol or Drug Dependence. *H. Zhang[1,2], H.R. Kranzler[3], BZ. Yang[1,2], J. Gelernter[1,2]* 1) Dept Psychiatry, Yale Univ Sch Med, New Haven, CT; 2) VA CT Healthcare System, West Haven, CT; 3) Dept Psychiatry, Univ CT Sch Med, Farmington, CT.

We investigated whether variation in the genes encoding the human and -opioid receptors and G-protein coupled receptor 7 (*OPRD1*, *OPRK1* and *GPR7*) could influence risk for substance dependence (SD). Eight single nucleotide polymorphisms (SNPs) spanning *OPRD1* were examined in a sample of 621 European Americans (EAs) affected with SD [557 with alcohol dependence (AD), 225 with cocaine dependence (CD), and 111 with opioid dependence (OD)] and 443 EA healthy controls. Statistically significant associations of SNP1 (F27C in exon 1) with OD only (both allelic and genotypic), SNP4 (in intron 1) with AD (genotypic), CD (genotypic) and OD (allelic), and SNP5 (in intron 1) with AD (genotypic) and CD (genotypic) were observed. The association of SNP1 with OD remained significant after correction for multiple testing. Although haplotype frequency distributions did not differ significantly between cases and controls, haplotype trend regression (HTR) analyses indicated that a specific haplotype GCAATACT, which harbors susceptibility alleles of SNP1 (G-allele), SNP4 (A-allele) and SNP5 (T-allele), may confer vulnerability to SD. The frequency of haplotype GCAATACT was 3.7% (control subjects), 6.4% (AD subjects), 8.1% (CD subjects) and 12.4% (OD subjects). When confounding factors, sex and age, were considered, the haplotype GCAATACT still showed a risk effect for OD (=3.20, *P*=0.011), but not for AD and CD. Population structure analyses with genotype data from 38 ancestry informative markers excluded population stratification artifact. Additionally, seven SNPs covering *OPRK1* and two SNPs covering the intronless *GPR7* were examined in most of the above samples. No significant difference in allele, genotype or haplotype frequency distributions was found between cases and controls. Nevertheless, HTR analyses revealed a possible risk effect of a specific *OPRK1* haplotype GGCTTCT on AD (=0.69, *P*=0.007). In summary, our findings suggested a positive association between *OPRD1* variants (especially the functional F27C) with OD, and a possible haplotypic association between *OPRK1* and AD in EAs.

A genome-wide scan of non-synonymous SNPs in a phase III clinical trial identifies variants influencing outcome in chronic lymphocytic leukemia. *G.S. Sellick[1], R. Wade[2], M.F. Rudd[1], S. Richards[2], D. Catovsky[3], R.S. Houlston[1]* 1) Section of Cancer Genetics, Institute of Cancer Research, Sutton, Surrey, UK; 2) Clinical Trial Service Unit, University of Oxford, Oxford, UK; 3) Section of Haemato-Oncology, Institute of Cancer Research, Sutton, Surrey, UK.

B-cell chronic lymphocytic leukemia (B-CLL) is a heterogeneous disease with a variable clinical course. Staging systems are useful for predicting survival and treatment requirements, however, even for patients with same-stage disease there is variability in clinical outcome. Germline sequence variation may influence disease prognosis. We undertook a genome-wide scan of non-synonymous SNPs (nsSNPs) in 425 CLL patients participating in a phase III trial (UK LRF CLL4) established to compare the efficacy of fludarabine, chlorambucil, and fludarabine and cyclophosphamide as a first-line treatment for patients with Binet stage B, C and A-progressive disease. The 990 nsSNPs in 870 genes were selected for their relevance to cancer biology and were strongly biased towards those likely to be functionally deleterious. Genetic data was linked to individual patient outcome and response to chemotherapy. The effect of genotype on progression free survival (PFS) and overall survival (OS) was assessed and the relationship between nsSNPs and PFS and OS was evaluated. Our study shows that germline variation influences CLL survival and identifies a number of variants that provide insight into the biological determinants of prognosis.

Maturation process of SUMO1 and SUMO4 in different cell lines. *W.Z. WEI, Y. WANG, C.Y. WANG* CBGM, Medical College of Georgia, Augusta, GA.

Sumoylation carried out by the small ubiquitin-related modifiers (SUMO1-4) is a newly identified mechanism contributing to the dynamic regulation of protein functions, implicated in DNA repair and synthesis, RNA processing, protein degradation, and glucose metabolism. SUMO proteins are synthesized as precursors that need the maturation process to expose the C-terminal glycine-glycine residues. This di-glycine motif is prerequisite for covalent conjugation of its substrates. Maturation process of SUMO1-3 by SUMO specific peptidase has been demonstrated, except for the newly identified isoform, SUMO4, which could be a susceptibility gene for human type 1 diabetes. In this work, in vitro maturation process of full-length SUMO1, SUMO1-P94, SUMO4 and SUMO4-Q90 were studied in different cell types, including HEK293, human monocytes, DC2.4, Hela, CM, and Jurket cells. Our results demonstrated that in HEK293 cells, the rates and efficiency of full-length SUMO4 and SUMO4-Q90 mutant were similar, but both of them are much slower or lower than those of full-length SUMO1, while SUMO1-P94 could not be processed. In addition, SUMO1 and SUMO4 maturation showed cell-type specificity by comparative studies among different cell types. Therefore, different SUMO peptidases should be responsible for SUMO1 and SUMO4 maturation, respectively.

Ciliary disfunction underlies cystic kidney in Oral-Facial-Digital Type I (OFD1) syndrome. *A. Zullo[1], A. Barra[1], A. Indrieri[1], A. Cantone[2], N. Messaddeq[3], G. Capasso[2], P. Dollé[3], P. Igarashi[4], B. Franco[1,5]* 1) Telethon Institute of Genetics and Medicine, Naples, Italy; 2) Nephrology Dept., Second University of Naples, Naples, Italy; 3) Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France; 4) Internal Medicine Dept., University of Texas Southwestern Medical Center, Dallas, Texas, USA; 5) Medical Genetics, Department of Pediatrics, Federico II University, Naples, Italy.

Oral-facial-digital type I syndrome is characterized by a distinctive X-linked dominant male-lethal pattern of inheritance, craniofacial and limb abnormalities, and cystic kidney of glomerular origin (30% of cases). We previously demonstrated that the responsible gene *Ofd1* is a centrosomal/basal body protein. More recently, we generated *Ofd1*-knockout animals, which reproduced the main features of the human disease. Renal cysts of glomerular origin were observed starting from E16.5 in heterozygous females and immunofluorescence and ultrastructural studies demonstrated defective cilia formation implicating ciliogenesis as a mechanism underlying cyst development. To overcome the problem of embryonic male and perinatal female lethality we generated a mouse line with kidney-specific inactivation of *Ofd1* by crossing the *Ofd1*-floxed line with a transgenic line (cre^{Ksp}) that expresses cre recombinase in renal tubular epithelial cells. Kidney-specific inactivation of *Ofd1* led to viable offspring that developed kidney cysts of tubular origin from P14. By P35 the renal parenchyma was completely replaced with large cysts and by P70 the mutant mice exhibited lethargy and growth retardation. Progressive GFR impairment was observed from P28. Specific tubular markers staining revealed that the cysts originated from the distal nephron and we demonstrated the absence of primary cilia exclusively in cells that lined the cysts. These data show that OFD1 is important in different segments of the nephron and that *Ofd1* inactivation results in cyst formation in the distal tubules as well as in glomeruli. The animal model generated represents an additional tool to dissect the molecular basis of cystic kidney disease and the relationship between renal cysts and primary cilia dysfunction.

A novel statistic to test for gene-environment interaction. *X. Zhou[1], M. Xiong[2]* 1) Dept Internal Medicine, University of Texas Health Science Center at Houston,TX; 2) Biostatistics, University of Texas Health Science Center at Houston, TX.

Most phenotypic variations, including those involved in complex diseases and differences in drug response, are generated by integrated actions of multiple genetic and environmental factors, through dynamic, epigenetic, and regulatory mechanisms. Despite growing consensus on the importance of testing for gene-environment interactions in genetic studies of complex diseases, there is still lack of powerful test statistics for detection o gene-environment interaction. A comprehensive delineation of the complicated interplay between genetic and environmental factors that influences complex traits will require complete characterization of DNA variation in the population and the development of mathematical tools for unraveling the interaction between genetic variation and environmental exposures. In this report, we study how gene-environment interactions create the linkage disequilibrium (LD) in the disease populations and develop a statistic to test for gene-environment interaction by comparing differences in LD between cases and controls. We perform simulations to investigate null distribution of the proposed statistic and calculate its type 1 error rates. We compare the power of the newly developed statistic and existing statistics for detection of gene-environment interactions. To further evaluate its performance the proposed statistic is applied to several real data sets. The results are encouraging. They demonstrate that the P-values of the proposed statistic are much smaller that that of other approaches.

High-throughput genotyping for detecting structural genomic variation in healthy African Americans. *S.W. Scholz[1], M. Matarin[1], H.C. Fung[1], J. Simon-Sanchez[1], D. Hernandez[1], A. Britton[1], J.R. Gibbs[1], A. Singleton[1], A.B. Zonderman[3], M.K. Evans[2]* 1) Laboratory of Neurogenetics, National Institute on Aging, Bethesda, MD, USA; 2) Health Disparities Research Section, Clinical Research Branch, National Institute on Aging, 5600 Nathan Shock Drive, Baltimore, MD 21224, USA; 3) Cognition Section, Laboratory of Personality and Cognition, National Institute on Aging, 5600 Nathan Shock Drive, Baltimore, MD 21224, USA.

Objective: To determine the frequency, distribution and size of structural variation in healthy African-Americans using whole genome genotyping.

Background: Illumina SNP genotyping technology has emerged as a powerful technique to study genomic variation in Caucasian populations. However, the applicability of this technology to study structural variation in non-Caucasian populations is unclear.

The African American population represents 13.4% of the population of the US and is known to be an admixture of Africans, Caucasians, and Native Americans. Studying genomic structure and regions of extended homozygosity in this non-Caucasian population may provide valuable insights into the role of these variations in human disease and clinical phenotypes.

Methods: A cohort of 147 healthy African-Americans was studied. Over 400,000 SNPs were genotyped for each individual using Sentrix HumanHap 300 Genotyping and Sentrix Human-1 Genotyping Beadchips (Illumina Inc., San Diego, CA).

Results: We identified 179 large-scale structural changes (111 duplications, 66 deletions) ranging in size from 47kb to 2.61Mb. Extended tracts of regions of homozygosity and V(D)J recombination in the T-cell receptor alpha locus were also detected.

Conclusion: This is the first whole genome SNP genotyping study of healthy African Americans. The data demonstrates the ability of SNP chip technology to detect structural genomic alterations (40kb in size) and extended regions of homozygosity in African-Americans.

Correcting for the winners curse in genetic association studies. *R. Xiao, M. Boehnke* Department of Biostatistics and Center for Statistical Genetics, University of Michigan, Ann Arbor, MI.

Studies of gene-disease association are now commonly used to localize genetic loci that impact disease susceptibility. It is also of interest to estimate the genetic effect of each identified locus. It is known that the initial positive findings of the genetic effect estimate tend to be upwardly biased, a phenomenon known as the winners curse. In our study, we model the winners curse in the context of case-control genetic association studies. We quantify its impact on the naïve estimators of the allele frequency difference between cases and controls as a function of several factors including sample size, minor allele frequency in controls and cases, and the chosen statistical significance level. We also propose a maximum likelihood method to improve the estimate of the allele frequency difference corrected for the ascertainment. Initial analytical and simulation results indicate that our method substantially reduces the observed overestimation, allowing better estimation of locus-specific effect, and more appropriate design for follow up studies.

Association study of CRP gene polymorphisms with serum CRP level and cardiovascular risk in the NHLBI Family Heart Study. *Q.W. Wang[1], S.C. Hunt[2], Q. Xu[1], M.A. Province[3], J.H. Eckfeldt[4], J.S. Pankow[5], Q. Song[1, 6]* 1) Cardiovascular Research Inst, Morehouse School of Medicine, Atlanta, GA; 2) Cardiovascular Genetics Division, University of Utah, Salt Lake City, UT, USA; 3) Division of Biostatistics, Washington University, St. Louis, MO, USA; 4) Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN, USA; 5) Division of Epidemiology and Community Health, University of Minnesota, Minneapolis, MN, USA; 6) Clinical Research Center, Morehouse School of Medicine, Atlanta, Georgia, USA.

Objective: Recent epidemiological studies indicated that baseline C-reactive protein (CRP) levels may have value in prediction of cardiovascular risk. Methods and results: Using 6 TagSNPs selected from our complete list of single nucleotide polymorphisms (SNP) on the CRP gene, we investigated the association of CRP genotypes with plasma CRP levels and cardiovascular risk in the NHLBI Family Heart Study (FHS) cohort (1296 Caucasians, male 48.5%, age 54.712.8 years). There was a significant trend towards association of CRP haplotypes with CRP levels (p=0.045). Single-SNP analysis indicated a highly significant association of SNP-757 (rs3093059, p=0.0004) and SNP-286 (rs3091244, p=0.0065) and a borderline association of SNP-7180 (rs1341665, p=0.06) with CRP levels. Neither CRP haplotypes nor individual SNP genotypes were associated with IMT of the common carotid arteries, internal carotid arteries, or at the bifurcations of the carotid arteries. Conclusion: These results indicated a strong impact of local SNPs of the CRP gene on plasma CRP levels, but there was no direct evidence that these genetically-controlled CRP elevations by local CRP SNPs contributed to cardiovascular disease phenotypes.

Mutation analysis approach to uncover the gene responsible of an autosomal recessive spastic ataxia associated with frequent white matter changes that map to 2q33-34. *I. Thiffault[1], M. Tetreault[1], L. Loiselle[1], J. Mathieu[2], M. Vanasse[3], G.A. Rouleau[4], J.P. Bouchard[5], J. Lessage[6], B. Brais[1,2,3]* 1) Laboratoire de neurogénétique , Center for the study of brain CHUM-Notre Dame Hosp, Montreal, PQ, Canada; 2) Clinique des maladies neuromusculaires, Carrefour de la Santé de Jonquière, Saguenay, QC, Canada; 3) Clinique des maladies neuromusculaires, Centre de réadaptation Marie Enfant, CHU Mère Enfant Sainte-Justine, Montreal, QC, Canada; 4) Laboratoire de neurogénétique, Center for the study of brain diseases, Centre de recherche du CHUM, Montreal, QC, Canada; 5) Service de neurologie, Hôpital de lEnfant-Jesus, Université Laval, Quebec, Qc, Canada; 6) Radiology Department, Centre hospitalier de lUniversité de Montréal, Montreal, QC, Canada.

Recessive ataxias are a heterogeneous group of diseases. To date, the mutated genes for ten recessive ataxias have been uncovered and two others have been mapped. The combination of pyramidal and cerebellar signs has been observed in a few recessive ataxias, complicated paraplegias, leukodystrophies and cerebral palsy. We recently reported the identification of a group of 23 French-Canadian cases from 17 families affected by an autosomal recessive spastic ataxia associated with frequent white matter changes (ARSAL) that map on 2q33-34. Candidate gene mutation analysis was performed in parallel with the fine mapping. The UCSC March 2006 freeze predicts that 23 genes lie in the conservative 2.51 cM haplotype-defined candidate region. A total of eight candidate genes on chromosome 2q33-34 were studied for mutations: ALSIN, EEF1B2, NRP2, NDUFS1, ALS2CR19, GPR1, KLF7 and ADAM23 (entire coding and a minimum of 30 bp of intronic flanking sequences. Primers used to amplify exonic and flanking sequences were designed using ExonPrimer. Preliminary results suggest that these candidate genes are unlikely the genetic cause of ARSAL. Further gene screening is on-going to identify the causal mutations. The uncovering of the mutated gene may point to a common pathway for pyramidal and cerebellar degeneration as both are often observed in recessive ataxias and complicated paraplegias.

Expression pattern of *Lgi1* gene in mouse brain during development suggests a possible function. *P.A.O. Ribeiro*[1], *L. Sbragia*[2], *R. Gilioli*[3], *F. Cendes*[4], *I. Lopes-Cendes*[1] 1) Medical Genetics, UNICAMP, Campinas, Brazil; 2) Pediatric Surgery, UNICAMP, Campinas, Brazil; 3) CEMIB, UNICAMP, Campinas, Brazil; 4) Neurology, UNICAMP, Campinas, Brazil.

Mutations in *LGI1* gene were described in patients with autosomal dominant lateral temporal lobe epilepsy and preliminary functional studies point to a possible involvement of *LGI1* with migration and/or neuronal proliferation. However, the precise function of *LGI1* remains unknown. The objective of the present study was to determine the expression pattern of the *Lgi1* gene in mice brain during development and in adult animals. Programmed mating was carried with Balb/c mice in order to obtain embryos of different ages. The brains of three animals at the following ages were removed: E15, E17, E18 days (E: embryo), P1, P7, P14, P28, P42 and P56 days (P: post-natal). Gene expression assays were carried out using real time PCR with the TaqMan system. In addition we used an endogenous control (*Gapdh* gene) and all experiments were performed in duplicates. *Lgi1* gene expression was significantly low during the intrauterine ages increasing gradually until P56 (adult animal). Samples from P28, P42 and P56 presented a seven fold increase in expression as compared to E15 samples. The pattern of *Lgi1* gene expression that we observed suggests a predominantly inhibitory function during development of the central nervous system (low expression during embryonic stages). In addition, we may speculate that in more advanced ages, when neurons are already differentiated, its inhibitory function could be also essential, which can be suggested by the high expression in adult mouse brain.

Proximal 10q trisomy syndrome resulting from a paternally inherited insertion of 10q11.2 to q21.3 with normal growth and ophthalmologic findings: implications for possible localization of gene(s) involved in growth regulation and/or eye development at 10q22. *M.N. Strecker, A.M. Slavotinek* Division of Genetics, Department of Pediatrics, UCSF, San Francisco, CA.

There have been ten reported cases of proximal 10q trisomy syndrome with varying cytogenetic breakpoints within the region of 10q11-10q22. The phenotypic consequences of this trisomy include mild to moderate developmental delay, postnatal growth retardation, microcephaly, long slender limbs, and craniofacial dysmorphism characterized by a prominent forehead, deep-set eyes, epicanthic folds, an upturned nose, dysplastic helices, a bow-shaped mouth and micrognathia. We report an 18 year-old male with an extremely mild phenotype constituting mild developmental delay (categorized as a sensory integration disorder), long thin limbs, and subtle facial dysmorphisms, including down-slanting palpebral fissures, flattened helices, a narrow maxilla, and a bow-shaped mouth. His karyotype by high resolution GTG banding was 46,XY,der(17)ins(17;10)(p13;q11.2q21.3), resulting from an interstitial insertion of chromosome 10q11.2-10q21.3 into chromosome 17p13. Parental chromosome analysis revealed that the father was a balanced carrier of this insertion, with a karyotype of 46,XY,ins(17;10)(p13;q11.2q21.3). In contrast to previous cases, our patient has a smaller and more proximal duplication without involvement of chromosome band 10q22, whereas all of the previously reported proximal 10q trisomy patients have had duplication of 10q21 to 10q22. Clinically, our patient also differed from the majority of other patients in that he had normal growth without microcephaly and was without ocular abnormalities. Our patient's karyotype and mild phenotype imply the possible localization of gene(s) involved in growth regulation and/or eye development at chromosome 10q22 that contribute to the proximal 10q trisomy phenotype. Candidate genes include COL13A1, a gene expressed in connective tissue of unknown function, and P4HA1, a component of the enzyme prolyl 4-hydroxylase involved in collagen synthesis.

Associations of gene expression variation with SNPs and copy number variants (CNVs) in the HapMap samples.

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Gene expression variation is likely a major determinant of phenotypic variation and susceptibility to disease in humans. The goal of this work is to identify and characterize functionally variable regions that contribute to gene expression variation. We have screened mRNA levels for ~48,000 transcripts in lymphoblastoid cell lines from 270 individuals of the HapMap Project. Approximately 15,000 transcripts gave a detectable expression signal relative to background. We used SNP genotypes and a recent set of copy number variants (CNVs) genotypes for the HapMap samples to correlate with gene expression. We used methodologies that consider each population separately, and are implementing methods that consider all populations together. We have detected significant associations for about 800 and 60 genes per population with SNPs and CNVs in regions nearby the genes. About 75% of the SNP and CNV associations are population-specific, while 25% of the associations are replicated in at least 2 populations. About 15% of the CNV associations are captured by SNP associations suggesting that phenotypic variation caused by CNVs may be tractable by SNPs in some cases. We have also identified genomic associations with genes whose expression exhibit significant population differentiation. Finally, we infer that the CNV effects on gene expression are of similar magnitude as the SNP effects. This is the first detailed analysis of associations of gene expression variation with both SNPs and CNVs in multiple human populations and is proving to be highly informative for the structure of genetic variation in human populations.

Familial Noonan syndrome with high intelligence associated with an isoform-specific KRAS mutation. *S. Unger[1,2], D. Horn[3], K. Lehmann[3], M. Zenker[4], C. Kratz[5]* 1) Institute for Human Genetics, Univ Freiburg, Freiburg, Germany; 2) Division of Clinical and Metabolic Genetics, Sick Kids, Toronto, Canada; 3) Institute for Medical Genetics, Humboldt University, Berlin, Germany; 4) Institute of Human Genetics, University of Erlangen-Nuremberg, Erlangen, Germany; 5) Department of Pediatrics and Adolescent Medicine, University of Freiburg, Germany.

Noonan syndrome (NS) (MIM163950) is an autosomal dominant disorder whose main features are mild short stature, congenital heart disease (pulmonic valve stenosis), and webbed neck. Developmental delay of a variable degree is seen in one third of the patients. Approximately 50% of Noonan syndrome individuals have a mutation in the PTPN11 gene encoding for SHP-2, a phosphatase that relays growth signals from activated growth factor to other signaling molecules including Ras. Downstream partners in the same signaling pathway as SHP-2 were natural candidates genes for the remaining NS patients. Recently, we have identified de novo missense mutations in the KRAS oncogene in several sporadic cases of NS. We report a multigeneration family with typical features of NS. All affected individuals had mild short stature and facial features consistent with NS and all were of normal/high intellect. Several affected individuals had scoliosis, some had multiple lentigines and one person had pulmonic valve stenosis. The proband, who has an IQ of 133, was found to carry a G179S KRAS mutation. This change is located in exon 5 and is thus expressed only in the A isoform of KRAS, which is in contrast to other mutations reported thus far. The precise mechanism by which this mutation disrupts Ras function is currently unknown. The G179S mutation is likely to interfere with Ras processing and cellular localization, but does not alter a residue known to be involved in the GTPase activity of Ras. We speculate that the location of this mutation is responsible for the relatively mild phenotype, in particular for the absence of developmental delay.

Identification of three potential candidate genes for autosomal dominant mental retardation. *A.K. Srivastava[1], S.D. Menon[1], G.F. Guzauskas[1], V.S. Vervoort[1,2], M.R. Vayalapaty[1], F. Zhang[1], S. Ladd[1], K.C. Ukadike[1], A. King[1], B.R. DuPont[1], K. Clarkson[1], R.J. Schroer[1]* 1) Greenwood Genetic Center, Greenwood, SC; 2) Present address: Developmental Neurobiology Department, Burnham Institute for Medical Research, La Jolla, CA.

Mental retardation (MR) is the most common developmental disability, affecting cognitive function in about 2-3% of the human population. The etiology of MR remains poorly understood in the majority of cases. MR may result from alterations in any of a number of genes involved in various pathways and these genes are presumably distributed throughout the human genome. Although the role of X-linked genes in MR is relatively well known, little progress has been made in identifying autosomal MR genes partially due to enormous genetic heterogeneity and the lack of large families for linkage analysis. We utilized patients with MR and balanced autosome;autosome translocations to circumvent some of these obstacles and to identify candidate autosomal MR genes for intensive mutation screening. Here we report the identification of three potential candidate autosomal MR genes: 1) ZNF161, a zinc finger gene at 17q23, was physically disrupted in a female patient with profound MR, 2) C10orf45, a novel gene at 10p13, was disrupted in a female patient with profound MR; and 3) a novel isoform of the CLDN14 gene was disrupted at 21q22 by the translocation breakpoint in a male patient with moderate MR and aggressive behavior. Both chromosomal breakpoints of the translocations were mapped and no additional genes were found to be affected by the second chromosomal breakpoints in these patients. Furthermore, no additional mutations of these genes were identified in the patients with the translocations indicating that the disruption of one copy of the gene in the respective patients does not unmask a recessive mutation in these genes. All three genes are expressed in adult and fetal brain. The three candidate MR genes were further characterized and are presently being screened in a large cohort of patients (500 females and 500 males) with MR of unknown etiology to investigate their potential involvement in some cases of autosomal MR.

Novel mutations in two large Saudi families affected with L-2-Hydroxyglutaric aciduria. *M. Ul Haque[1], E. Faqieh[2], A. Alduraihem[1], N.M. Saleh[1], H.A. Abalkhail[1], M. Al-Owain[2], A. Tbakhi[1], M. Al-Sayed[2]* 1) Molecular Genetics Lab, DPLM, King Faisal Specialist Hosp, Riyadh, Riyadh, Saudi Arabia; 2) Department of Medical Genetics, King Faisal Specialist Hospital & Research Centre, P.O.Box# 3354, MBC# 75, Riyadh 11211, Saudi Arabia.

Here we report 2 extended consanguineous families from different geographical regions of Saudi Arabia, displaying the typical features of L-2-Hydroxyglutaric aciduria encoded by L-2-hydroxyglutarate dehydrogenase (L2HGDH), a rare autosomal recessive neurometabolic disorder characterized by mild psychomotor delay in the first years of life, followed by progressive cerebellar ataxia, dysarthria and moderate to severe mental deterioration. We studied 13 patients, from 2 families with L2HGDH gene and sequenced the entire coding regions and exon-intron boundaries which revealed two different homozygous mutations in both families. In 1st family, we identified a novel single bp deletion A1015 in exon 8, resulting in a frameshift in the translated protein and replacement of 12 novel amino acids before a premature termination. The mutation was found in a homozygous state in all 6 affected individuals and was heterozygous in all unaffected carrier parents. In 2nd family, mutation analysis in 7 affected individuals revealed a homozygous C1319A transition at codon 440, which changes Serine a hydrophilic acidically charged residue to Tyrosine a basically charged residue in a conserve low complexity region of gene. To our knowledge, this is the first report of mutation analysis of the L2HGDH gene from Saudi Arabia.The two mutations are located in a highly conserved area across the multiple species suggest that the substituted residues are important for protein folding and/or enzyme catalysis and may effect the pre- processing and folding mechanism of the protein inside the mitochondria.

Clinical applications of rapid chromosome aneuploidy detection by quantitative fluorescent PCR. *Y. Yang, S. Hondorp, A. Haque, K. Thompson* Molecular Laboratory, Center for Medical Genetics, Houston, TX.

Common fetal chromosome aneuploidies compatible with live births are trisomies 13, 18 and 21 as well as sex chromosome anomalies (such as Klinefelter and Turner syndromes). Typically, detection of these anomalies can be accomplished by conventional cytogenetic analysis of prenatal cells from chorionic villi or amniotic fluids, which usually takes more than seven days. Two rapid approaches are available to provide preliminary results: fluorescence in situ hybridization (FISH) and quantitative fluorescent PCR (QF-PCR). While both methods can be used to detect aneuploidies for chromosomes 13, 18, 21, X, and Y within 24-48 hours, QF-PCR, a molecular genetics based test, has the advantage of being less expensive and requiring less amniotic fluids.
We are reporting here our experience of performing QF-PCR for rapid aneuploidy detection in the past two years. A total of 475 samples have been tested, including 378 amniotic fluid, 8 CVS and 89 newborn blood samples. Two tiers of multiplex QF-PCR were designed. The first tier includes two highly polymorphic STR markers for each chromosome studied and the Amelogenin gene. Trisomies can be detected by the tri-allelic pattern or 2:1 peak ratio of the PCR amplification products. Samples uninformative in the first-tier (about 5%) will be retested using the second-tier STR markers. Of the 475 samples, the abnormal results include 36 trisomy 21, 3 trisomy 18, 2 trisomy 13 and 3 cases strongly suggesting Turner syndrome (45,X). Our results have been compared to those of chromosome analysis and showed excellent agreement with the cytogenetics results: 100% sensitivity and specificity for trisomy detections. Overall our studies showed that QF-PCR is an accurate, efficient and inexpensive method for preliminary aneuploidy detection. By this method, clinicians and patients can have the preliminary results in as early as one day. It should be noted that both QF-PCR and FISH should be used in conjunction with routine chromosome analysis before any irreversible decisions are made.

Treatment of multiple sulfatase deficiency with recombinant human arylsulfatase B (galsulfase, Naglazyme). *S. Sandberg[1], L. Charnas[1], E. Braunlin[1], K. Bjoraker[1], M. Deanching[2], G. Hoganson[2], F. Rimell[1], C. Whitley[1]* 1) University of Minnesota, Minneapolis, MN; 2) University of Illinois, Chicago, IL.

C-alpha-formylglycine (FGly) is the catalytic residue at the active site of eukaryotic sulfatases, is created from cysteine by FGly-generating enzyme, coded by the SUMF1 gene. Mutations result in multiple sulfatase deficiency (MSD), a disorder with features of several lysosomal diseases, eg, increased glycosaminoglycans (GAG), sulfolipids, and steroid sulfates. We encountered a 13-year-old girl whose progressive MSD was life-threatening. Examination showed dysostosis multiplex, hepatomegaly, and ichthyosis. IQ was normal. MRI revealed cervical cord compression but no leukodystrophy. Urine GAG and sulfatides were elevated. Several lysosomal sulfatases were measured at low levels. The patients family declined hematopoietic stem cell transplant, a treatment that would replace several enzymes presumed necessary to halt MSD disease. Lacking other treatment, we considered enzyme replacement therapy (ERT) with human recombinant arylsulfatase B (ASB). After 4 months of galsulfase (Naglazyme) 1 mg/kg/week, urine GAG decreased from 1,023 mg GAG/g creatinine to 80. Liver volume decreased (745 to 581 cc) while spleen size was unchanged (172 to 170, respectively). There was rehabilitation from full-time bedrest, to part-time sitting. The patient became independent of respiratory assistance, by 5 months being free of oxygen and mechanical pressure support. In this patient, ERT demonstrated that deficiency of ASB was causing clinical pathology, and that some features were responsive analogous to Maroteaux-Lamy syndrome. Other features persisted: ichthyosis and sulfatiduria. Arylsulfatase A activity was immeasurable, but not low enough to cause neurologic disease (cf metachromatic leukodystrophy). Other sulfatases were low (ie, iduronate-2-sulfatase deficiency, heparan sulfate sulfamidase) but not so low as to cause Hunter syndrome or Sanfilippo syndrome type A. We speculate that clinical MSD may be the manifestation of only 1, 2 or 3 enzymes which are below a crucial threshold. Naglazyme may be efficacious in patients for whom ASB activity is a critical factor.

Combining information from multiple common susceptibility polymorphisms increases the predictive power of genetic information: a study of replicated type 2 diabetes variants. *M.N. Weedon[1], M.I. McCarthy[2], G. Hitman[3], M. Walker[4], C.J. Groves[2], E. Zeggini[2], N.W. Rayner[2], B. Shields[1], K.R. Owen[1], A.T. Hattersley[1], T.M. Frayling[1]* 1) Peninsula Medical School, Exeter; 2) Oxford Centre for Diabetes, Oxford; 3) Centre of Diabetes & Metabolic Medicine, University of London; 4) School of Medicine, Newcastle upon-Tyne.

A limited number of studies have assessed the risk of common diseases when combining information from several predisposing polymorphisms. In most cases, individual polymorphisms only moderately increase risk (~20%) and they are thought to be unhelpful in assessing subjects risk clinically. The impact of looking at multiple alleles simultaneously is not well studied. This is often because, for any given disease, there are very few confirmed, common risk alleles. Three common variants (K23 of KCNJ11, P12 of PPARG, and the T allele at rs7903146 of TCF7L2) predispose to type 2 diabetes mellitus (T2D) across many large studies. Risk allele frequencies range from 0.30 to 0.88 in controls. To assess the combined effect of multiple susceptibility alleles we genotyped these variants in a large case/control study (3668 controls v 2409 cases). Individual allele odds ratios (OR) ranged from 1.14 (95% CI: 1.05,1.23) to 1.48 (95% CI: 1.36, 1.60). We found no evidence of gene-gene interaction and the risks of multiple alleles were consistent with a multiplicative model. Each additional risk allele increased odds of type 2 diabetes by 1.28 (1.21, 1.35) times. Subjects with all 6 risk alleles had an OR of 5.71 (95% CI: 1.15, 28.3) compared to subjects with 0 risk alleles. The 8.1% of subjects double homozygous for the risk alleles at TCF7L2 and Pro12Ala had an odds ratio of 3.16 (95% CI: 2.22, 4.50), against the 4.3% of subjects with no TCF7L2 risk alleles and either 0 or 1 E23K or Pro12Ala risk alleles. In conclusion, combining information from several known common risk polymorphisms allows the identification of subgroups of the population with markedly differing risks of developing type 2 diabetes compared to when using single polymorphisms. This approach may have a role in future preventative measures for common, polygenic diseases.

E-Selectin Ligand 1 Negatively Regulates TGF in the Golgi during Skeletogenesis. *T. Yang[1], R. Mendoza-Londono[1], H. Lu[1], K. Li[1], B. Keller[1], M. Jiang[1,2], Y. Chen[1,2], T.K. Bertin[1], B. Dabovic[4], D.B. Rifkin[4], J. Hicks[3], A.L. Beaudet[1], B. Lee[1,2]* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Howard Hughes Medical Institute; 3) Department of Pathology, Baylor College of Medicine and Texas Childrens Hospital; 4) Department of Cell Biology, New York University Medical Center.

Transforming growth factor (TGF) signaling plays critical roles regulating growth and differentiation during development and disease. Its context dependent action is specified by numerous control mechanisms at the extracellular level and downstream of ligand-receptor interactions, but little is known about the regulation of its post-translational trafficking. E-Selectin Ligand-1 (ESL-1), the cysteine rich protein originally isolated as a ligand for E-Selectin, was also found to interact with FGFs and to be co-purified with TGF1 in a large protein complex. To elucidate its in vivo function, we generated Esl-1 mutant mice. The newborn Esl-1-/- mice are notably smaller with narrow chests and generalized shortening and thinning of all bony elements. This severe but proportionate growth retardation was observed from embryonic day 15.5 (E15.5) to maturity. By histological analysis, P1 Esl-1-/- mice showed shortening of the growth plates in both the proliferating zone and hypertrophic zone. Moreover, the notable less bone density was also detected in the adult mutant mice. Further molecular assays show that ESL-1 acts as a negative regulator of TGF production by binding TGF precursors via its laternt activation domain (LAP) in the Golgi in a cell autonomous fashion. In vivo, loss of ESL1 function causes increased TGF signaling resulting in decreased cell proliferation and delayed terminal differentiation in the cartilaginous growth plate, independent of effects on BMP and FGF signaling. Moreover, in vivo genetic models of gain vs. loss of TGF signaling in the growth plate confirm this effect. Our data not only identified ESL-1 as a critical regulator for skeletogenesis, cartilage and bone homeostasis, but also revealed a novel mechanism for regulating TGF intracellular pool in these processes.

A novel de novo frameshift mutation of the EDA gene in a Chinese family with hypohidrotic ectodermal dysplasia. *Xin. Tu, Qinbo. Yang, Changzhen. Huang, Tie. Ke, Qing. Wang, Mugen. Liu* Center for Human Genome Research, Huazhong University of Science and Technology, Wuhan, Hubei, China.

Hypohidrotic ectodermal dysplasia is characterized by severe hypohidrosis, hypotrichosis, and hypodontia. It can be inherited in autosomal dominant, autosomal recessive, or X-linked patterns. Mutations in the EDA gene, which encodes ectodysplasin-A (EDA), are responsible for X-linked hypohidrotic ectodermal dysplasia (XLHED). In the present study, we identified a Chinese family with X-linked hypohidrotic ectodermal dysplasia. By linkage analysis we revealed that the causative gene was linked to EDA locus in the family. Direct DNA sequence analysis of whole coding regions and exon -intron boundaries of EDA gene showed this is the only de novo INSERTION mutations of EDA described, and c.573-574 ins T in EDA gene existed in all affected males and carrier females. This mutation resulted in a frameshift leading to altered amino acid structure and early termination of EDA. Further PCR-RFLP analysis suggested that the c.573-574 ins T mutation of EDA gene is a cause for X-linked hypohidrotic ectodermal dysplasia in the family.

Genetic variation in the endothelin converting enzyme-like 1 gene is associated with type 2 diabetes (T2DM) in the Old Order Amish. *Y. Wang[1], X. Shi[1], P.F. McArdle[1], C.M. Damcott[1], Y.C. Chang[1], A.R. Shuldiner[1,2], B.D. Mitchell[1], N.I. Steinle[1]* 1) Department of Medicine, University of Maryland, Baltimore, MD; 2) GRECC, Veterans Administration Medical Center, Baltimore, MD.

T2DM is a polygenic disorder, characterized by gene-gene and gene-environment interactions with onset in adulthood. The endothelin converting enzyme-like 1 (*ECEL1*) gene is located on chromosome 2q37, within a region on the distal arm of chromosome 2 that has identified as a locus of diabetes susceptibility. *ECEL1*, whose substrate is unknown, is highly expressed in skeletal muscle, pancreas and brain, and is localized in the plasma membrane and the endoplasmic reticulum (ER). We tested the hypothesis that variation in *ECEL1* is associated with T2DM and related traits by genotyping 4 common (MAF>0.1) haplotype tagging SNPs (htSNPs), selected from the CEU HapMap database and one rare nonsynonymous SNP Tyr328His (frequency 0.03) in participants from the Amish Family Diabetes Study (AFDS) (n=1205). In our case-control study, we found that the three intronic htSNPs (rs909431, rs1190430 and rs746379) were significantly associated with either T2DM (p=0.005-0.02) or the combined T2DM/impaired glucose tolerance (IGT) trait (p=0.0002-0.02). Furthermore, these three intronic SNPs as well as the promoter htSNP (rs2742072) were strongly associated with higher glucose and insulin area under the curve during an oral glucose tolerance test in a subset of nondiabetic subjects (n=654) ($p<0.0009$ and $p<0.03$, respectively), suggesting that these individuals have impaired insulin sensitivity. Haplotype analysis showed that the intronic SNPs are in one linkage disequilibrium block with one common haplotype (ACG, frequency 0.293) showing strong association with increased risk of T2DM (p=0.002) and the combined T2DM/IGT trait (p=0.00003). Our data provide the first evidence that genetic variation in *ECEL1* is associated with T2DM, IGT and insulin resistance, through mechanisms that are unknown, but possibly related to ER stress.

Molecular characterization of a case with CHARGE syndrome like phenotype and partial 6p trisomy. *I. Ragoussis[1], G. K. Mirza[1], L. Schimmenti[2], R. Clark[2]* 1) Genomics, Wellcome Trust centre for Human Genetics, Oxford, United Kingdom; 2) USC/Keck School of Medicene, Cancer Genetics Units, Norris Comprehensive Cancer Center, Los Angeles, CA 90089.

We present a case diagnosed with CHARGE syndrome based on initial clinical findings (parents were normal). Cytogenetic analysis identified a normal karyotype in lymphocytes but revealed a *de novo* duplication of 6p in fibroblasts and therefore mosaicism. Molecular analysis was carried out using over 40 BAC and PAC clones that had been previously mapped for FISH on nuclei obtained from fibroblast cultures. The analysis confirmed a terminal duplication karyotype: 46,XY,dup(6)(22), with the region of duplication estimated to be 15Mb. Our case presented with heart defects, choanal atresia, bilateral sensorineural and conductive hearing loss, ocular coloboma, submucus cleft palate, and mild retardation, which are pertinent to the phenotypic characteristics of CHARGE syndrome. Of these, heart defects and choanal atresia are also found in 6p duplications. Additional clinical features included facial dysplasia, myopia, ptosis, strabismus, and skin hypopigmentation, which are consistent with 6p duplications. Although hearing defects are associated with CHARGE syndrome, they have not been previously reported in 6p trisomy cases. External ear abnormalities are associated highly with individuals affected with CHARGE syndrome, but no structural defects of the ears were reported in our case. Interestingly the phenotype is more complex than a reported mosaic case with 6p22-pter trisomy, where the trisomy is found in a small proportion of peripheral blood and fibroblast cells (Giardino et al Am. J. Med. Genet. 108:36-40 (2002)). Most affected individuals with CHARGE syndrome, have mutations or deletions involving the chromodomain helicase DNA-binding protein-7 (CHD7) gene on chromosome 8.We have excluded deletion of the CHD7 gene and mutation analysis is in progress in an effort to delineate the phenotype further. In conclusion, this would be the first time that CHARGE syndrome is possibly linked to 6p.

Identification of Novel Deletions of 15q11q13 in Angelman Syndrome by Array-Based Comparative Genomic Hybridization (CGH): Large Segmental Duplicons Flank the Breakpoints. *S. Peters[1], T. Sahoo[1], J.R. German[1], C.A. Shaw[1], L.M. Bird[2], V. Kimonis[3], A.L. Beaudet[1], C.A. Bacino[1]* 1) Dept Pediatrics, Baylor Col Medicine, Houston, TX; 2) Pediatrics, Childrens Hospital San Diego, San Diego, Ca; 3) Boston Childrens Hopsital, Boston, Ma.

Angelman syndrome (AS) is a neurodevelopmental disorder characterized by severe mental retardation, ataxia, and a happy disposition. Deletions of the 15q11q13 region are found in approximately 70% of AS patients. The deletions are sub-classified into Class I and Class II based on their size (~6 to ~6.8 Mb), and their breakpoint involvement, with two different proximal breakpoints and a single common distal breakpoint. Utilizing a chromosome 15-specific genomic microarray, we have determined the deletion sizes and mapped the breakpoints in a cohort of cases (n=44) to identify a common underlying mechanism and derive precise genotype-phenotype correlations. Interestingly four patients of the 44 studied (9.1%) had novel and unusually large deletions. This is the first report of extremely large deletions of 15q11q13 resulting in Angelman syndrome; the largest being >10.6-Mb. These novel deletions involve three different distal breakpoints, two of which have been shown to be involved only in the formation of isodicentric (15)(q11.2). Additionally, precise determination of the deletion breakpoints reveals the presence of segmental duplications in the vicinity of all the recurrent and novel breakpoints identified so far. This genomic organization provides strong evidence for a mechanism that generates the common as well as rare deletion events. Detailed phenotypic characterization of the probands indicates large deletions result a much more severe phenotype. The large deletions result in a loss of a number of genes outside the common AS-PWS critical interval. The impact of the loss of these additional genes requires further characterization. Array-based CGH thus provides very precise information regarding the nature of the recurrent and rare deletion events that result in Angelman syndrome and identifies genomic structures that most likely are involved in the abnormal recombination events resulting in 15q11q13 deletions.

Enhanced Oversight of Genetic Testing Laboratories Needed and Supported: Results of a Survey of Laboratory Directors. *J. Scott[1], G. Javitt[1], J. Murphy[1], D. Kaufman[1], S. Katsanis[2], K. Hudson[1]* 1) Genetics & Public Policy Ctr, Johns Hopkins Univ, Washington, DC; 2) DNA Diagnostic Laboratory, Johns Hopkins Univ, Baltimore, MD.

Introduction: To ensure high quality laboratory testing, the Clinical Laboratory Improvement Amendments (CLIA) mandate proficiency testing (PT) for high complexity tests within CLIA-defined specialty areas. Genetic tests are considered high complexity, but no specialty area exists for molecular and biochemical genetic tests, and no PT is specified. A few voluntary PT programs exist. The federal government has repeatedly recommended creation of a genetic testing specialty under CLIA. A survey of U.S. genetic testing lab directors assessed the relationship between participation in PT and lab quality, and attitudes about oversight. **Methods:** U.S. molecular and biochemical genetic testing lab directors were asked to complete a confidential online survey between December 2005 and March 2006. Relationships between use of PT and lab errors encountered was explored using regression. **Results:** Of 345 eligible lab directors, 190 (55%) responded, and 95% were at CLIA certified labs. Common specialty certifications included pathology (48%) and chemistry (46%)- 16% were not specialty-certified. Nearly two-thirds conducted some PT on all tests offered, however, 26% conducted PT on 50-99% of their tests, 9% on <50% of tests, and 3% conduct no PT. As the percent of tests covered by PT increased, PT deficiencies in the lab declined significantly ($p=0.004$), and as the number of PT deficiencies declined, the number of incorrect test reports issued also declined significantly ($p=0.03$). Labs performing PT on <100% of tests were also more likely to identify an analytic error as their most common error($OR=3.1$ $p=0.02$). Most lab directors (92%) felt that PT improves the quality of genetic testing, and 73% felt a genetic testing specialty should be created under CLIA. **Conclusions:** Lab directors support the creation of a genetic specialty by CLIA, including specific PT programs which 92% find useful. PT testing is strongly correlated with quality-related outcomes, further supporting creation of the CLIA specialty.

MATN3 (Matrilin-3) sequence variation (p.T303M) is a risk factor for osteoarthritis of the CMC1 joint of the hand, but not for knee osteoarthritis. *A. Tagariello[1], O. Pullig[2], A. Schweizer[1], B. Swoboda[2], P. Schaller[3], A. Winterpacht[1]* 1) Inst Human Genetics, Univ Erlangen-Nuremberg, Erlangen, Germany; 2) Department of Orthopaedics, Univ Erlangen-Nuremberg, Erlangen, Germany; 3) Department of Hand Surgery and Plastic Surgery, Kliniken Dr. Erler, Nuremberg, Germany.

Osteoarthritis (OA) is the most common musculoskeletal disorder, characterized by progressive degeneration of joint cartilage resulting in joint pain and stiffness. The disorder has a multifactorial etiology. Hand OA is a subtype of OA, for which a strong genetic basis was suggested. Recently, it has been reported that a single nucleotide polymorphism (SNP) in the matrilin-3 (MATN3) gene encoding T303M at a highly conserved position in an EGF-like domain is associated with an increased risk [frequency of 2 %, relative risk of 2.1] of idiopathic hand OA in the Iceland population (Stefansson et al., 2003). We have investigated the SNP in a small but heterogeneous German cohort of patients with radiographic and symptomatic hand OA of the first carpometacarpal joint (CMC1) [n = 50], 177 patients with radiographic and symptomatic knee OA and 356 unrelated Caucasian control individuals from Germany by direct sequencing. We analysed the frequency of the SNP in hand OA patients and in 356 controls and observed a difference in allele frequency (^{2} = 10.84, p = 0.00099). The rare T allele was present in 10 % of the hand OA patients and in 2.5 % of the healthy controls. The estimated relative risk is 4.28 (95% CI, 1.18-14.8), but this value is uncertain due to the small number of patients. No difference in allele frequency and no significant association could be observed between this allele and the occurrence of knee OA in our cohort (Table 1, ^{2} = 0.03, p = 0.85755). Although our cohorts were fairly small, the present data are significant and we could replicate the results for hand OA reported by Stefansson et al. (2003). These data support the importance of the analysed SNP for this specific form of OA, but not for knee OA where no association could be found.

Association Studies Of Oncogenes In Invasive Epithelial Ovarian Cancer. *L. Quaye[1], H. Song[2], S.J. Ramus[1], I.J. Jacobs[1], B.A.J. Ponder[2], A.S. Whittemore[3], E. Høgdall[4], S. Krüger-Kjær[4], P.D.P. Pharoah[2], S.A. Gayther[1]* 1) Gynaecological Oncology, University College London, London, United Kingdom; 2) Department of Oncology, University of Cambridge, UK; 3) Department of Health Research and Policy, Stanford University, USA; 4) Department for Virus, Hormones and Cancer, Danish Cancer Society, Denmark.

Epithelial ovarian cancer (EOC) represents 5% of all cancers in women worldwide, but it is poorly understood. Approximately 5-10% of ovarian cancer cases are caused by highly penetrant susceptibility, primarily BRCA1 and BRCA2. However, these genes are responsible for only 30% of the excess familial EOC risk. The remaining familial risks are thought to be caused by a combination of susceptibility genes of lower risk (i.e. moderate-low penetrance). We are using candidate gene association studies in EOC case-control populations in order to find low penetrance genes. In this study, we have used a single nucleotide polymorphism (SNP) tagging approach to identify SNPs in candidate oncogenes that have previously been shown (or are predicted) to be involved in ovarian cancer development: BRAF, KRAS, ERBB2, PIK3CA, and the NMYC-CMYC interactor gene, NMI. SNPs were selected with an r2 0.8, minor allele frequency 0.05. Genotyping was performed using the multiplex genotyping approach iPLEX (Sequenom) in two different epidemiological based EOC case-control series from Denmark and the UK. Together, these populations comprise approximately 1200 invasive EOC cases and 2100 unaffected, female controls. We identified two SNPs (rs3771882 and rs3854012) in the NMI gene that were significantly associated with decreased EOC risk. For rs3771882 HetOR=0.77 (95% CI 0.65-0.92), HomOR=0.86 (CI 0.70-1.06) P=0.0136 and for rs3854012 HetOR=0.79 (CI=0.66-0.94), HomOR=0.88 (CI 0.72-1.08) P=0.0236. Both of these SNPs are within the same linkage equilibrium (LD) block with an r2=0.08. A multi-centre consortium study with a further 2500 cases and 3500 controls will be conducted to confirm if these SNPs are associated with decreased EOC risk.

Semi-parametric test based on spline smoothing for genetic association study under structured populations. *Q. Zhang, J. Luo, R. Chakraborty, R. Deka* Dept Environmental Health, Univ Cincinnati, Cincinnati, OH.

Statistical methods have been proposed using markers to control for population stratification in genetic association studies. However, these methods either have low power when population stratification becomes strong or can not control for population stratification well under admixture population models. Zhang et al. (Genet Epidemiol 2003, 24: 44-56) and Chen et al (Ann Hum Genet. 2003, 67 : 250-64) proposed semi-parametric tests based on kernel function to detect the association between candidate markers and quantitative traits, and binary traits, respectively. However, their semi-parametric tests based on kernel smoothing are complex and difficult to implement. Here, we propose another semi-parametric test based on penalized spline smoothing to detect association between candidate markers and qualitative traits. Penalized spline smoothing can be expressed in a linear mixed model framework, which allows such model to be fitted using standard mixed models software, and also allows us to fit complex models, such as proportional odds model for ordinal data. Another advantage of penalized spline smoothing is that the smoothing parameter is the ratio of variance component and can be selected in a natural fashion using restricted maximum likelihood estimation (REML). We are conducting coalescence simulations to compare the power and type I error of this test with other existing method. Our initial results show that the type I error of our method is low with acceptable power.

Genomic convergence in Alzheimer disease. *M. Slifer[1], E. Martin[1], H. Munger[1], J. Haines[2], J. Gilbert[1], M. Pericak-Vance[1]* 1) Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Ctr Human Genetics Research, Vanderbilt Univ Medical Ctr, Nashville, TN.

Late-onset Alzheimer disease (LOAD) is the most common cognitive disorder affecting more than four million Americans. Over the past two decades, hundreds of variations in candidate genes have been reported as associated with LOAD. However, only the apolipoproteinE4 allele has been widely accepted as a risk factor. The deluge of reported susceptibility alleles, limited resources, lack of follow-up, small sample sizes, varying datasets, and differing experimental designs have complicated the search for confirmed LOAD genetic variants. In this study, we use the genomic convergence approach to select candidate genes for systematic follow-up. A gene is declared convergent if significant effects are published from at least three independent experimental methods (e.g. genetic linkage, genetic association, and gene expression). Nineteen candidates (comprising 1.5 Mb of genomic DNA) meet full convergent criteria and have been interrogated in a large independent case-control dataset (n=1000). All cases meet NINCDS/ADRDA criteria for Alzheimer disease as agreed upon by consensus conferences. Controls have no history of cognitive impairment, no family history of LOAD in a first degree relative or and no evidence of dementia on psychometric testing. Using dense SNP genotyping (approximately 1 SNP per 2.5 kb) we examined candidate variants for association with LOAD. Nine of the nineteen convergent genes have SNPs significantly associated with LOAD ($p<0.05$) including multiple SNPs within 3 regions of the amyloid beta (A4) precursor protein gene ($p=0.0002$), and a region proximal to the 3 end of the major histocompatibility complex, class 1, A gene ($p=0.0001$).

Comprehensive analysis of causative and related genes for sporadic ALS using a high throughput DNA microarray-based resequencing system. *Y. Takahashi, J. Goto, S. Tsuji* Dept Neurology, Univ Tokyo, Tokyo, Japan.

[Background] The causes of sporadic amyotrophic lateral sclerosis (ALS) remains unknown. However, recent reports have demonstrated that mutations of causative genes for familial ALS have been identified in a portion of sporadic ALS patients. The molecular dissection through comprehensive analysis of causative genes should contribute to elucidating molecular epidemiology and providing insight into pathogenesis of sporadic ALS. In addition, comprehensive screening for candidate genes may reveal some variations related to genetic risks of ALS. With this background, we have developed a DNA microarray-based high throughput resequencing system for ALS, TKYALS01. [Objectives] To conduct molecular dissection of sporadic ALS and to identify novel variations potentially associated with disease risks or phenotypic variations of ALS. [Methods] Thirty-three sporadic ALS patients were included in this study, consisting of 18 definite ALS, 9 probable ALS, 3 possible ALS, and 3 ALS-plus patients based on the El Escorial and the revised Airlie House diagnostic criteria. We have screened all the exonic and flanking intronic sequences of 3 causative genes (*SOD1* , *ALS2* , and *DCTN1*) and 7 related genes (*SLC1A2*, *SMN1*, *LIF*, *RNF19*, *ADAR2* , *CNTF* , and *VEGF*) for ALS using TKYALS01. [Results] Two causative mutations were identified, including 1 novel *DCTN1* putative mutation in a possible ALS patient and 1 previously known *SOD1* mutation in a definite ALS patient. In addition, 6 novel heterozygous nonsynonymous substitutions, 5 *ALS2* substitutions in 4 definite and 2 probable patients and 1 *VEGF* substitutions in 1 ALS-plus patient, and 1 heterozygous substitution in the 5UTR of *DCTN1* in 1 definite patient, were identified. The remaining novel heterozygous substitutions included 4 synonymous substitutions in *ALS2* , *DCTN1* , *RNF19* , and *VEGF* . [Conclusion] The present study revealed two causative mutations in 6% of the sporadic ALS patients (2/33), suggesting that a portion of sporadic ALS patients have mutations in the causative genes with reduced penetrance or de novo mutations.

The role of NF-B in TNF-regulated transcription of the human COMT in astrocytes. *I. Tchivileva[1], R. Sitcheran[2], A.S. Baldwin[2], W. Maixner[1], L. Diatchenko[1]* 1) Ctr for Neurosensory Disorders, University of North Carolina at Cahpel Hill, Chapel Hill, NC; 2) Lineberger Comprehensive Cancer Ctr, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Catechol-O-methyltransferase (COMT) plays a crucial role in the metabolism of catecholamines. Reduction in COMT activity resulted in increased pain sensitivity in animal models. The main sources of the enzyme in the nervous system are neurons and astrocytes, and astrocytes are activated under conditions associated with enhanced pain responses. In this research, we hypothesized that regulation of COMT in astrocytes could contribute to development of inflammatory and neuropathic pain. We cloned a distal promoter of human COMT (P2-COMT) which is the only one active in the brain into a luciferase reporter vector and transiently transfected the construct into H4 astroglioma cells. We showed that proinflammatory cytokine tumor necrosis factor (TNF) down-regulated the activity of P2-COMT construct. Real-time RT-PCR and Western blot analyses of cell lysates confirmed decreased level of endogenous COMT after TNF treatment at both mRNA and protein levels. We then proposed that this TNF-dependent regulation of COMT expression was mediated by activation of nuclear factor B (NF-B), the pivotal regulator of inflammation and one of the major targets of TNF. In support of this hypothesis, cotransfection of P2-COMT reporter construct with either p65 NF-B subunit, or IKK subunit expression constructs, led to strong repression of luciferase activity. Furthermore, selective NF-B inhibitor PDTC blocked TNF-mediated down-regulation of the P2-COMT construct. Functional deletion analyses of P2-COMT showed that this TNF-dependent effect on COMT expression was mediated by a 155 base pair fragment in the promoter region of COMT that contained the putative EB binding site. Finally, TNF-dependent suppression of endogenous COMT expression and P2-COMT vector activity was abrogated in H4 cells stably expressing IB super-repressor. Collectively, these data strongly suggest that COMT is down-regulated under inflammatory conditions through the NF-B pathway and NF-B activation can lead to persistent pain states.

Dysregulation of Alternative Splicing of Coagulation Factor V Results in Bleeding Disorder, East Texas Type.

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Proper control of alternative splicing is speculated to contribute more to human disease than currently recognized. We identified a large family (16 affected) from East Texas with autosomal dominant inheritance of a novel bleeding disorder characterized by easy bruising, excessive bleeding with trauma/surgery requiring transfusions, and menorrhagia in affected women. Laboratory studies demonstrate prolonged PT and/or aPTT and normal activities/levels of all coagulation factors. Linkage analysis maps the defective gene to 1q23-24 (LOD 7.33), which contains coagulation factor V (FV). An alteration(A2440G) in FV in exon 13 segregates with disease and was not present in 62 controls. Exon 13 of FV is highly polymorphic and non-functional with respect to procoagulant function of FV. Interestingly, this alteration falls on the 5 splice site(TA/GT) of a splicing variant confirmed by RT-PCR of control liver and leukocytes. Furthermore, immunoblot analysis demonstrates that this splice variant produces a low abundance, 250kD isoform of FV in control plasma. The A2440G alteration forms a higher efficiency consensus site for splicing(TG/GT). Semi-quantitative RT-PCR confirms this improved efficiency with an increase of splicing seen in patients RNA from leukocytes(n=3) versus controls(n=3). This translates into a 20-fold increase in the 250kD isoform of FV seen in patients plasma(n=6) versus conrols(n=5). Precipitation of Vit-K dependent coagulation factors suggests that this isoform interacts with known FV-interacting proteins. Plasma mixing studies indicate dose-dependent correction, thus suggesting a dominant negative mechanism of impeding coagulation. **These data indicate that A2440G up-regulates an alternatively spliced transcript of FV, and increases a FV isoform that hinders coagulation as opposed to promoting it like its wildtype counterpart.** As verified by this unique mutation, alternative splicing is an important physiological event that can result in disease if its delicate balance is disrupted.

Molecular Diagnostics in Dual Site Ovarian and Endometrial Cancers. *S.J. Ramus[1], K. Elmasry[1], J. Whittaker[2], Z. Luo[3], A. Gammerman[3], N. Singh[4], W.G. McCluggage[5], A. Ayhan[6], K. Lu[7], I.J. Jacobs[1], S.A. Gayther[1]* 1) Dept Gynaecological Oncology, University College London, UK; 2) London School of Hygiene & Tropical Medicine, UK; 3) Royal Holloway, University of London, UK; 4) St.Bartholomew`s and The Royal London, UK; 5) Royal Victoria Hospital, Belfast, UK; 6) Seirei Mikatahara Hospital, Japan; 7) MD Anderson Cancer Center, USA.

Five to eight percent of patients undergoing laparotomy for suspected ovarian malignancies are found to have synchronous tumours of the ovary and endometrium. Patients with dual primary (DP) tumours have a better prognosis than a single primary tumour with metastasis (SPWM). The benefits of a correct diagnosis include the individualisation of patient management, the avoidance of unnecessary treatment and a more accurate prognosis. Currently, the distinction between DP and SPWM involves pathological interpretation and there is often uncertainty when the tumours have the same histological subtype. We have established a collection of 90 synchronous ovarian and endometrial tumours, in which tumour pairs have the same histological subtype. We have developed a molecular genetic test to distinguish between DP and SPWM cases, based on the analysis of microsatellite markers for allelic imbalance and microsatellite instability at 22 microsatellite markers. We compared genetic data with working pathology diagnoses and detailed histopathology review. Of the 39 patients previously diagnosed as DP cases, 31 had the same results for pathology and genetic tests. A further 7 cases were not informative and one case was given a diagnosis of SPWM. Of 25 patients diagnosed as SPWM by pathology, genetic tests identified 17 as SPWM and 3 as not informative. Significantly, 5 cases previously thought to be SPWM by pathology were diagnosed as DP by genetics suggesting these cases may have had more rigorous adjuvant chemotherapy than necessary. There were 26 patients in this study for whom a diagnosis by pathology was uncertain. Genetics analyses diagnosed 20 of these cases as DP, 4 as SPWM and 2 were uninformative. These data indicate that genetic methods can be used as powerful tools to complement existing histopathology for the diagnosis of synchronous cancers.

Functional and genetic analysis of hnRNPA1 as modifier gene in spinal muscular atrophy (SMA) discordant siblings. *A. Vielle-Canonge[1], I. Bagni[1], L. Vallo[1], L. Alias[2], E. Also[2], E. Tizzano[2], M. Bertoli[1], G. Novelli[1], P. Spitalieri[1], E. Bonifazi[1], A. Botta[1]* 1) Department of Biopathology and, University of Roma Tor Vergata, Rome, Italy; 2) Department of Genetics and Research Institute, Hospital Sant Pau, Barcelona, Spain.

Spinal muscular atrophy (SMA), the second most common autosomal recessive disorder, is caused by the absence of or mutations in the Survival Motor Neuron 1 (SMN1) gene, which encodes an essential protein. A nearly identical copy of the gene, SMN2, fails to compensate for the loss of SMN1 because exon 7 is alternatively spliced, producing a truncated, unstable protein. SMN1 and SMN2 differ by a critical C>T substitution at position 6 of exon 7 in SMN2, that causes skipping of this exon producing only low levels of functional SMN protein. The C>T transition creates an exonic splicing silencer (ESS) in SMN2 and functions as a binding site for a known repressor protein, hnRNPA1. The reduction of hnRNPA1 mRNA and protein levels in minigenes assays showed an efficient SMN2 exon 7 inclusion, making this gene an attractive modifying factor in SMA discordant families. We have first studied the effect of hnRNPA1 depletion in culture of CVS derived from SMA patients using synthetic siRNAs. Analysis conducted 48h after lipofection, showed a significant increase of the SMN protein levels and GEMS numbers in nuclei of SMA patients cells, compared to untreated controls. We have therefore analyzed the sequence of the hnRNPA1 gene and of its binding site on the SMN2 gene, in 10 SMA families with sibling showing identical 5q13 haplotypes and a variable clinical phenotypes. DHPLC/SSCP and sequencing analysis has been used to screen the hnRNPA1 gene and the SMN2 exon 7 in SMA discordant siblings. We were able to identify 3 nucleotides differences (c.17-57G>A, c.913+33C>T, c.1066-62C>T) in 3 exons of the hnRNPA1 gene, which have no association to the SMA clinical outcome. No sequence variations was observed in the sequence of the SMN2 genes where the hnRNPA1 protein binds. Our findings suggest that the hnRNPA1 gene, although playing a role in SMN2 exon 7 skipping, does not directly acts as modifier gene of the SMA phenotypes (FIS05-2416).

Allele Frequencies of CYP2C9, CYP2C19 and CYP2D6 in the Ashkenazi Jewish Population. *S.A. Scott, L. Edelmann, R. Kornreich, R.J. Desnick* Department of Human Genetics, Mount Sinai School of Medicine, New York, NY, 10029.

The polymorphic cytochrome P450 (CYP) isoenzymes are involved in the oxidative metabolism of a number of commonly used drug classes and xenobiotics. Their polymorphisms result in marked individual and ethnic variability in the metabolism and disposition of these drugs and are partly responsible for differences in clinical responses to some drugs. We determined the allele frequencies of 29 major CYP2C9, CYP2C19, and CYP2D6 polymorphisms in 250 anonymous, unrelated Ashkenazi Jewish (AJ) individuals from the greater New York metropolitan area. Genotyping of CYP2C9, CYP2C19, and CYP2D6 was performed using Tag-It Mutation Detection Kits (Tm Bioscience) that simultaneously detect nucleotide variants in a multiplex format. Genotype frequencies for each CYP were in Hardy-Weinberg equilibrium. CYP2C9 and CYP2C19 have extensive, intermediate, and poor metabolizer genotypes. In the AJ population there were 60.4% (151) extensive, 33.2% (83) intermediate, and 6.4% (16) poor CYP2C9 metabolizers, and 69.2% (173) extensive, 27.6% (69) intermediate, and 3.2% (8) poor CYP2C19 metabolizers. Similarly, CYP2D6 has ultrarapid, extensive, intermediate, and poor metabolizer genotypes and 12.0% (30) ultrarapid, 78.8% (197) extensive, 4.0% (10) intermediate, and 5.2% (13) poor CYP2D6 metabolizers were identified. CYP2C9 and CYP2C19 allele and genotype frequencies in the AJ subjects were comparable to those in other Caucasian populations and distinct from those previously reported in African and Asian populations. In contrast, the ultrarapid metabolizer frequency in the AJ population (12.0%), which results from a functional CYP2D6 duplication, was 2-3 times higher than that observed in most Caucasians (4-5%). Given the individual, ethnic, racial, and demographic variability in CYP metabolism status, these results support the incorporation of CYP2C9, CYP2C19, and CYP2D6 genotype information into individualized pharmacologic dose recommendations. Moreover, the higher frequency of the ultrarapid metabolizer genotype suggests that AJ individuals should be screened to identify those at risk for adverse drug reactions.

Constriction of the Region for Familial Combined Hyperlipidemia on 11p. *C.L. Plaisier[1], C.J. van der Kallen[2], T.W. de Bruin[3], P. Pajukanta[1]* 1) Dept Human Genetics, Univ California, Los Angeles, Los Angeles, CA; 2) Department of Medicine, CARIM, Academic Hospital, Maastricht, The Netherlands; 3) GlaxoSmithKline, Translational Medicine and Genetics, Research Triangle Park, North Carolina, USA.

Patients with Familial Combined Hyperlipidemia have high levels of serum total cholesterol (TC) and triglycerides (TGs). FCHL is a complex heterogeneous disorder that predisposes these patients to coronary heart disease. The genome-wide linkage scan of Dutch FCHL families identified a locus for FCHL on chromosome 11p with a multipoint LOD score of 2.6. No LOD scores > 3.0 were obtained for this Dutch genome scan, making it unlikely that a single major gene would predispose to FCHL in the Dutch FCHL families. The chromosome 11 finding was replicated in British FCHL families with a LOD score of 2.7 for TGs. This region has also been linked to TC, low-density lipoprotein cholesterol, as well as to obesity and glucose-related traits in multiple studies. In order to fine map the 40cM LOD -1 region defined by the overlapping Dutch and British peaks, we genotyped an additional 33 microsatellites in Dutch FCHL families bringing the marker density down to 1 marker per 2cM. SimWalk parametric multipoint linkage analysis of the microsatellites restricted the LOD -1 to a 9-cM region on 11p between markers D11S1761 and D11S1780. Importantly, this fine mapping approach reduced the number of candidate genes from 745 to 31 known genes. Our future plan is to use the CEPH HapMap data for these 31 genes to select tagging SNPs for association testing in Dutch FCHL families and other Caucasian combined hyperlipidemia study samples for replication.

BBS proteins are necessary for proper function of the regulatory center of the centrosome and spindle pole and for regulation of -tubulin levels. *J. Wei[1], H.J. Yen[1], A. Fedler[3], Q. Qian[2], C. Searby[1], M. Andrews[1], D.Y. Nishimura[2], S. Patil[2], V.C. Sheffield[1,2]* 1) Howard Hughes Medical Institute, Iowa City, IA; 2) Dept of Pediatrics, Univ of Iowa, Iowa City, IA; 3) Univ of Iowa, Iowa City, IA.

Bardet-biedl syndrome (BBS) is a pleiotropic genetically heterogeneous disorder characterized by obesity, retinopathy, polydactyly, renal malformations, learning disabilities, and hypogenitalism. To date, 11 BBS genes have been identified. Some BBS proteins have been reported to localize to the centrosome. A knockin mouse model of BBS1 (*Bbs1*$^{M390R/M390R}$) and knockout mouse models of BBS2 (*Bbs2*$^{-/-}$), BBS4 (*Bbs4*$^{-/-}$) and BBS6 (*Bbs6*$^{-/-}$) have been developed in our laboratory. These mouse models have features of human BBS. In this study, we utilize cultured mouse renal cells to demonstrate that BBS2 is a microtubule-associated protein and concentrated at the converging sites of microtubules during interphase and at the spindle poles during mitosis. In cultured *Bbs2*$^{-/-}$, *Bbs4*$^{-/-}$, and *Bbs1*$^{M390R/M390R}$ renal metaphase cells, we observed that -tubulin is aberrantly associated with spindle microtubules (MTs). Moreover, these mutant renal mitotic cells have aberrant asters that are associated with misalignment of chromosomes. Further investigation of these phenomena revealed that absence of BBS2, or BBS4, as well as the M390R BBS1 mutation, leads to the excessive accumulation of -tubulin. The cause of excessive accumulation of -tubulin was investigated using a MT depolymerization and repolymerization assay. Abnormal regulation of the level of -tubulin was observed in cultured mutant renal cells. In addition, we uncovered the existence of an induced proteolytic pathway that is responsible for the rapid degradation of -tubulin during MT reassembly after the depolymerization of MTs by nocodazole treatment. We propose that BBS proteins are necessary for assembly, maintainence, and disassembly of the regulatory center in the centrosome and spindle poles for rapid regulation of the level of -tubulin. Failure of regulation of level of -tubulin leads to chromosomal misalignment and aneuploidy during mitosis.

A progress report on suspension FISH combined flow cytometry applied to detection of chromosomal abnormalities. *X. Wu[1,2], Z. Chen[3], J.N. Lucas[1]* 1) ChromoTrax, Inc, Frederick, MD, USA; 2) Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China; 3) Cytogenetics Laboratory, Division of Medical Genetics, Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, UT, USA.

The broad goal of this research is to develop a novel approach of hybridizing chromosomes in suspension with fluorescently-labeled DNA probes, for flow cytometric analysis in order to sensitively, precisely and rapidly quantify chromosomal abnormalities. In this work we have established interphase suspension FISH. We demonstrated the efficacy of our approach by detecting aneuploidy in trisomy 21 human cells with a DNA probe specific for chromosome 21q. After hybridization in suspension, the majority of interphase cells showed distinct signals. In both the normal and patient groups, the signal distribution patterns between FISH in suspension and on slides were very similar. Recently, we applied suspension FISH to interphase lymphocytes combined with flow cytometry to detect trisomy cells from normal cells. Our results showed a peak shift to the right for trisomy cells relative to that for normal cells, consistent with expected fluorescence density increase for trisomy. Finally, we developed a standard protocol for hybridization of chromosomes in suspension. Using the probes 1q21-SO and 3q26-SO, we observed bright, consistent signals after suspension FISH. Currently, we are applying metaphase suspension FISH combined with flow cytometry for detection of metaphase genome abnormalities.

Plasma Adiponectin is associated with Ghrelin receptor in African-Americans. *X. Zhu[1], H.N. Lyon[2,3], T. Bersaglieri[2,3], M. Egyud[4], D. Kan[1], R.S. Cooper[1], J.N. Hirschhorn[2,3]* 1) Preventive Med & Epidemiology, Loyola Univ Medical Ctr, Maywood, IL; 2) Divisions of Genetics and Endocrinology, Childrens Hospital Boston, Massachusetts and Department of Genetics, Harvard Medical School, Boston, Massachusetts; 3) Broad Institute of Harvard and MIT, Cambridge, Massachusetts; 4) Department of Biology, Boston College.

We previously performed linkage analysis and identified strong linkage evidence of 3q27 to obesity in African-Americans. We are now testing SNPs in 3q27 for a role in obesity and on hormone levels, with a particular focus on growth hormone secretagogue receptor (GHSR, Ghrelin receptor) as the best candidate gene in the region. GHSR has been considered to play an important role in signaling energy insufficiency, and was expected to have a significant effect on feeding behavior and body fat deposition. Plasma adiponectin is inversely correlated with body mass index (BMI). We genotyped 28 SNPs located in GHSR in 606 individuals from 170 African-American families. These SNPs were chosen to capture most of common variation in GHSR (77% in HapMap CEU sample and 56% in YRI data). Linkage disequilibrium reveals this gene consists of four 2-SNP haplotype blocks, with other weak correlated SNPs. We tested the association of the four blocks and the weak correlated SNPs with the residual of log transformation of plasma adiponectin level using family based association test (FBAT) and identified a two SNP haplotype that is significantly associated with plasma adiponectin ($p=0.000008$). Although BMI itself was not significantly associated with the identified haplotype, the association evidence was reduced by adjusting for BMI ($p=0.001$). This is the first study that demonstrates variation in GHSR associated with plasma adiponectin modified by obesity. However, further independent studies are needed to confirm the association.

Development of therapeutic siRNAs for treatment of the skin disorder pachyonychia congenita. *F.J.D Smith[1], R. P. Hickerson[2], H. Liao[1], J. Sayers[1], S. A. Leachman[3], D. Leake[4], R. L. Kaspar[2], W.H.I McLean[1]* 1) Human Genetics Unit, University of Dundee, Dundee, Tayside, United Kingdom; 2) Transderm, Santa Cruz, CA, USA; 3) Department of Dermatology, Univeristy of Utah, Salt Lake City, UT, USA; 4) Dharmacon Inc., Lafayette, CO, USA.

Pachyonychia congenita (PC) is a rare autosomal dominant keratin disorder characterised by hypertrophic nail dystrophy, palmoplantar keratoderma, and oral leukokeratosis. PC is divided into two main types; PC-1 is caused by mutations in keratin K6a or K16 and PC-2 by mutations in K6b or K17. Here, we report 8 recurrent and 2 novel mutations in K6a, and present preliminary studies for a therapeutic approach. Currently there is no treatment for PC and the most painful aspect of the disorder is the plantar keratoderma which can result in wheelchair use. RNAi offers a novel approach for treating genetic disorders such as PC. We have designed four siRNAs against the unique 3UTR region of K6a to target K6a in patients with mutations in this gene. We predict that treatment with K6a siRNA resulting in reduction/knockout of both K6a alleles (mutant and wildtype) could alleviate the symptoms of PC at treated sites. We propose that loss of K6a would be compensated for by other keratins, such as K6b, as demonstrated by transgenic mouse models. Here, in a tissue culture model, we were able to knockdown endogenous K6a in the HaCaT keratinocyte cell line by transient transfection of the four independent K6a siRNAs, resulting in almost 100% reduction of K6a as visualised on protein gels and by immunoblotting. Other keratins present in HaCaT cells were unaffected. These siRNAs were shown to be specific for K6a and not for K6b in a cell culture model. Previously, we have shown that specific siRNAs (but not non-specific controls) strongly inhibit reporter gene expression in vivo (intradermal injection into mouse footpads). These 3UTR K6a siRNAs are being tested in similar animal studies.

Whole genome SNP linkage screen for successful aging loci in the Amish. *W.K. Scott[1], P.J. Gallins[1], H.J. Munger[1], J.L. McCauley[2], L. Jiang[2], P.C. Gaskell[1], A.E. Crunk[2], M. Creason[1], L. Caywood[1], D. Fuzzell[2], C. Knebusch[2], M.C. Morey[1], E.R. Hauser[1], C.E. Jackson[3], J.R. Gilbert[1], M. A. Pericak-Vance[1]* 1) Duke University, Durham, NC; 2) Vanderbilt University, Nashville, TN; 3) Scott & White, Temple, TX.

Successful aging (SA) involves avoiding disease and disability, maintaining cognitive and physical function, and being socially engaged throughout the lifespan. Several components of SA have demonstrated heritability in different samples: longevity, upper extremity strength, lower extremity function, and retention of cognitive ability. The oldest Amish communities of Indiana and Ohio were founded in the mid-1800s by few individuals and remain socially and genetically isolated. Isolation and a relatively homogeneous environment make the Amish a suitable population for identifying complex trait loci. We surveyed cognitively intact Amish age 80 and over, collecting DNA, subjective and objective measures of function, cognition, life satisfaction, and social support. Over 150 individuals have been enrolled in the study and 107 were included in a whole-genome SNP linkage screen (Illumina Linkage Panel IVb) of 460 individuals clustered in 13 kindreds of closely related members. Nine kindreds had two or more of the 43 adults meeting our criteria for SA (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 on a lower-extremity physical function battery, and having adequate social support). 5,902 SNPs were analyzed for linkage to SA using SUPERLINK and affecteds-only dominant (q=0.01) and recessive (q=0.3) models. SNPs on 6 chromosomes generated 2-point lod scores meeting criteria for suggestive linkage (lod > 1.5) under the dominant model: 1q24, 1q32, 4q28, 15q23, 16p12, 17q21, 17q25, and 19q13. The region on chromosome 4q28 is within 20 Mb of the region previously linked to longevity, the 19q13 region is 6 Mb from APOE, and 1q32 contains several genes related to musculoskeletal function. These results suggest that several regions of the genome might harbor loci that influence successful aging in the Amish.

Relationship of peroxisome proliferation-activated receptor-gamma C161-T gene polymorphism with coronary artery disease in Han Race Chinese. *Jing. Wan[1], Jianghu. Ren[2], Yexin. Ma[1], Xin. Tu[1], Maoyin. Cao[2], shixi. Xiong[2]* 1) Cardiology department, Tongji Hospital of Huazhong Science and Technology University, Wuhan city, Hubei Province, China; 2) Zhongnan Hospital of Wuhan university, Wuhan City.

Objective: To investigate the relationship between peroxisome proliferation-activated receptor-gamma (PPAR) C161T gene polymorphism and coronary artery disease (CAD). Methods: 292 subjects were investigated in this study, including 89 healthy persons, 203 cases diagnosed as CAD. PPARC161T gene polymorphism was determined by polymerase chain reaction and restriction fragment length polymorphisms, the blood glucose and the blood lipoprotein were detected. The coronary artery lesions were detected by coronary angiography and analysed quantitatively by Gensini score method. The risk factors of CAD were estimated, and the frequencies of PPARC161T genotypes and the T allele in the CAD and healthy groups were observed. Results: 1, In healthy group, T allele frequency was 0.213, and in CAD group T allele frequency was 0.192. There was no significant difference between the two groups. 2, There was a significant association between PPARC161T genotypes and the number of significantly disease vessels. T allele carriers were far more frequent in patients without than those with significantly diseased vessels ($P<0.05$), and the CAD risk in the T allele carries (OR: 0.56, 95%CI: 0.24-0.63) was much lower than that in the CC homozygote (OR: 1.92, 95%CI: 1.09 -2.54). The results showed that Gensini score in patients with CC genotype was markedly higher than that in patients with Tallele ($p<0.05$). 3, apoB was obviously higher in patients with CC homozygote than those with T allele carriers (1.020.22 and 0.940.23, $P<0.05$).Conclusion: In the Han race Chinese, the distributing trend of PPARC161T gene polymorphism in the healthy group is as the same as that in the patients with CAD group; The Gensini score of the coronary artery angiography in persons with CC genotype are significantly higher than that in the persons with T allele. It means that there is a lower risk of CAD in the patients with T allele.

Progressive CNS lesions in (GCase) null with skin rescued mice and conduritol B epoxide injected Gaucher mice.

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Gaucher disease types 2 and 3 are neuronopathic, life-threatening disease variants. We developed neuronopathic mouse models, to facilitate understanding of the molecular mechanism of progressive primary CNS involvement. Since complete knock-outs are lethal in mice, a conditional reactivation model, kn-9h, was developed in which a low activity enzyme (<3% wt activity) was reactivated in skin from a neo-containing D409H allele that was null. The floxed neomycin marker in the neo-D409H allele was excised in keratinocytes using Cre driven by the K14 promoter. All other tissues/cells retained the neo-D409H, i.e., null alleles. kn-9h mice survived for 14 days, but developed progressive lethal CNS disease by 10 days. To mimic this phenotype, CBE, a covalent inhibitor of GCase, was injected daily into wild-type or D409V homozygotes. The kn-9h CNS phenotype was recapitulated in either mouse model with seizures, tail arching, shaking, tremor, and quadra-paresis, with death by 14 days. Histologically, the mouse models had storage cells and glucosylceramide in multiple tissues and extensive CNS neuronal degeneration. Neuronal loss and apoptotic neurons were in spinal cord and brain. Importantly, progressive neuronal degeneration continued for >2 months in D409V homozygotes, but not in wild-type mice after discontinuations of CBE injections. Untreated D409V homozygotes do not spontaneously develop CNS disease or histologic lesions. The CNS signs and pathology in these mouse models mimic Gaucher disease types 2 or 3. These results indicate that CNS deterioration can be progressive even after the initiating insults were stopped and have significant implications for therapy of CNS lesions in Gaucher disease.

Discordance between interphase FISH and cytogenetic results of CVS. *G.S. Sekhon, J.B. Ravnan, E.N. McDonald, D.M. Stenberg, A.N. Lamb* Dept Cytogenetics, Genzyme, Santa Fe, NM.87505.

Interphase fluorescence in situ hybridization (FISH) testing has become a commonly used method of screening direct preparations of prenatal specimens for the common aneuploidies involving chromosomes 13, 18, 21, X, and Y, followed by complete cytogenetic analysis on cultured cells. Because the interphase FISH testing is done on direct preparations of the CVS, there is the potential for discordant results with the chromosome results from cultured preparations, just as discordant results are found between direct cytogenetic preparations and the cultured results. Four cases are presented with discrepant results between the FISH from a direct CVS preparation and cytogenetic analysis on cultured cells out of a total of 2743 FISH tests performed. In all four cases, the initial FISH results were confirmed by repeating the FISH testing on a second slide from the same direct preparation. In two of the four cases, additional FISH testing of the separated layers of the chorionic villus showed that results from the outer layer of the villi (cytotrophoblast) were consistent with the initial direct FISH results, while results from the inner portion of the villi (mesodermal core) were consistent with the cultured cytogenetic results. These findings are consistent with confined placental mosaicism as an explanation for the discordant results. Discordant results between direct and cultured CVS preparations are found in approximately 0.5 % of chromosome analyses performed on both tissue types. Our four cases with discordant results represent 0.1 % of the interphase FISH tests performed on CVS specimens. The lower rate of discordance of interphase FISH is most likely due to the limited nature of the abnormalities that can be detected by FISH in comparison with a full chromosome analysis. Interphase FISH has its limitations in prenatal diagnosis of CVS but in conjunction with chromosomes it can provide useful information. Although FISH can provide preliminary information, caution is necessary when rapid analysis is performed on one layer and chromosomes on the other, just as with conventional cytogenetics on direct and culture preparations in CVS.

Long-term renal stabilization and sustained GL-3 clearance in Fabry patients treated with agalsidase beta. *S. Waldek[1], D. Germain[2], R. Abichandani[3], M. Banikazemi[4], P. Lee[5], W. Wilcox[6], N. Guffon[7]* 1) Hope Hospital, Manchester, United Kingdom; 2) Hôpital Européen Georges Pompidou, Paris, France; 3) Genzyme Corporation, Cambridge, MA; 4) Mount Sinai School of Medicine, New York, NY; 5) National Hospital for Neurology and Neurosurgery, London, UK; 6) Cedars-Sinai Medical Center, Los Angeles, CA; 7) Hôpital Edouard Herriot, Lyon, France.

BACKGROUND: Fabry disease, an X-linked lysosomal storage disorder caused by deficient alpha-galactosidase A (-GalA), results in progressive lysosomal accumulation of globotriaosylceramide (GL-3) and ultimately premature death from renal, cardiac, and cerebrovascular manifestations. This study was conducted to determine long-term safety and efficacy of treatment with recombinant human (rh) -GalA (agalsidase beta) in patients (pts) with Fabry disease. METHODS: This was a multicenter, open-label, phase III extension study of 58 pts with Fabry disease who completed a 20-week, double-blind phase III study and transitioned to an extension study to receive biweekly 1 mg/kg rh--GalA infusions for up to 54 months. GL-3 accumulation was evaluated in capillary endothelium of the skin, kidney and heart. Renal function was also assessed. RESULTS: After 54 months of agalsidase beta therapy, continued, (near) complete clearance of the capillary endothelium of the skin was demonstrated. In those pts (N=8) with kidney biopsies similar GL-3 clearance in renal capillary endothelial cells was shown, and clearance was also observed in other renal cell types. Mean plasma GL-3 levels were quickly normalized, and remained in the normal range at Month 54 (N=44). Median serum creatinine and estimated GFR remained stable (normal) at Month 54 (N=41). Six pts had renal disease progression; 4/6 were over 40 years and 5/6 had significant proteinuria and/or >50% glomerular sclerosis at baseline. Infusion-associated reaction was the most common drug-related AE. CONCLUSIONS: Long-term rh--GalA therapy in pts with Fabry disease results in sustained clearance of capillary endothelial cells, multiple renal cell types, and reduction of GL-3 in the plasma,. Long-term rh--GalA therapy stabilized renal function in patients without advanced renal disease at baseline.

Epidemiology of Congenital Malformations in Scotland. *A.D. Rasalam[1], J.C. Dean[1], D. Clark[2], R. Dobbie[2], D. Tennyson[2]* 1) Department of Medical Genetics, Aberdeen, Scotland; 2) Information Services Division, Edinburgh.

Most large prospective or population based studies have suggested a malformation rate in children exposed to Anti-Epileptic Drugs (AEDs) before birth of around 6-9%, compared with a background risk of 3%. In our previous population based study in Aberdeen, the malformation rate was 14% in those exposed to AEDs, and 5% in unexposed siblings. We suggested this higher rate might be due to the longer follow-up period in our study. A recent register based study reported a substantially lower malformation rate of 2.2 - 6% for exposed children, but used a different definition (Eurocat) of major malformation. In Scotland, all malformations collated from routine administrative data are notified to the Information Services Division (ISD) at birth and during subsequent hospital admissions. Maternal illnesses are similarly coded and notified. We undertook a records linkage study using the ISD Scottish Linked Congenital Anomalies Register to determine malformation rates in all singleton births (565731) in Scotland between 1992 and 2001, according to whether mothers had epilepsy, bipolar disorder (BPD) or neither of these. 2001 was chosen as the latest year of birth in the study, so that at least 5 years follow-up data would be available for the children. Disease coding used ICD9 or ICD10 definitions. Malformations were diagnosed in 12.3% of all deliveries (6.6% by 28 days of age), in 14.6%; of children of mothers with epilepsy (7.6% by 28 days) and 12.2%; of children of mothers with BPD (6.6% by 28 days). When Eurocat definitions were used, the background rate was 5.9% (4.3% by 28 days), the rate for children of mothers with epilepsy was 7.2% (5.1% by 28 days) and 6.6% (4.9% by 28 days) for children of mothers with BPD. These data show that the background malformation rate is higher than expected, Eurocat definitions underestimate malformation rate, many malformations are diagnosed after 28 days of age, and that maternal epilepsy is associated with significantly increased malformation rates compared with background and with maternal BPD. Part of this additional risk may be due to teratogenic effects of AEDs.

A second MHC susceptibility locus for multiple sclerosis. *S.J. Sawcer for the International Multiple Sclerosis Genetics Consortium (IMSGC)* Clinical Neurosciences, University of Cambridge, Cambridge, United Kingdom.

Variation in the Major Histocompatibility Complex (MHC) on chromosome 6p21 influences susceptibility to multiple sclerosis, with the DR15 haplotype (HLA-A3-B7-Cw7-DR15) being the most consistently identified risk factor. The high gene content, extreme polymorphism and extensive linkage disequilibrium (LD) that characterise the MHC have confounded efforts to resolve the nature of this association. The strongest effect originates in the class II region, with the DRB1 gene identified as the primary susceptibility locus. The possibility that other genes in the MHC independently influence susceptibility to multiple sclerosis has been suggested but remains unconfirmed. Here we show that the class I Human Leukocyte Antigen (HLA) gene, HLA-C, exerts an independent effect on susceptibility to multiple sclerosis. After excluding effects attributable to the DR15 haplotype we found residual evidence for association, maximal in the region of HLA-C, in independent simple nuclear family cohorts from the US (n=450) and UK (n=480). By extending analysis of the classical loci into 721 additional sporadic cases, and utilizing data from 3660 controls, we refined this secondary signal and show that it is partly due to allelic heterogeneity at the HLA-DRB1 locus, and partly due to an independent effect from the HLA-C locus ($p=5.9x10^{-5}$). Our results enhance understanding of the established DRB1 association in multiple sclerosis and identify a novel second susceptibility locus for this disease. The identification of HLA-C as a risk factor in multiple sclerosis implicates innate immune mechanism in particular the killer cell immunoglobulin-like receptors (KIRs). These results open up new opportunities for future research activities.

Association Study of Reading Disabilities and Genes in the Chromosome 15q21.3 region. *K. Wigg[1], J. Couto[1], Y. Feng[1], B. Anderson[2], T. Cate-Carter[2], R. Tannock[2], M. Lovett[2], T. Humphries[2], C.L. Barr[1,2]* 1) Cell & Molecular Div, Toronto Western Hosp, Toronto, ON, Canada; 2) Brain and Behaviour Programme, The Hospital for Sick Children, Toronto, ON, Canada.

Reading disabilities (RD) have been linked to a number of chromosomal regions including 15q. Recently a gene in the 15q region, EKN1, was identified via a translocation breakpoint in a family cosegregating with RD (Taipale et al, 2003). Several single nucleotide polymorphisms (SNPs) in this gene were found to be associated with reading disabilities in a small sample of families from Finland. In our sample of 148 families identified through a proband with reading difficulties, we found evidence for allelic association for one of these SNPS in EKN1 to the phenotype of RD identified as a categorical trait marker (chi-squared=5.586, 1 d.f., p=0.018) and to the reading component processes using quantitative analysis (Wigg et al., 2004). However, association was observed with different alleles and haplotypes than those reported to be associated in the Finnish sample. Several other follow up studies have not replicated the original findings (Meng et al, 2005, Marino et al, 2005) This indicates that the SNPs identified as associated are unlikely to be the functional DNA change contributing to reading disabilities. It may be in linkage disequilibrium with another variation, possibly in the regulatory region of EKN1 or in another gene in the region. We have expanded our study of the 15q region to include other possible candidate genes: Phosphatidylinositol glycan (PIGB), cell cycle progression 1 isoform 1 (CCPG1), pygopus homolog 1 (PYGO1), protogenin (PRTG) and neural precursor cell expressed (NEDD4). SNPs across these genes are being genotyped in our RD sample, and preliminary results show suggestive evidence of linkage in three of the SNPs genotyped ($p<0.09$). These results suggest that further studies of the genes in this region is warranted.

Noncompaction of the ventricular myocardium and hydrops fetalis in cobalamin C deficiency. *P. Tanpaiboon[1], P.F. Callahan[2], J. Sloan[1], D. Zand[3], U. Lichter-Konecki[3], C.P. Venditti[1]* 1) National Genome Research Institute, NIH, Bethesda, MD; 2) Child Cardiology Associates, Fairfax,VA; 3) Division of Genetics and Metabolism, Childrens National Medical Center, DC.

Noncompaction of the ventricular myocardium (NCV) is a rare congenital cardiomyopathy, believed to be caused by arrest of normal compaction process of endomyocardium. The phenotypes observed in affected individuals are highly variable ranging from asymptomatic to hydrops fetalis. Mutations in genes involving muscle structures and functions have been reported. Other than Barth syndrome, no inborn errors of metabolism associated with this condition have been described. We report a 2-year-old girl who presented with hydrops fetalis at gestational age 22 weeks. Fetal echocardiography revealed possible tricuspid atresia and poor biventricular function. The patient was born at 37 weeks. Neonatal echocardiography performed shortly after birth demonstrated noncompaction of the left ventricle and mild to moderate left ventricular dysfunction. At 3 days of age, metabolic evaluations revealed homocysteinemia and methylmalonic aciduria. Complementation studies assigned the patient to the cblC group and mutation analysis revealed a homozygous c.271dupA in the *MMACHC* gene. A repeat echocardiogram at 6 months of life showed improved left ventricular function with a low normal range ejection fraction. Ejection fraction at 30-month-old was sixty percent and fractional shortening was twenty seven percent. Cardiac function continued to stabilize and digoxin initiated in the first few days of life was discontinued at 18 months. This is the first report of cobalamin C deficiency associated with noncompaction of the ventricular myocardium. The findings seen in this patient expand the spectrum of prenatal and cardiac manifestations seen in this disorder, and raise the question of whether noncompaction of the ventricular myocardium will be seen in other cblC patients, especially those who have been described as having cardiomyopathy.

A simplified medium-throughput 48-plex SNP genotyping method. *L. Pawlikowska[1], C. Chu[1], Z. Jiang[2], P.-Y. Kwok[1]* 1) Ctr for Human Genetics and Cardiovascular Research Institute, University of California - San Francisco, San Francisco, CA; 2) Beckman Coulter, Fullerton, CA.

Current genotyping methods based on primer extension typically employ 6 sets of dye-labeled terminators to cover the 6 possible SNP allele combinations. For multiplex genotyping, the requirement that all SNPs in a panel have the same allele combination poses a design challenge for smaller projects with fewer than 400 SNPs, resulting in multiple incompletely filled panels and reduced efficiency. To overcome this limitation, we have explored the use of 2 fluorescent labels in a reagent set containing all 4 terminators (labeling 2 terminators with each dye). We have applied this strategy to the SNPstream 48plex genotyping system (Beckman Coulter), which uses multiplex PCR and single base primer extension chemistry on 384-well tag-array plates to genotype 48 SNPs simultaneously. The platform thus occupies a useful experimental space for mid-size (40-400 SNPs) genotyping projects, such as candidate gene studies and follow-up studies to larger scans. We have adapted the SNPstream 48plex to genotype mixed panels containing 4 SNP extension types using existing terminators labeled with two fluorescent dyes (TAMRA and Bodipy). Combining Bodipy-labeled A and T with TAMRA-labeled C and G allows for genotyping of CT, AG, GT and AC, i.e. all but the rarest (AT and CG), SNP allele combinations together. Alternatively, combining Bodipy-labeled C and T with TAMRA-labeled A and G permits genotyping of TG, CA, TA and CG SNPs.

We have genotyped 3 different mixed 48-plex SNP panels on a cohort of 95 human DNA samples with 81.3%3.6% average conversion rates and 96.4%1.3% average call rates. Genotyping accuracy was 100% using 5 duplicates included in the DNA panel, and 99.7%0.13% as compared to HapMap genotypes genotyped by singleplex primer extension. The mixed panels thus performed comparably to standard single-allele combination 48plex panels. We are implementing this streamlined medium-throughput genotyping method in several human candidate gene association and mouse mapping studies.

Detection of copy number changes in patients with mental retardation using high density SNP microarrays. *J. Wagenstaller[1], S. Spranger[2], B. Heye[5], B. Kazmierczak[2], M. Cohen[3], P. Freisinger[4], T. Meitinger[1,5], M. Speicher[1,5], T.M. Strom[1,5]* 1) Institute of Human Genetics, GSF National Research Center, Munich, Germany; 2) Praxis für Humangenetik, Bremen, Germany; 3) Kinderzentrum, Munich, Germany; 4) Children's Hospital, Technical University; Munich, Germany; 5) Institute of Human Genetics, Technical University; Munich, Germany.

Whole genome analysis using high-density SNP oligonucleotide arrays allows identification of yet unknown microdeletions, microduplications and uniparental disomies. We collected a cohort of 70 children and, when available, their parents with unexplained mental retardation with and without additional symptoms. High resolution banding analysis and metabolic investigations were inconspicuous. The genomic DNAs were analyzed using the Affymetrix GeneChip 100K array. Data analysis was performed with median normalization and genotype-specific dosage calculation using R-scripts and revealed 11 de novo copy number changes (CNCs), 8 deletions and 3 duplications. These CNCs varied in size from 500 kb to 10 Mb. Four of the CNCs were flanked by low-copy number repeats. A 1 Mb deletion in 17q21.3 has also been found deleted in other studies and encompasses a known inversion polymorphism of 900 kb. These deletions may define a new microdeletion syndrome. We also looked for known and new copy number variations (CNVs) in 100 healthy parents. Our data revealed 7 known and 68 new CNVs, most of them were unique. This supports the previous notion that at least the larger CNVs have a low population frequency. We compared the 100K Affymetrix platform with platforms that promise a higher resolution. The signal-to-noise ration (SNR) of the 500K Affymetrix array was considerable lower than the SNR of the 100K Affymetrix arrays and did not significantly increase the overall resolution. The SNR of the 300K Illumina arrays was somewhere in between. In summary, the resolution of copy number variation detection with 100K Affymetrix arrays is currently comparable with the resolution of BAC-array CGH. The increase of the number of features and the new design of feature localization should allow detection of single-gene deletions and known microdeletion syndromes.

A rapid and sensitive method for RNA integrity determination on capillary electrophoresis systems. *A.B. Shah[1], S. Karudapuram[1], L.K. Joe[1], M. Rhodes[1], Y. Lou[1], J. Keefe[1], J. Briggs[1], M. Wenz[1], C. Waldron[1], C. Carver[2], S. Bass[2]*
1) Applied Biosystems, Foster City, CA; 2) Gene Logic Inc., Gaithersburg, MD.

RNA quality is directly correlated to the success of various applications, such as microarray or real time qPCR-based gene expression analyses, cDNA library construction, Northern analyses, and RNAse protection assays, which utilize RNA samples from various organisms, tissues, cell lines, and precious biological samples. We present a novel method for examining RNA integrity using capillary electrophoresis that is more cost-effective and scalable than current standard methods. In addition, the sensitivity of the technique allows for use of less material and for the detection of impurities following RNA purification and degradation from nuclease contamination. Total RNA, derived from various tissues and cell lines, were stained with the dye SYBR-Green II and run through a custom polymer formulation on a capillary electrophoresis platform. Using downstream analysis software, we resolved RNA species and their relative quality based on parameters such as sizing, profile, peak area, and peak height. Our results highlight the potential for high-throughput capillary electrophoresis as a much more discriminatory and cost-saving method in evaluating RNA purity.

Discovery and association of gamma-glutyml carboxylase (GGCX) polymorphism with warfarin dosing. *M.J. Rieder* Genome Sciences, University of Washington, Seattle, WA.

Gamma-glutyml carboxylase (GGCX) is a component of the vitamin K cycle and carries out the carboxylation of protein glutamic acid (Glu) residues to gamma-carboxyglutamic acid (Gla), and results in the activation of several vitamin K dependent clotting factors (e.g. F2, F7, F9, F10). Given the critical role of GGCX in mediating this reaction, we studied the effect of GGCX polymorphisms that may influence the dosing requirements of patients stabilized on the anticoagulant warfarin. We performed complete resequencing in 16 kb encompassing GGCX in a discovery population of European-Americans (n=23), identifying 29 single nucleotide polymorphisms (SNPs) and indel polymorphisms. LD-Select was used to cluster these polymorphisms into six tagSNP bins ($r^2 = 0.65$). TagSNPs for GGCX were tested for an association with warfarin dosing in a well-studied clinical population (n = 186), using univariate, multivariate, and haplotype-based linear regressions. After adjusting for covariates such as other genetic (i.e. CYP2C9 and VKORC1) and patient factors (i.e. age, sex, amiodarone, losartan), all models revealed a single, noncoding tagSNP (GGCX-12970; C/G-0.11/0.89 allele frequency) associated with warfarin dose. No other polymorphisms in GGCX were found to be linkage disequilibrium with this tagSNP. Under the univariate, dominant effects model, homozygous carriers of the 12970-G allele had a higher warfarin dose requirement than 12970-C carriers (5.42.6 vs 4.62.3 mg/d). Full multivariate regression analysis showed that the GGCX-12970 polymorphism explained approximately 2% of the variance in warfarin dose (permutation p-value <0.02, dominant model). Haplotype analysis, used to infer and test for a warfarin dose association using a generalized linear model, revealed a single haplotype effect ($p < 0.03$, dominant model). This distinctive haplotype was the only carrier of the GGCX-12970/C allele. Both single SNP and haplotype associations suggest that GGCX-12970 contributes independently to the variability in warfarin dosing, and work is ongoing to understand the mechanism by which this SNP acts on this widely prescribed anticoagulant.

Variable functional impairment of RMRP mutations explain genotype - phenotype correlation in Cartilage hair hypoplasia and Anauxetic dysplasia. *C.T. Thiel[1], G. Mortier[2], I. Kaitila[3], A. Rauch[1]* 1) Institute of Human Genetics, University of Erlangen-Nuremberg, Erlangen, Germany; 2) Department of Medical Genetics, Ghent University Hospital, Ghent, Belgium; 3) Clinical Genetics Research, University of Helsinki, Helsinki, Finland.

Mutations in the RMRP gene have been demonstrated to cause a wide phenotypic spectrum from Anauxetic dysplasia (AD) and Cartilage hair hypoplasia (CHH) to Metaphyseal dysplasia without hypotrichosis (MDWH). The RMRP gene encodes the RNA subunit of the ribonucleoprotein endoribonuclease, RNase MRP. So far, 5 mutations causing Anauxetic dysplasia, at least 62 mutations causing CCH and MDWH, and 8 SNPs have been identified. We recently demonstrated that RMRP gene mutations affect growth regulation by altering cyclin dependent cell cycle regulation and impaired ribosomal assembly (Thiel et al. 2005 Am J Hum Genet). Mutations located between the TATA box and the transcription starting site are reducing the transcription level of the RNA subunit. To clarify the influence of different base substitutions within the transcript on the clinical phenotype, 13 CHH/MDWH and 2 novel AD mutations each in different functional domains of the RMRP gene were tested for rRNA and mRNA cleavage activity. Whereas the AD mutations g.14GA and c.256_265delCAGCGCGGCT revealed a lack of expression, all remaining mutations demonstrated a variable decrease in cleavage activity. The degree of short stature and skeletal dysplasia correlated with the overall functional impairment, but mainly of rRNA cleavage activity (ribosomal assembly). Whereas significantly diminished mRNA cleavage activity (cyclin dependent cell cycle regulation) was a prerequisite for immunodeficiency. Thus, the clinical phenotype emerges in most cases of the combined effect of either a hypomorphic allel, hyploinsufficiency, or the respective effect on the cleavage activity.

Linkage analysis identifies a novel locus for Restless Legs Syndrome on chromosome 2q in a South Tyrolean population isolate. *I. Pichler[1], F. Marroni[1], C. Beu Volpato[1], S. Pedrotti[1], J.F. Gusella[2], C. Klein[3], G. Casari[4], A. De Grandi[1], P.P. Pramstaller[1,3,5]* 1) Institute of Genetic Medicine, European Academy, Bolzano, Italy; 2) Molecular Neurogenetics Unit, Center for Human Genetic Research, Massachusetts General Hospital, Boston MA, USA; 3) Department of Neurology and Human Genetics, University of Lübeck, Germany; 4) Vita-Salute University and San Raffaele Scientific Institute, Milan, Italy; 5) Department of Neurology, General Regional Hospital, Bolzano, Italy.

Restless legs syndrome (RLS) is a common neurological condition with three loci (12q, 14q, 9p) described so far though none of these genes has yet been identified. Isolated populations provide a powerful approach for mapping genes associated with complex disease phenotypes due to reduced genetic heterogeneity and environmental noise. We systematically assessed a population isolate in South Tyrol (Italy) for the presence of RLS. A 4-cM genome-wide scan was performed, including all idiopathic RLS patients (n=37) who originated in a small isolated village. Using both nonparametric and parametric analyses, we initially obtained suggestive evidence for a novel RLS locus on chromosome 2q. Follow-up genotyping yielded significant evidence for linkage (NPL score = 5.5, P value = .0000033; HLOD score = 5.1; = 1.0). Model-based linkage analysis was performed under the assumption of an autosomal dominant mode of inheritance. Haplotype analysis revealed a shared disease-haplotype between two families (Fam.S01 and Fam.S05) defining a candidate region of 8.2 cM; in addition, the affected individual in Fam.S016 shared three linked alleles at neighboring markers, suggesting a reduced candidate interval of only 1.6 cM. The three families were shown to descend from a common founder couple living in the 18th century resulting in a ten-generation pedigree. Two-point linkage analysis of this pedigree further supported the novel RLS locus on chromosome 2q (LOD score = 4.1). These findings re-emphasize the genetic heterogeneity of the disorder and strongly support the identification of a novel locus for RLS on chromosome 2q. Sequence analysis of the region to identify the causative gene is in progress.

The proposed association of ovarian cancer with Birt-Hogg-Dube syndrome. *S. Randall Armel, A. Pintzov, M. Howe, B. Rosen* Familial Ovarian Cancer Clinic, Princess Margaret Hospital, Toronto, Ontario, Canada.

Birt-Hogg-Dube syndrome (BHD) is an autosomal dominant condition characterized by multiple neoplasms that exhibits variable expressivity both within and between families. To date, more than 60 affected families have been identified from various populations. The most common manifestations of BHD are skin abnormalities, specifically fibrofolliculomas, acrochordons and trichodiscomas, which first appear in the patients thirties or forties. Other common symptoms include the appearance of lung cysts, spontaneous pneumothorax, and renal tumors. Less common symptoms that have been reported include colonic polyps and hereditary medullary thyroid carcinoma (MTC). We present a case of a 46-year-old woman affected with BHD presenting with freckles, breast lumps beginning in her thirties, enlarged thyroid and ovarian cancer (malignant mixed mullerian tumor). Full genetic analysis of BRCA1/2 in this patient was negative; however, she was found to carry a 1285insC mutation in the FLCN gene, which codes for folliculin. This mutation occurs in a C8 tract in exon 11, where 44% of all BHD mutations occur. Family history was remarkable, with at least one confirmed diagnosis of BHD in the probands niece, who presented with a colon adenoma, multiple (>80) trichodiscomas, breast lumps and a spontaneously resolving ovarian cyst. Several first and second-degree relatives presented with various cancers, including thyroid, colon and melanoma. In the literature, there has been one additional report of an ovarian cyst that was considered unrelated to the diagnosis of BHD at the time. To date, there are no other reported associations of ovarian cancer with a mutation in the FLCN gene. These findings suggest that a patient with BHD may be at an increased risk for developing ovarian cancer above that of the general population. We propose that women diagnosed with BHD should be offered ovarian cancer screening, consisting of annual transvaginal ultrasounds and CA125 blood tests.

Absence of ataxin-3 causes transcriptomic de-regulation of the ubiquitin-proteasome pathway, signal transduction, transcription regulation, and cell-structure/motility genes in *C. elegans*. *A.J. Rodrigues[1], G. Coppola[2], C. Santos[3], M.C. Costa[1], M. Aillion[4], J. Sequeiros[3], D. Geschwind[2], P. Maciel[1]* 1) ICVS/ECS,Univ. of Minho,Braga,Portugal; 2) Neurogenetics,Dept of Neurology,UCLA,Los Angeles,USA; 3) UnIGENe,IBMC,Porto,Portugal; 4) Dept of Biology,Univ. of Utah,Salt Lake city,USA.

Machado-Joseph disease is the most common dominant spinocerebellar ataxia and is caused by a CAG expansion in the *ATXN3* gene which encodes for ataxin-3. Ataxin-3 has been proposed to i) act as a deubiquitinating (DUB) enzyme in the ubiquitin-proteasome pathway, ii) be involved in transcriptional repression and iii) be related to structural and motility-related proteins.

To gain further insight into the function of ataxin-3, we have identified the *Caenorhabditis elegans* orthologue of the *ATXN3* gene and characterized its pattern of expression, developmental regulation and subcellular localization of the respective protein. We also demonstrate that, analogous to its human orthologue, *C. elegans* ataxin-3 has DUB activity *in vitro* against polyubiquitin chains. Mutations in the putative catalytic cystein C20 inhibit DUB activity and the josephin domain alone was capable of cleaving the polyubiquitin chains. Mutations in the two ubiquitin-interacting motifs did not interfere with ataxin-3 DUB activity.

We also characterized the first known ko animal models for ataxin-3 and found that the two strains were viable and displayed no gross phenotype. However, a microarray analysis of gene expression in both ko strains revealed a significant de-regulation of core sets of genes involved in the ubiquitin-proteasome pathway (7.5%), structural/motility components (50%), signal transduction (20.4%) and transcription regulation (4%). Interestingly, of these 290 differentially expressed genes, 253 were upregulated and 37 were downregulated. This gene identification provides important clues that can help elucidate the specific biological role of ataxin-3 and unveils some of the physiological effects caused by its absence or diminished function.

Performance of putatively functional variant assays in drug metabolism genes across multiple control populations using the Applied Biosystems TaqMan DME panel. *R. Welch[1], K. Haque[1], T. Harkins[2], F. Hyland[2], K. Lazaruk[2], F.M. De La Vega[2], M. Yeager[1]* 1) Core Genotyping Facility, DCEG, NCI. SAIC-Frederick, Inc., NCI-Frederick, Frederick, Maryland; 2) Applied Biosystems, Foster City, CA, USA.

A number of candidate genes encode enzymes responsible for the metabolism of drugs and other xenobiotics. Genetic variation in these Drug Metabolism Enzyme (DME) genes accounts for some of the variability in the effect of drugs in humans, and may contribute to complex disease. The CGF has genotyped N=2394 individual TaqMan assays in 220 DME genes, and has examined genetic variation among two groups of populations, 1) the International HapMap populations; and 2) the SNP500Cancer populations (http://snp500cancer.nci.nih.gov). In addition, the CGF has sequenced / genotyped, by alternative methodologies, 500 of the same SNPs in overlapping sets of individuals. We present an analysis of the DME variants typed and assay performance among various populations. 62% of these putative functional variants were polymorphic in at least one population. In/dels, splice site, missense, and nonsense SNPs were more likely to appear monomorphic (or have lower MAF than this study can detect) than the other types of variants. MAF in these variants were low (e.g. mean MAF of 0.16 in Europeans when polymorphic) as expected, since alleles likely to be under strong purifying or directional selection. Completion and concordance rates (internal duplication) for all assays were 99%. Further, genotype comparisons examining data generated at the CGF and previously published genotype / sequencing data for the same SNPs shows high concordance. However, interesting differences exist between CGF-generated data and the HapMap published data. Mendelian transmission analysis for the trios and relateds shows little inconsistency. Ongoing analyses include relative coverage of the data generated by the HapMap and studies in population genetics. In summary, this panel of assays performed extremely well on controls, and the CGF continues to investigate these SNPs in pharmacogenetic studies relevant to cancer. Funded by NCI Contract N01-CO-12400.

A Novel Glutamic Acid-358 to Glutamine Substitution Mutation in Ectodysplasin A causes X-linked Dominant Incisor Hypodontia Not X-linked Hypohidrotic Ectodermal Dysplasia. *P.S. Tarpey[1], T.J. Pemberton[2], D.W. Stockton[3], P. Das[4], V. Ninis[4], S. Edkins[1], P.A. Futreal[1], R. Wooster[1], S. Kamath[5], R. Nayak[6], M.R. Stratton[1], P.I. Patel[2]*
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The congenital absence of one or more teeth, or hypodontia, is classified as syndromic when it is associated with anomalies in other organs and non-syndromic when the anomaly is confined to teeth. We ascertained a large Asian Indian family that presented with a unique and rare apparently non-syndromic X-linked dominant hypodontia of their incisor teeth. Linkage analysis using 45 markers on the X-chromosome identified a 22.7 Mb interval on Xp11.22-Xq13.2 that contains 175 known and predicted genes. DNA from two affected males was included in an automated high-throughput mutation detection screen to systematically search for mutations within these genes. A c.1072C>G missense mutation in exon 8 of the ectodysplasin A (EDA) gene, mutations within which have been previously implicated in X-linked hypohidrotic ectodermal dysplasia (XLHED), was identified as the causative mutation in this family. This mutation predicts a substitution of glutamic acid for glutamine at amino acid residue position 358 (Q358E) in the TNF domain of the encoded EDA protein. Based on reported structural and functional observations for EDA, we hypothesize that the relatively conservative Q358E substitution only partially disrupts the interaction of the EDA homotrimers and their target receptors, affecting their function significantly only in the dental tissues, resulting in the observed unique hypodontia phenotype rather than the full XLHED phenotype associated with other mutations in the EDA gene. (Supported by NIH grant DE01412).

Sensitivity and specificity of detecting minor allele fractions through High-Resolution melting analysis. *G.H. Reed, Y. Wang, C.T. Wittwer* Pathology, University of Utah, Salt Lake City, UT.

Background: Detection of minor allele fractions is important in cancer diagnosis, detection of recurrence, detection of fetal DNA in the maternal circulation, and population studies of low frequency genes. The sensitivity of sequencing is limited to an allele fraction of around 20%. Methods: Detection of allele fractions ranging from 0.25% to 50% were evaluated using a double stranded DNA dye (LCGreen Plus), real-time PCR (LightCycler) and high resolution melting analysis (LightScanner and HR-1). For PCR templates we used mixtures of genomic DNA for HFE H63D, paroxysmal nocturnal hemoglobinuria, RhD, and the 2 bp deletion in the NCF-1 gene associated with chronic granulomatous disease. In addition, three different plasmids differing in their average GC content (40, 50, 60%) were used. Homozygous fractions were mixed to achieve various allele fractions. The presence or absence of variation was assessed by a blinded investigator using melting curve shape after fluorescence normalization and temperature overlay. Two unknown melting curves were always compared to two known homozygous melting curves for assessment of sensitivity and specificity. Results: Over 2,500 different blinded calls on various targets indicate that we can identify allele fractions as small as 0.25% with a sensitivity of 95-100% and a specificity of 83-97%. Accuracy depended on the allele fraction, with lower allele fractions more difficult to call correctly. The position of the mutation, the GC content of the PCR product and the choice of instrument did not appear to affect the results. Conclusion: Allele fractions down to 0.25% can be distinguished from the wild type through high resolution melting analysis. In addition to being superior to sequencing and most existing methods in detecting minor allele fractions, our method has the added advantage that it is a simple, rapid and inexpensive closed-tube alternative, which makes sample separation unnecessary.

Barriers and Motivators to Family-Based Screening for Hereditary Hemochromatosis (HH). *D.K. Wagener[1], K. Bandel[1], M. Reyes[2], D. Dunet[2], M. Trisolini[1]* 1) RTI International, Rockville, MD; 2) Centers for Disease Control and Prevention, Atlanta, GA.

Health care providers can facilitate detection of hereditary hemochromatosis (HH) and associated iron overloading in the body by counseling patients about the need for their family members to have a diagnostic test for iron overload. This qualitative research study used interviews and focus groups with HH patients (n=60), siblings of patients (n=25), and health care providers who treat patients with HH (n=10) to understand barriers and motivators to: (a) communication about HH and the need for diagnostic testing; and (b) the actions taken by siblings in response to being told they are at risk for HH. This report focuses on patients and siblings. Most HH patients recalled being told by their physician that family members should be advised to have diagnostic testing. HH patients passed this information to their siblings. HH patients identified factors which motivated communication with their siblings including: a high perceived seriousness of HH; perceived benefit of a diagnostic test; and a doctor's recommendation or Internet health information suggesting that siblings be tested. Barriers included estranged relationships with siblings; and, in one case, the perception that diagnostic testing was of little benefit to a sibling who was already ill and disabled. Our analyses indicate that the following factors motivated siblings of HH patients to seek diagnostic testing: perception that HH is serious; perceived benefit of screening; and knowing someone--especially a family member--who had a related complication or died of HH. Siblings who did not seek diagnostic testing identified these barriers: perception that personal risk of HH is low; belief that doctor would have mentioned HH if it was serious; perception that personal risk is low due to lack of symptoms; lack of understanding that HH is hereditary; costs of testing; worries about future insurability; and lack of routine doctor visits.

Overexpression of CUGBP suppresses the Fragile X CGG premutation repeat induced neurodegeneration in Drosophila melanogaster. *O. Sofola[1], R. Duan[2], P. Jing[2], D. Nelson[1], J. Botas[1]* 1) Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Dept Molec & Human Genetics, Emory University, Atlanta, GA.

Fragile X Syndrome is the most common form of hereditary mental retardation. It is caused by a large expansion of the CGG trinucleotide repeat (>200 repeats) in the 5 untranslated region of FMR1 that leads to transcriptional silencing of the gene. Individuals with CGG repeat expansions between 60 and 200 are referred to as premutation carriers. These premutation carriers are able to transcribe FMR1 and hence are phenotypically normal with respect to the features of Fragile X syndrome. However, a neurodegenerative disorder has recently been described in premutation carriers. A transgenic fly model expressing the 5 UTR of the human FMR1 gene with 90 CGG repeats was designed by Jin and colleagues to examine whether the premutation length repeat could cause neurodegeneration. The repeats are transcribed into RNA but are not translated into protein; flies expressing these CGG repeats in the eye display a disorganized eye phenotype. Furthermore, the eye phenotype could be suppressed by over expressing hsp70, a chaperone involved in protein folding. These results suggested that transcription of the CGG90 repeats lead to an RNA mediated neurodegenerative disease, possibly via influencing RNA binding proteins. We set out to identify these RNA binding proteins by carrying out a genetic screen on the CGG90 eye phenotype. The screen involved mating the CGG90 transgenic flies with other candidate mutant flies that expressed either a reduced or an increased level of different proteins. The progeny were examined for potential suppression or enhancement of the disorganized eye phenotype seen in control CGG90 transgenic flies. We show that overexpression of human CUGBP1, an RNA binding protein is able to suppress the neurodegenerative phenotype of the CGG90 transgenic fly. Furthermore, we show that the CUGBP1 protein is able to interact with the CGG repeats via hnRNPA2/B1, another RNA binding protein. These findings suggest potential pathways that are disturbed by expression of expanded CGGs that may contribute to neuronal degeneration.

Identification of Novel Asthma Susceptibility Loci Using a Family-Based Screening Approach. *K.G. Tantisira[1], A. Murphy[2], A.A. Litonjua[1], J. Lasky-Su[1], B.A. Raby[1], R. Lazarus[1], B. Klanderman[1], E.K. Silverman[1], C. Lange[2], S.T. Weiss[1]* 1) Channing Laboratory, Brigham & Women's Hospital and Harvard Medical School, Boston, MA; 2) Harvard School of Public Health, Boston, MA.

Introduction: The evaluation of genetic datasets to identify asthma susceptibility loci has been limited by multiple comparisons and failure to replicate. We hypothesized that a screening algorithm in the program FBAT would help to overcome these problems. The algorithm screens using the between family-component in order to identify candidates with the greatest power for association within the informative families, limiting the number of tests performed within the families. **Methods:** We tested the association of 861 single nucleotide polymorphisms (SNPs) in 116 candidate genes with asthma affection status in 400 Caucasian trios consisting of parents and Childhood Asthma Management Program probands. The screening algorithm identified and ranked SNPs in order of statistical power. Only the most powerful SNPs were formally evaluated by FBAT testing. **Results:** 570 SNPs in 62 genes had never previously been analyzed. Of SNPs ranked in the highest quintile of statistical power, SNPs in 20 genes were significantly associated with asthma affection status within the trios (p-values 0.0007-0.047). Among these were four genes that we have previously identified as asthma susceptibility genes (IL10, TBX21, TLR10, and VDR), supporting the validity of this approach. Three additional genes (NOS3, PLN, and BDKRB2) have been associated with asthma by others. Of the remaining genes, PTGIR, ITPR2, NDFIP1, ADCYAP1, CREB5, and RGS12 were both associated with asthma and within the top 5% of power, suggesting the greatest potential for replication. **Conclusions:** Novel asthma susceptibility genes have been identified using the FBAT screening approach, which may help address problems of multiple comparisions and poor likelihood of replication. Formal replication studies are planned.

Investigation of the *TNF receptor-associated factor (TRAF)* genes with rheumatoid arthritis. *C. Potter, J. Worthington, A. Barton* ARC-EU, Univ Manchester, Manchester, United Kingdom.

Background: The TNF receptor-associated factor (TRAF) family is an important group of intracellular adapter proteins for a wide variety of receptors including the TNF and IL-1 receptor superfamilies. Collectively, these molecules are essential for transducing extracellular cytokine signals which culminate in a variety of TNF- and IL1-induced disease-relevant processes. Thus, these genes represent strong candidate susceptibility factors for inflammatory diseases such as rheumatoid arthritis (RA).

Aim: To investigate association of RA with single nucleotide polymorphisms (SNPs) spanning the *TRAF1-6* genes.

Methods: Twenty three haplotype-tagging (ht) and 26 random SNPs were identified from phase 1 of the Hapmap dataset (www.hapmap.org). Genotyping was preferentially performed using MassARRAY as recommended by the manufacturer (www.sequenom.com/). Single point and haplotype analyses were performed in STATA and Helixtree software (Golden Helix, Inc), respectively. Samples were genotyped in three stages, only those SNPs showing association ($p<0.05$), either singly or by haplotype were carried forward.

Results: Forty-three SNPs were successfully genotyped and conformed to Hardy Weinberg expectations. Eight SNPs across the *TRAF2* and *5* genes were associated at single and/or haplotype analysis in the first subset of 351 cases and 368 controls. Following genotyping in an additional 594 cases and 368 controls, only one SNP, rs7514863, remained associated. This SNP was genotyped in the remaining samples and demonstrated a significant association across the entire cohort (1469 cases and 2760 controls; OR for minor allele = 1.2 (1.06-1.37), $p= 0.004$).

Conclusion: We have found evidence for association of a SNP upstream of a strong candidate RA susceptibility gene, *TRAF5*, in a large cohort of patients and controls. Further association and functional studies are required to investigate the role of this SNP in RA disease causation.

Association Analysis of CTLA4 Polymorphisms in Mexican Population with Childhood-Onset Systemic Lupus Erythematosus. *G. Salas[1], V. Baca[2], R. Velazquez[1], F. Espinoza[3], S. Jimenez[1], G. Jimenez[1], L. Orozco[1]* 1) Laboratory of Research, National Institute of Genomic Medicine, Mexico City, Mexico; 2) Department of Pediatric Rheumatology, CMN-Siglo XXI, IMSS. Mexico City, Mexico; 3) Department of Immunology, INP,SS. Mexico City, Mexico.

Introduction. Susceptibility to systemic lupus erythematosus (SLE) has been attributed to complex interaction between genetic and environmental factors. The gene region 2q33 has been identified as a susceptibility region for SLE in genome-wide scans. This region harbors the Cytotoxic T Lymphocyte Antigen 4 (CTLA4) gene that encodes an immunoinhibitory receptor expressed on activated T cells, acting as a negative feedback regulator of T-cell activation. Polymorphisms in CTLA4 gene have been implicated in the development of several autoimmune disorders, including SLE. However, results obtained in different studies on the association of SLE with CTLA4 polymorphisms have been inconsistent. Objective. The aim of this study was to investigate the possible association of the -318C/T, +49A/G and the CT60A/G polymorphisms in the CTLA4 gene with childhood-onset SLE in Mexican population. Methods. We performed a case-control association study in 245 pediatric patients with SLE and 360 unrelated, healthy Mexican controls. Genotyping of these polymorphisms was carried out by the 5 nuclease assay (TaqMan). The genotypes and allele frequencies were compared between cases and controls by X2 test. Results. There were no significant differences in the distribution of genotypes and allele frequencies in the -318C/T and +49A/G polymorphisms between SLE patients and controls. SLE patients had a higher frequency of the CT60A allele with a marginal statistical significance (47.35%; versus 41.67%;, P = 0.051; OR 1.26, 95%; CI = 0.99-1.59), which was no longer statistically significant after correcting the P value (P = 0.10). Conclusion. Our results suggest that these polymorphisms in CTLA4 gene do not confer a relevant role in the susceptibility of childhood-onset SLE in Mexican population. Genotyping of the polymorphism in the CTLA4 gene promoter region (-1722T/C) is underway, as well as the haplotype analysis.

Molecular analysis of a large FMR1 gene deletion. *R. Polli, A. Casarin, L. Anesi, E. Leonardi, M. Martella, A. Murgia* Dept Pediatrics, Univ Padua, Padua, Italy.

Most of the cases of fragile X syndrome result from expansion and hyper-methylation of the CGG tract in the FMR1 gene. Deletions and point mutations of FMR1 are much less common. We report the case of a female, member of a fragile X syndrome pedigree, in whom the molecular analysis allowed the identification of a large deletion involving the 5UTR region and part of the coding sequence of FMR1. Her brother, previously tested, was found to carry a reverse-mutation with presence of fully mutated fragments together with a deletion within the repeat tract. Southern blot, performed with the use of StB 12.3 as a probe and EcoRI/EagI double digestion, showed in the female two fragments, of 5.2 and 2.8 Kb, nevertheless the relative intensity of the bands appeared slightly unbalanced and the amount of DNA loaded on the lane suggested a reduced FMR1 gene dosage. PCR analysis of the FMR1 CGG repeats allowed the identification of a normal allele with 31(CGG)s and of a premutation with approximately 70 repeats in the mother, while the father carried a 30 repeats allele. The daughter presented only the paternal 30 (CGG)s. In order to confirm the loss of the mutated maternal allele and to map the deletion breakpoints, we performed a haplotype analysis with the use of polymorphic markers FraxaC1, FraxaC2, DXS548 and several SNPs located within the FMR1 coding sequence, and a Real Time quantitative PCR analysis. We have located the 3breakpoint within intron 9 of the FMR1 gene while the position of the 5 breakpoint is currently under investigation. The extreme conditions of mosaicism seen in Fragile X affected individuals are due to expansions and deletions commonly contained within the unstable trinucleotide CGG repeats or including immediately flanking sequences. The occurrence of deletions extending beyond the region of repeats must be due to the presence of other rearrangement-prone sequences that might be somehow influenced by the FMR1 expansion/methylation.

Improving Power in Genome-Wide Association Studies: Weights Tip the Scale. *K. Roeder[1], L. Wasserman[1], B. Devlin[2]* 1) Dept. Statistics, Carnegie Mellon Univ., Pittsburgh, PA; 2) Dept. of Psychiatry, Univ. of Pittsburgh SOM, Pittsburgh, PA.

Genome-wide association is considered a promising approach for identifying genetic variants that are associated with human disease. Scanning the genome for association between markers and complex disease typically requires testing hundreds of thousands of genetic polymorphisms. When testing such a large number of hypotheses, various strategies are available for balancing the trade-off between power to detect meaningful associations and the chance of making false discoveries. One recent suggestion is to apply differential weights to the hypotheses so as to increase the power to detect likely signals. Weights can be incorporated into any multiple testing proceedure that relies on P-values, such as false discovery or Bonferroni. Roeder et al. (2006;78:243-252) investigated the potential for using weights based on an externally derived source, such as a linkage trace. Their results suggest that if the weights are informative, the weighted procedure improves power considerably; remarkably, the loss in power is small even when the weights were uninformative. This result holds provided the tests that are up-weighted are relatively sparse. We investigate this premise further and quantify the gain function for a binary system of weights. We find that, if a given fraction of tests are up-weighted, then a unique choice of weights exists to optimize the gain function. For a 2-stage design, internally derived weights are also an appealing choice. For instance, suppose that a fraction of the samples is genotyped on a large number of markers in stage 1, and then only the most promising subset of the markers is genotyped on the remaining samples in stage 2. With this 2-stage design, stage 1 can be used to formulate the weights, and both stages can be used in the analysis. This strategy yields weights that are nearly optimal. We compare this approach to the one proposed by Skol et al. (Nat Genet 2006;38:209-213).

Biotin changes multiple proteins expression in Saccharomyces cerevisiae grown under fermentative and aerobic conditions. *A. Velázquez-Arellano[1], V. Pérez-Vázquez[1], A. Hernández-Mendoza[1], M. Hernández[2], G. Martínez-Batallar[2], S. Encarnación[2], S. Uribe[3], D. Ortega-Cuéllar[1]* 1) Nutritional Genetics, Instituto de Investigaciones Biomédicas, UNAM & Instituto Nacional de Pediatría, Mexico City, D.F., Mexico; 2) Centro de Ciencias Genómicas UNAM, Cuernavaca, Mor., Mexico; 3) Instituto de Fisiología Celular UNAM, Mexico City, Mexico.

In rat and human cells, the vitamin biotin acts as regulator of reportedly over 1000 diverse proteins, this role being different from prosthetic group of carboxyl transferring enzymes. The regulatory functions are enigmatic, without apparent relation with its structure. To find clues for these effects, we have turned to yeast Saccharomyces cerevisiae and chose a proteomic approach, using low (2 nM), intermediate (200) and high (20,000) media concentrations, with glucose or lactate as carbon sources. Proteins were separated by 2D electrophoresis, stained by Coomassie and identified by MALDI-TOFF MS. We observed approximately 650 proteins (11% of total yeast proteins) in cultures containing glucose (fermentative); 42 of them changed their expression: 22 diminished when biotin was intermediate or high, whereas 11 were increased. Some others did not change their amount but their position in the 2D gel, suggestive of a post-translational change such as phosphorylation. On cultures grown on lactate (aerobic), approximately 590 proteins were observed (10.6 % of the total yeast); 48 of them changed their expression: 33 diminishing and 2 increasing their amounts when biotin was present. Those proteins were classified according to their function: 4 were associated with carbohydrate metabolism, 2 were DNA binding proteins, 4 were stress-responsive proteins, 3 were associated with purine and pyrimidine synthesis, 1 with DNA replication, 3 with protein synthesis, 1 was structural and 3 others were of miscellaneous or unknown functions. Therefore, the pleoitropic regulatory effects of biotin seem to extend along the eukaryotes, and the easily manipulable yeast may serve as a model organism to study these effects and their regulation. (Supported by PAPIIT IN228605 from DGAPA-UNAM).

Fmr1 gene family regulates circadian clock in mammals. *J. Zhang[1], Z. Fang[1], K. Kaasik[2], C.C. Lee[2], B.A. Oostra[3], D.L. Nelson[1]* 1) Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Dept of Biochemistry, UT-Houston Medical School, TX; 3) Dept of Clinical Genetics, Erasmus University, Rotterdam, The Netherlands.

Fragile X syndrome, the most common heritable cause of mental retardation, results from loss-of-function mutations in the fragile X mental retardation (FMR1) gene that lead to absence of its gene product (FMRP). FMRP belongs to a family of proteins that includes the Fragile X related proteins 1 and 2 (FXRP1 & FXRP2). FXRPs share high similarity in their functional domains with FMRP. It is likely that FXRPs play redundant roles with FMRP. Fruit fly *Drosophila* models carrying loss-of-function alleles of *dfxr* have shown defects in circadian activity. We therefore study circadian rhythm in mice with knockout mutations in the *Fmr1* and *Fxr2* genes. We examined circadian wheel running activity in *Fmr1* knockout, *Fxr2* knockout and *Fmr1*/*Fxr2* double knockout animals using a circadian controlled locomotor activity assay. We observed that *Fmr1* and *Fxr2* single knockout mice showed shorter period lengths than wildtype littermates in the absence of light cues, while double knockout mice displayed a total loss of rhythmicity even in the presence of light input. This arrhythmic phenotype is unique among mouse models with circadian defects studied to date. We have examined the circadian mRNA expression of 6 known central clock component genes in liver. We found that the patterns of cyclical behavior for the *Per1*, *Per2*, *Cry1* and *Npas2* genes were altered and that the peak of *Per2* mRNA expression is abnormally low in both *Fxr2* and double knockout mice. Using an *in vitro* transfection assay to measure luciferase output from Per promoter constructs, we observed that *Fxr2* alone or *Fmr1* and *Fxr2* together can increase *Per1* or *Per2* induced *Bmal1-Npas2* dependent activation of both the *Per1* and *Per2* promoters, and that the effects are dependent on the dose of FXRP2. Together, our data suggest a role for the *FMR1* gene family in regulation of the mammalian central circadian clock, which could be significant to further understand the learning and memory related deficiencies observed in fragile X patients.

Identification of a cryptic 11;17 translocation resulting in a novel *NUP98-PHF23* fusion in acute myeloid leukemia. *J.C. Reader[1], J.S. Meekins[2], I. Gojo[3,4], Y. Ning[1,2]* 1) Program in Human Genetics; 2) Dept Pathology; 3) Dept Hematology Oncology, University of Maryland School of Medicine, Baltimore, MD; 4) Marlene and Stewart Greenebaum Cancer Center, Baltimore, MD.

Chromosomal abnormalities on 17p are associated with a variety of leukemias. We recently identified a patient with acute myeloid leukemia (AML) that presented with a possible abnormality on 17p by standard karyotype analysis. Fluorescence *in situ* hybridization (FISH) was performed using a *p53* probe to confirm the involvement of 17p, but a normal signal pattern was observed. Further FISH analysis with a *LIS1* probe distal to *p53* revealed a cryptic balanced 11;17 translocation. We attempted to identify the translocation breakpoints. Considering the *NUP98* gene located at 11p15 is commonly involved in translocations associated with AML, we constructed a breakapart probe set using bacteria artificial chromosome (BAC) clones containing the telomeric and centromeric ends of *NUP98*. With this probe we demonstrated the involvement of *NUP98* in our patient. The breakpoint on 17p occurred in the region between *p53* and *LIS1*. After constructing and testing a series of BAC clone probes between the two genes we narrowed down the breakpoint to within 100 Kb. Using a candidate gene approach we were able to amplify a fusion transcript by RT-PCR. Sequence analysis revealed a novel in-frame fusion of *NUP98* exon 13 with *PHF23* exon 4. *PHF23* is a novel gene encoding a hypothetical protein containing a plant homeodomain (PHD) type zinc finger which is thought to play a role in transcriptional regulation. *NUP98* is one of the most promiscuous fusion partner genes in hematological malignancies with more than 20 different fusion partners on various chromosomes; however, the 11;17 translocation and the resultant *NUP98-PHF23* fusion is a novel finding in AML. The FISH probes developed in this study have not only provided accurate cytogenetic diagnosis, but also made it possible to monitor therapy and disease progression. In addition, further characterization of the new fusion gene will aid in understanding the mechanism of leukemic transformation.

Megalencephaly, thick corpus callosum, dysmorphic facial features, seizures, enamel defect and mental retardation in two unrelated patients: a new syndrome? *M. Rio[1], I. Desguerre[2], N. Boddaert[3], M. Le Merrer[1], A. Munnich[1], V. Cormier-Daire[1]* 1) Medical Genetics, Necker Hospital, Paris, France; 2) Pediatric Neurology, Necker Hospital; 3) Pediatric Radiology, Necker Hospital.

Macrocephaly associated with mental retardation is a part of various syndromes including overgrowth conditions, cutis marmorata telangiectatica, Cowden disease, and neuro-metabolic disorders. We report here a new association characterized by megalencephaly, thick corpus callosum, mental retardation, seizures, specific facial features, and enamel defect in two unrelated boys. Case 1 is the second child of unrelated parents. He was able to walk alone at 3 years of age, developed seizures at the age of 5 and had limited speech. The physical examination at the age of 9 revealed normal weight and length but a large head circumference (+3SD) and dysmorphic features including downslanting palpebral fissures, long and expressionless face, small mouth, enamel defect, and strabismus. He also had distal mucle atrophy. Case 2 is the first child of first cousin parents. Parameters at birth were above the normal range. Head circumference curve was above + 4 SD whereas length and weight were in the normal range at 3 years of age. Seizures appeared at the age of 3. He was able to walk at 8 and had only a few words. He had the same dysmorphic features (long expressionless face, downslanting palpebral fissures, small mouth,enamel defect, and distal amyotrophy). Extensive metabolic and cytogenetic studies were normal in the two boys. Myotonic dystrophy and fragile X were excluded in case 1. In addition, molecular screening of NSD1, GPC3 and the 11p15 region was negative in case 2.In the two children, brain MRI detected bilateral megalencephaly, a thick corpus callosum, an enlarged white matter and normal ventricles.This association of megalencephaly and thick corpus callosum has been previously reported by G. Göhlich-Ratmann in 1998 in 3 children who had in addition pachygyria and complete lack of motor development, features not observed in our cases. We hope that ongoing cytogenetic and molecular studies will further define this new entity.

FINLAY-MARKS Syndrome: Thirty Year Follow-up in the affected one of two Dyzigotic Twins. *A. Zeffiri[1], M. Ottaviani[1], M. Isoldi[1], S. Stagi[1], L. Di Medio[1], M. Levi[1], L. Carosi[1], L. Giunti[2], U. Ricci[2], M.L. Giovannucci Uzielli[1]* 1) Dept. Paediatrics, Genetics, University of Florence, Florence, Italy; 2) Childrens Hospital A. Meyer, Florence, Italy.

Finlay-Marks syndrome, or Scalp-Ear-Nipple syndrome (SEN, OMIIM 181270) is a very rare Autosomal Dominant disorder, first described in 1978 by Finlay and Marks in a family with 10 affected individuals over five generations. The clinical diagnosis is especially based on the occurrence of scalp defect, dysplastic external ears, and absent or rudimentary nipples and breasts. Other clinical features were reported in the few additional cases described in the literature: in particular, urinary tract malformations and/or disfunctions, and diabetes. We report the follow-up study performed in our Genetics Unit, in the affected one of two dyzygotic female twins, since the age of 2 years and during 30 years. The periodical clinical evaluation, with the diagnostic laboratory and instrumental tests, performed in order to follow the natural history of the phenotype, revealed a progressive number of additional abnormal features. In the same time, we were able to exclude other features reported in this disorder: in particular, no hydrocephaly or large ventricle, no cataract nor hearing impairment. Normal intelligence and behaviour. The stature is reduced if compared with the twin sister, the brother, and both parents. The patient was submitted to surgery correction of the congenital bald nodules over the scalp, reduction of the abnormal eye spacing, correction of the prominent and dysplastic ears, correction of the bilateral athelia and amastia by reconstruction of nipples and breasts. Unilateral renal hypoplasia was revealed by ultrasounds at age 5 years. Only recently the patient developed severe renal hypertension. Normal 46,XX karyotype. By using a battery of polymorphic DNA markers we confirmed the DZ twinning.

Variation in the Human Matrix Metalloproteinase-9 (MMP-9) Gene is Associated with Arterial Stiffness in Healthy Individuals. *Y. Yasmin[1], C.M. McEniery[1], K.M. O'Shaughnessy[1], P. Harnett[1], A. Arshad[1], S. Wallace[1], K. Maki-Petaja[1], B. McDonnell[2], M.J. Ashby[1], J. Brown[3], J.R. Cockcroft[1], I.B. Wilkinson[1]* 1) Clinical Pharmacology Unit, University of Cambridge, Cambridge, UK; 2) Department of Cardiology, University of Wales College of Medicine, Cardiff, UK; 3) Trinity College, Cambridge, UK.

Background Arterial stiffness is an important determinant of cardiovascular risk. Elastin, is the main elastic component of the arterial wall, and can be degraded by a number of enzymes including serine proteases and matrix metalloproteinases (MMPs). Serum MMP-9 levels correlate with arterial stiffness and predict cardiovascular risk. Polymorphisms in the MMP-9 gene are also associated with large artery function in subjects with coronary artery disease. Therefore, we investigated the influence of known MMP-9 (-1562C>T, R279Q) polymorphisms on arterial stiffness in a large cohort of healthy individuals (n=865). Methods Aortic pulse wave velocity (PWV) and augmentation index were assessed using SphygmoCor. Supine blood pressure, biochemical markers, serum MMP-9 levels and serum elastase activity (SEA) were determined by standard methods. Genomic DNA was extracted and genotyping performed using PCR-RFLP and a validated Taqman assay respectively. Results Aortic PWV, serum MMP-9 and SEA were higher in carriers of the rare alleles for the -1562C>T and R279Q polymorphisms. These polymorphisms were also associated with aortic PWV after correction for other confounding factors. Stepwise regression models with known or likely determinants of arterial stiffness revealed that ~60 percent of the variability in aortic PWV was attributable to age, mean arterial pressure and genetic variants ($P<0.001$). MMP-9 whole genotypes also had a significant dose-dependent effect on aortic PWV and serum MMP-9 levels. Conclusions We have demonstrated for the first time that aortic stiffness and elastase activity are influenced by MMP-9 gene polymorphisms. This suggests that the genetic variation in this protein may be involved in the process of large artery stiffening presumably through effects on turnover of matrix proteins in the vessel wall.

Distal arthrogryposis type 5 is caused by defects of myosin. *R. Toydemir[1], L.B. Jorde[1], M. Bamshad[2,3]* 1) Department of Human Genetics, Univ Utah, Salt Lake City, UT; 2) Departments of Pediatrics and Genome Science, Univ Washington, Seattle, WA; 3) Childrens Hospital and Regional Medical Center, Seattle, WA.

The distal arthrogryposes (DA) are a group of syndromes characterized by congenital contractures of the hands and feet, limited proximal joint involvement, autosomal dominant inheritance, reduced penetrance, and variable expressivity. To date, 10 different DA syndromes have been characterized. Among the DAs, DA5 is unique since in addition to contractures of the skeletal muscles, affected individuals have ocular abnormalities such as ptosis, ophthalmoplegia, and strabismus. Based on our previous findings, which showed DAs are caused by mutations that encode proteins of contractile apparatus of myofibers, we hypothesized that DA5 might be caused by contractile proteins that are expressed in both skeletal and extraocular muscles. Two such proteins are myosin heavy chain IIa and myosin heavy chain 13 that are encoded by *MYH2* and *MYH13*, respectively. We screened the entire coding region of these genes in 8 independent cases of DA5. In two cases, we found missense mutations in *MYH2* that caused substitutions of highly conserved amino acid residues. Neither of these mutations was found in more than 200 chromosomes from controls matched for geographic ancestry. Additionally, one of the two DA5 cases with a *MYH2* mutation also had a mutation in *MYH13*. This mutation also alters a highly conserved amino acid residue and it is not found in the healthy population. Our results suggest that DA5 is genetically heterogeneous, and mutations in *MYH2* cause a subset of DA5 cases. In addition, mutations in other contractile proteins might modify the phenotype associated with *MYH2* mutations or, alternatively, *MYH2* might in some cases modify a phenotype caused by mutations in genes that encode other contractile proteins.

HNF4A variants predispose to high serum lipid levels and the metabolic syndrome. *D. Weissglas[1], A. Huertas-Vazquez[1], T. Tusie-Luna[2], C. Aguilar-Salinas[2], M-R. Taskinen[3], TW. de Bruin[4], CJ. van der Kallen[5], P. Pajukanta[1]* 1) Dept. of Human Genetics, UCLA, Los Angeles, USA; 2) INCMNSZ, Mexico City, Mexico; 3) Dept. of Medicine, University of Helsinki, Finland; 4) GlaxoSmithKline, Translational Medicine and Genetics, Research Triangle Park, USA; 5) Dept. of Medicine, CARIM, Academic Hospital, Maastricht, The Netherlands.

Hepatic nuclear factor 4, alpha (HNF4A) has been associated with type 2 diabetes mellitus (T2DM) in several populations. Considering the phenotypic overlap between T2DM and familial combined hyperlipidemia (FCHL), the most common dyslipidemia predisposing to coronary heart disease (CHD), we previously investigated HNF4A in FCHL families from two distinct populations, Finnish and Mexicans. We observed that common HNF4A variants are associated with high serum lipid levels and the metabolic syndrome (MS). Importantly, we found two common HNF4A haplotypes (rs6031558-rs745975-rs3212198) that were associated with elevated serum triglycerides (TGs) (P=.006) and total cholesterol (P=.005) in both Finnish and Mexican FCHL families. In the present study we sequenced subjects with the risk or protective haplotypes for the coding region of HNF4A to identify the functional variant(s). Although no coding variants differ between the haplotype groups, a variant adjacent to exon 5, was found in 10 of the risk-haplotype carriers and was absent in the protective-haplotype group (P=.03). We are currently analyzing this variant for a possible role in FCHL. We also examined the HNF4A variants implicated in the Finnish and Mexican families, in an independent study sample comprising of 557 individuals from 33 Dutch FCHL Families. In the Dutch FCHL families, rs745975 was individually associated with both TGs and the MS (P=.04, .01). Haplotype analyses in the Dutch FCHL families also showed significant associations with FCHL, high TGs and with the MS for several HNF4A haplotypes (P=.01-.05). We are currently investigating the potential effect of all implicated HNF4A variants on the expression of the downstream genes. Taken together, these data imply that common HNF4A variants confer the susceptibility to high serum lipid levels and the MS.

Phactr2, Genomewide association and Parkinsons disease. *O.A. Ross[1], J.T. Stone[1], J. Aasly[2], T. Lynch[3], M.J. Farrer[1]* 1) Dept of Neuroscience, Mayo Clinic, Jacksonville, FL; 2) Department of Neurology, St. Olav's Hospital, Trondheim, Norway; 3) Department of Neurology, Mater Misericordiae Hospital & The Conway Institute, University College, Dublin, Ireland.

The first genomewide association study has been reported in Parkinsons disease (PD). Although the study nominated eleven chromosomal loci follow-up independent replication studies have not been able confirm the role of these in disease. The study involved two tiers with the second a case-control series design. Interestingly the SNP with the lowest p-value in Tier 2 was not one of the nominated loci. This SNP, rs11155313 ($P<0.000016$) is located in the Phactr2 gene on chromosome 6. The Phactr2 protein belongs to the Phactr protein family which are reported to regulate protein phosphatase 1 activity. Our study identified a significant association between this SNP and a case-control series from the Irish population. This association could not be replicated in a case-control series from Norway. Given the importance of phosphorylation and kinase genes in PD the role of the Phactr family of proteins in neurodegeneration deserves further investigation.

Clinical and radiographic delineation of Odontochondrodysplasia. *J. Spranger[1], F. Antoniazzi[2], M. Brugnara[2], Y. Alanay[3], K. Lachlan[4], S. Ikegawa[5], G. Nishimura[6], S. Unger[7,1], A. Superti-Furga[1]* 1) Dept of Pediatrics, Univ of Freiburg, Germany; 2) Dept of Pediatrics, Univ of Verona, Italy; 3) Div Clin Genet, Dept of Pediatrics, Hacettepe Univ, Ankara, Turkey; 4) Wessex Reg Genet Serv, Southampton, UK; 5) Lab Bone and Joint Dis, SRC, RIKEN, Tokyo, Japan; 6) Dept of Radiol, Tokyo Metrop Kiyose Childrens Hosp, Kiyose, Japan; 7) Inst Human Genet, Univ of Freiburg, Germany.

The association of dentinogenesis imperfecta with chondrodysplasia (odontochondrodysplasia; ODCD) has been reported in 5 individuals so far. We diagnosed ODCD in four unreported children and retrospectively in two brothers reported previously as metatropic dysplasia variant, bringing the total number of subjects with ODCD to eleven. ODCD manifests at birth with short stature, narrow thorax, and severe spondylometaphyseal dysplasia; three of the eleven individuals died in the first two years of life with respiratory insufficiency. In childhood, high forehead, flat nose, mesomelic limb shortening, joint laxity, scoliosis, and dentinogenesis imperfecta with small, brownish, and fragile primary teeth become apparent. Mental development is normal and eye or ear complications have not been seen so far. Radiographic features are marked spondylar dysplasia with coronal clefts in infancy; square iliac wings with wide lacy borders, horizontal acetabular roofs, and coxa valga; progressive metaphyseal splaying with enchondromatous changes; mesomelic shortening; short metacarpals and phalanges with round cone-shaped epiphyses; and osteopenia with expanded diaphyses and thin cortex. The differential diagnosis in the newborn is with SMD Sedaghatian (platyspondyly with spiked metaphyses) and with collagen 2 disorders (platyspondyly with coronal clefts); later, ODCD resembles metatropic dysplasia (narrow thorax and spondylar changes), Dyggve-Melchior-Clausen syndrome (SEMD with lacy iliac crests), or severe Shwachman-Diamond syndrome (narrow thorax and metaphyseal changes; the SBDS gene was normal in one case). The consistency of findings in 11 individuals confirms that ODCD is a distinct entity. Three pairs of affected sibs born to unaffected parents suggest AR inheritance.

A Novel Missense Mutation, p.R808W, in the *HOPA* Gene is Present in 10% of a Cohort of FG Syndrome Families. *H. Risheg[1], M.J. Friez[1], J.M. Graham Jr.[2], J.B. Moeschler[3], R.C. Rogers[1], J.M. Opitz[4], R.E. Stevenson[1], C.E. Schwartz[1]* 1) Greenwood Genetic Center, Greenwood , SC; 2) Cedars-Sinai Medical Center, Los Angeles, CA; 3) Dartmouth Medical School, Lebanon, NH; 4) University of Utah School of Medicine, Salt Lake City, UT.

FG Syndrome (MIM 305450) is an X-linked recessive form of mental retardation (XLMR) associated with hypotonia, macrocephaly, dysgenesis of the corpus callosum, and other anomalies. This syndrome is heterogeneous with 5 possible loci on the X chromosome. One locus (FGS1) is in Xq12-q22. The *HOPA* gene, located at Xq13, is a human opposite-paired gene involved in thyroid hormone signal transduction, making it a good candidate gene for XLMR given our understanding of the *MCT8* gene. Our initial study consisted of sequencing the 45 exons of *HOPA* in 24 probands from XLMR families with linkage covering the Xq13 interval. Sequence analysis identified a missense change in 2/24 XLMR male probands (one Caucasian/ one African American), both of which have similar clinical features of FG syndrome. This missense change (c.2422 C>T) in exon 21 results in an arginine to tryptophan change at amino acid 808 (p.R808W). Affected males and obligate carrier females were concordant for the alteration in the families of both FG males. The alteration was not found in 451 normal adult male controls, 343 consecutive male newborns and 64 normal adult female controls, making it unlikely that it represents a rare polymorphism. Simulated protein modeling indicates the alteration has a pathogenic effect on the structure of the *HOPA* protein. Subsequently, a panel of 48 samples from either males diagnosed with FG syndrome or mothers of boys diagnosed with FG syndrome, were tested for the c.2422 C>T alteration. Analysis revealed 3 additional individuals with the same change (1 male from a large family with 3 other deceased affected males, and 2 unrelated obligate female carriers). To date, a total of 3 hemizygous males affected with FG syndrome and 2 heterozygous females, from 5 apparently unrelated FG syndrome families, have been identified. Thus, the novel p.R808W missense mutation of *HOPA* has been found in 10% of the FG syndrome families tested.

Exon resequencing - the search for sequence variation in the human genome. *J. Rogers, J. Allen, H. Arbery, G. Bethel, R. Bennett, S. Bhaskar, A. Dunham, M. Bush, J. Burton, J. Durham, T. Eades, M. Earthrowl, S. Hunt, S. Leonard, K. McLay, D. Niblett, R. Norris, A. Sanderson, Y. Umrania, A. Coffey* Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

The primary aim of the ExoSeq programme is to obtain a near-complete catalogue of common variation in human genes. Initially, we are re-sequencing exons in 48 Caucasian individuals using high-throughput sequencing of PCR products. For all known protein-coding genes, novel coding sequences and transcripts, exons and their flanking sequence are extracted from the Vega database, which contains high-quality manually curated annotation to allow us to identify all exons for each gene. Primers are designed to encompass the exon and splice sites and are checked for uniqueness prior to synthesis. Primers are pre-screened with successful primer pairs being re-arrayed and grouped together for amplification. Bi-directional sequencing of amplicons is carried out using Big Dye chemistry with a success rate of over 95%. We are currently processing 3,000 STSs per week (1 million sequencing reads per month).

SNPs are called using a novel algorithm developed in-house, ExoTrace. All high confidence variants detected automatically or those passing manual review are deposited in dbSNP on a monthly basis. To date we have identified over 75,000 SNPs, approximately 41% of which are novel (66% of which are rare), and 10% of which represent non-synonymous changes.

We are developing methods for analysing variation in other regions of the genome of potential functional importance, including promoters and other regulatory elements identified as those showing varied levels of conservation between species.

Variations in disease studies and medical sequencing projects are also underway in areas including epilepsy, cardiovascular disease, platelet biology, deafness, diabetes and cognition. Exons are sequenced in both the Caucasian samples and patient samples to identify additional SNPs for association studies or direct detection of mutations.

Adenoviral-mediated correction of methylmalonyl-CoA mutase deficiency in mut human hepatocytes. *M.S. Tsai[1], R.J. Chandler[1], K. Dorko[2], J.L. Sloan[1], M. Korson[3], R. Freeman[4], S. Strom[2], C.P. Venditti[1]* 1) GDRB,NHGRI,NIH, Bethesda, MD; 2) Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA; 3) Metabolism Service,Tufts University, Boston, MA; 4) Department of Surgery, Tufts University, Boston, MA.

Mut class methylmalonic acidemia (MMA) is a metabolic disorder caused by deficiency of the mitochondrial-localized, adenosylcobalamin-dependent enzyme, methylmalonyl-CoA mutase. Liver transplantation in the absence of severe hepatic dysfunction has been used as a supportive therapy in severely affected patients, and provides significant increase in metabolic stability. As a first step to examine the effectiveness of gene and cell therapy in this disorder, viral correction experiments were performed on primary human hepatocytes derived from a mut MMA patient. The affected patient exhibited a severe clinical phenotype and harbored two early premature truncating mutations, E224X and R228X, in the methylmalonyl-CoA mutase gene. He underwent a combined liver-kidney transplant at the age of 5 years. Primary hepatocytes were isolated from the native liver by a three-step collagenase digestion method and used to test the efficacy of gene delivery using a bi-functional adenoviral vector that expressed the methylmalonyl-CoA mutase gene as well as EGFP. Viral infection and delivery were monitored by fluorescence. Measuring C-14 propionate incorporation into macromolecules assessed enzymatic correction, and Western analysis was used to demonstrate protein expression. Functional correction of the mut hepatocytes was comparable to the degree of correction achieved using MEF cell lines derived from Mut knock-out mice, and showed a restoration of propionate flux and prominent expression of the enzyme on Western analysis. The protein expression levels from the corrected cells were equal to or greater than that seen in extracts derived from a control human liver. These studies provide proof of principle for hepatocyte-mediated gene delivery in methylmalonic acidemia. In the future, MMA may be amenable to liver-specific gene and cell-based therapies as an alternative to liver transplantation, which consumes an already scarce resource.

Microdissection-based genomic array analysis: what you see is what you get. *J. Yu[1], Z. Qi[1], G.M. Rice[2], R. Selzer[3], T. Richmond[3], K. Thompson[2], S. Liao[4], R.M. Pauli[2], D. Albertson[1], D. Pinkel[1], J.E. Hearst[4]* 1) University of California San Francisco, San Francisco, CA; 2) University of Wisconsin - Madison, Madison, WI; 3) NimbleGen Systems, Inc., Madison, WI; 4) University of California Berkeley, Berkeley, CA.

Chromosome microdissection is a unique technique for in situ chromosome cloning - direct isolation and cloning of any chromosome or chromosome region of interest from a single cell or a few cells for genomic analysis. We show here that dissected DNA samples from marker chromosomes and abnormal chromosome regions that carry a deletion and a balanced translocation, respectively, can be readily amplified and analyzed with either oligo- or BAC-based genomic arrays. It demonstrates that virtually any chromosomal abnormality can be comprehensively characterized with microdissection-based DNA array analysis for chromosome origin, breakpoints, gene content and other genomic features. The analysis is straightforward, quick and reliable. In particular, the analysis is especially powerful for characterization of abnormalities that cannot be easily analyzed with Array-based comparative genomic hybridization (array CGH), such as balanced rearrangements and low-level mosaicism or mixed normal/abnormal cell populations that are frequently seen in cancer specimens.

The CHD7 protein, mutated in CHARGE syndrome, binds to specific sites on chromatin. *P.C. Scacheri[1], F. Tie[1], S.R. Lalani[2], J.W. Belmont[2], F.S. Collins[3]* 1) Genetics, Case Western Reserve University, Cleveland, OH; 2) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) National Human Genome Research Institute, NIH, Bethesda, MD.

Babies born with CHARGE syndrome have multiple birth defects including coloboma of the eye, heart malformations, choanal atresia, cleft lip and palate, retardation of growth, genital abnormalities, and ear abnormalities. In 2004 it was shown that de novo mutations in the CHD7 gene (coding for chromodomain helicase DNA-binding protein 7) cause most cases of CHARGE syndrome, but little information has been available about the normal function of the protein. Based on homology to other proteins in the CHD family, we hypothesized that CHD7 is located in the nucleus and is associated with chromatin. To address these hypotheses we made antibodies to CHD7 and tested them by subcellular fractionation and Western blot analysis as well as by chromatin immunoprecipitation on tiled microarrays (ChIP-chip). Our results reveal for the first time that: 1) CHD7 is a nuclear protein; 2) CHD7 is physically associated with chromatin; and 3) CHD7 binds to promoters, at or near transcriptional start sites. The ChIP-chip data also indicate that CHD7 does not bind globally to all promoters, but rather co-localizes to a subset of those specifically marked with methylation of lysine 4 on histone H3 (H3K4me), suggesting that CHD7 targets a specific subset of transcriptionally active genes. Some of the CHD7 target genes, including several genes within the HOXA cluster, suggest clues to mechanisms underlying the complex phenotype of CHARGE syndrome. We hypothesize that CHD7 is involved in transcription regulation, and that haploinsufficiency of CHD7 affects expression of multiple CHD7 target genes during development, leading to birth defects in several organ systems.

Coupling of UPR Induction with Aminoglycosides: A Potential Therapeutic Strategy for Genetic Disorders Caused by Nonsense Mutations. *N.A. Sharifi, H.C. Dietz* Inst of Genet Med, Johns Hopkins Univ Sch of Med and HHMI, Baltimore, MD.

It is estimated that nonsense mutations are seen in up to 20% of all human disease alleles. The presence of a PTC does not usually lead to production of truncated peptides since the majority of nonsense transcripts are recognized and degraded by a translation-coupled and highly efficient process known as nonsense-mediated mRNA decay (NMD). Recent attempts to treat diseases caused by nonsense mutations with readthrough-promoting agents (e.g. aminoglycosides) have shown limited and highly variable success in model systems and in people. A near uniform finding is that these agents do not increase the amount of nonsense transcripts. Thus, despite high readthrough potency for selected drugs, the opportunity is simply lacking in the face of ongoing NMD. While simultaneous inhibition of NMD and promotion of readthrough represents a potentially potent strategy, the toxicity of known drugs that inhibit NMD precludes their use in both animal models and people. Our microarray analysis of physiologic mRNA substrates for mammalian NMD revealed significant overlap with transcripts that are upregulated by the unfolded protein response (UPR). Initiation of the UPR in response to diverse forms of cellular stress induces phosporylation of eIF2 and the subsequent inhibition of bulk translation, while maintaining selective translation of proteins that would promote cellular survival (e.g. ATF3). It seemed possible that either inhibition of NMD leads to the production of truncated proteins that then initiate the UPR, or that the induction of UPR and subsequent relative inhibition of translation inhibits NMD. We provide definitive experimental evidence that only the latter is true. Furthermore, the inhibition of NMD was both potent and observed in all tested nonsense transcripts. Furthermore, we show that geldanamycin and its less toxic derivative 17AAG, which are in phase II clinical trials for anti-tumor activity in people, both induce the UPR and inhibit NMD. These agents maintain sufficient residual translation to support readthrough protein production in the presence of aminoglycoside, constituting a complete strategy for the treatment of diseases caused by PTCs.

Genetic vs. environmental hypotheses of disease causation: the case of autism. *H.K. Tabor, M. Mohindra, A. Boyce, M. Cho* Stanford Ctr for Biomedical Ethics, Stanford University, Palo Alto, CA.

The completion of the Human Genome Project has led to an increase in association studies of complex traits, or traits with both genetic and environmental components. The proliferation of these studies has led to conflict around how causation is determined and what it means if a disease is environmental or genetic in origin. To examine how causation is framed for complex traits, we examined a disease with a history of such discussions: autism. While the genetic component of autism is well established, no single genes have been conclusively identified that account for the majority of cases. Furthermore, potential environmental causes of autism have received a great deal of media attention, leading to public debate about disease causation. We are analyzing the report Immunization Safety Review: Vaccines and Autism published by the National Academy of Sciences in 2004. This report examined the hypothesis that vaccines are causally associated with autism. We analyzed the text of the report itself and of the public submissions to the committee, evaluating the evidence and language used to support causation models using the causal criteria originally proposed by the epidemiologist Austin Bradford Hill. Our preliminary analyses suggest that groups supporting environmental causes emphasize different causal criteria and evidence than those arguing against environmental causes. Environmental cause advocates tend to emphasize criteria of temporal sequence, biological gradient, and experimental evidence, while environmental cause opponents tend to cite strength of association and consistency of evidence. Both perspectives cited specificity, biologic rationale, coherence, and analogous evidence. Our preliminary analysis suggests that consensus in the scientific and public arenas may be less dependent on the strength or quantity of certain kinds of evidence and more on the ability to reconcile differing priorities for causal criteria. These differing priorities may reflect the social implications of different causal pathways and of the agendas of the groups that advocate for them.

Identifying schizophrenia-associated non-coding variants in the NOS1AP gene. *N. Wratten[1], H. Memoli[1], M. Azaro[1], J. Messenger[1], J. Hayter[1], S. Buyske[2], A. Bassett[4,5], E. Chow[4,5], L. Brzustowicz[1,3]* 1) Dept Genetics, Rutgers University, Piscataway, NJ; 2) Dept Statistics, Rutgers, NJ; 3) Dept Psychiatry, UMDNJ-NJMS, Newark, NJ; 4) Clinical Genetics Research Program, University of Toronto, ON, Canada; 5) Dept Psychiatry, University of Toronto.

The *NOS1AP* locus has been implicated by both linkage and association studies in pedigrees of European descendents as a schizophrenia susceptibility gene. Studies in the rat indicate that the NOS1AP protein functions in the NMDA receptor pathway and is therefore a good candidate gene for schizophrenia. Furthermore, genotype data from different populations suggest that multiple independent regions within the 300 kb *NOS1AP* genomic locus are associated with schizophrenia. In our pedigrees none of the identified coding SNPs are associated with disease, indicating that the mutations(s) are located in regulatory sequences. Evidence from expression studies of the various *NOS1AP* transcripts suggests that this gene contains multiple regulatory modules. We performed a comprehensive screen of the *NOS1AP* gene for schizophrenia-associated regions by genotyping almost 70 markers selected using a tag-SNP approach to minimize LD between markers. Genotype data was generated using a multiplex PCR-LDR assay and analyzed for association with schizophrenia. Approximately 40% of markers in the large second intron showed association with schizophrenia including a SNP within a 1 kb region that is highly conserved within mammals. This sequence was tested for regulatory function using a luciferase reporter assay and found to act as a repressor of the *NOS1AP* promoter in neuronal cell culture. Comparison of the two allelic variants of this regulatory sequence indicated some difference in the level of repression between alleles. However, the difference in repression conferred by the alleles was dependent on the neuronal cell line used and could reflect differences in transcription factor composition between cell lines. Future experiments will test whether this region and other schizophrenia associated regions from *NOS1AP* function in rat primary neuronal cell culture.

Successful bone marrow transplant in a child with mevalonic aciduria. *V. Valayannopoulos[1], B. Neven[2], P. Quartier[2], S. Romano[1], D. Rabier[3], MO. Rolland[4], L. Cuisset[5], AM. Prieur[2], A. Fischer[2], P. Delonlay[1]* 1) Metabolic Ped, Necker-Enfants Malades Hosp, Paris, France; 2) Ped ImmunoHematology; 3) Biochemistry Lab, Necker-Enfants Malades Hosp, Paris, France; 4) Biochemistry Lab, Debrousse Hosp, Lyon, France; 5) Genetics Lab, Cochin Hosp, Paris, France.

Mevalonic aciduria (MA) (OMIM +251170) is a rare inborn error of isoprene biosynthesis caused by a deficient activity of mevalonate kinase (MVK) resulting from mutations in the MVK gene. The neonatal phenotype is characterised by severe periodic fever attacks, ataxia, failure to thrive and cataracts. The prognosis is usually poor and children die in early infancy. The mechanism of recurrent inflammatory disease is unclear. Serum levels of pro-inflammatory cytokines are increased during attacks. We hypothesized that replacement of the haematopoietic system by a bone marrow transplant (BMT) from a healthy geno-identical donor may improve inflammatory symptoms. We report a 2.5 years old boy with mevalonic aciduria diagnosed at the age of 3 months upon elevated mevalonic acid in urine, confirmed by severely impaired activity of mevalonate kinase in lymphocytes and a homozygous mutation in the MVK gene. He presented at birth with low birth weight, severe anemia, hepatosplenomegaly and liver disease. Recurrent episodes of fever were noted every two weeks, with transient rash and abdominal pain. He had failure to thrive and mild cerebellar ataxia. The effect of several anti-inflammatory drugs on febrile attacks was very transient. Because of the very severe condition of the child, we proposed a BMT from a geno-identical heterozygous sister. Hematological recovery was achieved on day 21 post BMT with a 96% donor chimerism. We are now 5 months since the BMT and the child has presented no new fever attack and no complication. The volumes of liver and spleen have significantly decreased. Body growth is good and psychomotor development improved. Mevalonate aciduria has decreased significantly. Mevalonate kinase activity has not been tested yet due to lymphopenia. In conclusion, BMT is a feasible procedure in MA patients and may be efficient to prevent inflammatory attacks. However, these encouraging data are still preliminary.

Association Study of RANTES Polymorphisms in Childhood-Onset Systemic Lupus Erythematosus and Juvenile Rheumatoid Arthritis in Mexican Population. *J. Ramirez[1], V. Baca[2], R. Velazquez[1], M. Morales[1], F. Espinosa[3], L. Orozco[1]* 1) Laboratory of Research,National Institute of Genomic Medicine,Mexico City,Mexico; 2) Department of Pediatric Rheumatology, CMN-Siglo XXI,IMSS.Mexico City, Mexico; 3) Department of Immunology,INP,SS.Mexico City,Mexico.

Introduction. Recent studies in Asian populations have found two single nucleotide polymorphisms (SNPs) in the promoter region of RANTES gene (-403 G/A and -28C/G), to be associated with increased risk of developing systemic lupus erythematosus (SLE) and rheumatoid arthritis. RANTES -28C/G SNP was associated with childhood-onset SLE, while in adult SLE patients the two SNPs did not correlate with SLE as individual polymorphisms. However, a compound genotype (-403 G/G and -28C/C) was significantly more frequent in SLE than controls, and the -403 G/A SNP was associated with lupus nephritis. Otherwise, the -403A allele was associated with RA susceptibility. Objective. To asses the possible association between RANTES -403 G/A and -28C/G polymorphisms and childhood-onset SLE and juvenile rheumatoid arthritis (JRA) in Mexican population. Methods. We performed a case-control association study in 250 pediatric patients with SLE, 115 patients with JRA and 265 healthy Mexican controls. Genotyping was carried out by TaqMan. The genotypes and allele frequencies were compared between cases and controls by x^2 test. Results. There were no significant differences in the distribution of genotypes and allele frequencies in the RANTES promoter polymorphisms between SLE patients and controls. Neither association with lupus nephritis or SLE with compund genotypes was observed. Otherwise, there was a significant difference in the -403A allele frequency between JRA patients and controls (35% versus 27%, P = 0.03; OR = 1.4, 95% CI = 1.04-2.01), however the association was no longer statistically significant after correcting the P value (P = 0.06). Conclusion. Our results suggest that -403 G/A and -28C/G SNPs in RANTES gene are not associated with childhood-onset SLE susceptibility or lupus nephritis in Mexican population. However, we could not rule out completely the relationship of the -403A allele with JRA due to the small sample size.

Methyl Primer Express Software and influence of amplicon characteristics to success rate in sequencing of bisulfite treated DNA. *E.S. Vennemeyer[1], A.A. Pradhan[1], A. Rico[2], B. Finkelnburg[3], M.F. Fraga[4], C. Ferrero[4], M. Esteller[4]* 1) Applied Biosystems, Foster City, CA; 2) Applera France S.A; 3) Applera Deutschland, GmbH; 4) 4Cancer Epigenetics Laboratory, Spanish National Cancer Centre (CNIO), Madrid, Spain.

A well known method to study methylation patterns is to treat gDNA by sodium bisulfite to distinguish methylated cytosine (5mC) from unmethylated C, which is deaminated to uracil (U) and replaced by thymine (T) in subsequent amplification. 5mC still remains as C. Subsequent amplification can focus on selective amplification of methylation patterns in CpG islands (methyation specific PCR, MSP) or on amplification of bisulfite treated (converted) gDNA (Bisulfite treatment specific PCR, BIS). Selection of PCR focus is done by primer design. After PCR, sequencing can clarify the methylation pattern but several factors must be taken into account to ensure reliable data. During the bisulfite treatment base composition will undergo dramatical changes. This must be taken under consideration during primer and amplicon design for the initial amplification. Dependend on the base composition of the target region the design may change the originally focused strategy for sequencing. In first instance, strategy is depending on preferred outcome: 1. More or less methylated? Which CpG in target region is differentially methylated? Direct sequencing of PCR products. 2. Semi-quantitative results Cloning of PCR products and sequencing of multiple clones. In a second instance, the base composition of the amplicon itself will lead to the conclusion, whether direct sequencing of PCR products can be done or not. Here we use a new PC Software called Methyl Primer Express to design BIS oligos on different promotor-target regions to show examples for critical amplicons and recommendations for successful amplification and sequencing.

A QTL influencing both serum PAF-AH activity and LDL cholesterol concentration maps to the baboon ortholog of human chromosome 2p. *A. Vinson[1], M.C. Mahaney[1,2], L.A. Cox[1,2], J. Rogers[1,2], J.L. VandeBerg[2], D.L. Rainwater[1]*
1) Southwest Foundation for Biomedical Research, San Antonio, TX; 2) Southwest National Primate Research Center, San Antonio, TX.

Given their correlated contributions to cardiovascular disease (CVD) risk, biomarkers of inflammation and lipoprotein metabolism may share genes that influence risk of CVD. In this study, we analyzed variation in the activity of serum platelet-activating factor acetylhydrolase (PAF-AH, an enzyme associated with inflammation) and low-density lipoprotein cholesterol concentration (LDL-C, a risk factor for CVD), from 650 pedigreed baboons (*Papio hamadryas*) fed a low-cholesterol, low-fat basal diet. We estimated the genetic correlation between PAF-AH and LDL-C at 0.51 (p0.00001), indicating that approximately 26% of the additive genetic variance in each trait was attributable to the effects of the same gene(s). To locate genes with common effects on variation in serum PAF-AH activity and LDL-C concentration, we performed both univariate and multivariate multipoint linkage scans of the baboon genome. Our univariate analyses found significant evidence for a QTL affecting serum PAF-AH (LOD=2.79, genome-wide p=0.0395) and suggestive evidence for a QTL affecting LDL-C (LOD=2.16). Both QTLs were located at the same point on the baboon ortholog of human chromosome 2p, in a region corresponding to 2p24.2-23.3. We used bivariate linkage analysis to test the hypothesis that a single QTL explains some or all of the additive genetic correlation between serum PAF-AH activity and LDL-C concentration. This bivariate analysis improved evidence for the QTL (LOD=3.19, genome-wide p=0.0148) over that obtained in the univariate screens for each trait. Likelihood ratio tests of the QTL-specific genetic correlation between the traits ($_Q$=0.62) rejected hypotheses of co-incident linkage (P[$_Q$=0]=0.0465) and complete pleiotropy (P[$_Q$=1]=0.0217). We interpret this partial QTL pleiotropy to be consistent with sharing by PAF-AH and LDL-C of the effects of some, but not all, QTL polymorphisms. We conclude that a QTL mapping to human chromosome 2p24.2-23.3 affects variation in both serum PAF-AH activity and LDL-C concentration.

Single-exon deletion or a large deletion of 42 exons in dystrophin gene can be detected with multiplex ligation-dependent probe amplification (MLPA) in Chinese DMD patients. *X.Z. Wang[1], F. Yao[2], N. Zhong[1,3]* 1) Peking University Center of Medical Genetics, Beijing, China; 2) Peking Union Medical University, Beijing, China; 3) New York State Institute for Basic Research, Staten Island, NY.

Duchenne muscular dystrophy (DMD) is one of the most common X-linked recessive neuromuscular diseases. It is caused by genetic defects of dystropin gene with deletion, duplication, or point mutation that results in muscle fatigue and dystrophy. Usually, gene deletion of one or more exons of dystrophin accounts for about 55-65% of DMD/BMD patients, duplication for about 5-10% patients, and point mutation for 25% patients. Most of hotspot deletion of dystrophin can be detected by a classical multiplex PCR and the point mutation can be detected by PCR/sequencing analysis, however, it remains a challenge to detect duplication. The multiplex ligation-dependent probe amplification (MLPA) is an efficient procedure that can accurately analyze the copy number and deletion mutation of whole dystropin gene. We have developed a protocol with two reactions for simultaneous detection of entire dystropin gene. Each reaction may analyze 39.5 exons of dystrophin gene. The MLPA was applied to study 16 DMD/BMD patients who were collected in Beijing area. Deletion can be detected among 10 patients with MLPA. Seven patients were found to have deletion at previously reported hotspot regions from exon 45 to exon 55, which can be detected with a classical multiplex PCR. Two patients who had not been found deletion by the classical multiplex PCR could be detected with MLPA to have rare deletions of a single exon 18 or exon 43. A large fragment deletion spanning 42 exons from exon 3 to exon 44 was detected in one patient. Compared to the classical complex PCR, our MLPA protocol may increase the detecting rate and provide a simple, rapid and accurate method for both hotspot and non-hotspot regions.

When Autism Meets Neoplasia? - the *PTEN* Hamartoma Tumor Syndrome. *J. Stein[1], K. Holland[2], C. Eng[1], M.R. Natowicz[1]* 1) Genomic Medicine Institute, Cleveland Clinic, Cleveland, OH; 2) Division of Pediatric Neurology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Autism is an etiologically heterogeneous disorder; different chromosomal and monogenic causes are typically noted in <10% of cases and in most cases a specific etiology is not established. *PTEN* hamartoma tumor syndrome (PHTS) is an autosomal dominant disorder encompassing a variety of clinical syndromes including Bannayan-Riley-Ruvalcaba syndrome (BRRS). Clinically, BRRS is characterized by macrocephaly, lipomas, hemangiomas, hamartomatous intestinal polyposis, and pigmented macules of the penis. Neurological manifestations of BRRS include developmental delay and myopathy. We report a boy with macrocephaly, seizures, hypotonia, markedly delayed speech and cognitive development, lipomas, multiple intestinal polyps, pigmented macules of the penis, hemangiomas, scrotal tongue and autism associated with a de novo germline mutation in the *PTEN* (831-833insT). This case adds to the few published reports regarding a possible association of autism spectrum disorders in those with *PTEN* mutations. The only other published report of a family with classic Cowden syndrome with autistic features also had a germline *PTEN* truncating mutation. While our patient has classic features of BRRS, we have (Butler *et al.*, J Med Genet 2005) identified germline *PTEN* mutations in 3 of 18 children ascertained by macrocephaly and autism alone. In contrast to patients presenting with BRRS and autism, these germline *PTEN* mutations are all missense. Autism spectrum disorders as a true component of PHTS, is supported not only by of our patient-based observations, but by a neuron-specific *pten* knock-out murine model that appears to display features of autism (Kwon *et al.*, Neuron 2006). Therefore, PHTS needs to be seriously considered in individuals with autism who have even only a few major features associated with BRRS or with extreme macrocephaly, particularly in view of the genetic ramifications and medical surveillance implications of this diagnosis.

Copy number analysis and allele quantitation by 60,000 plex Molecular Inversion Probes (MIPs) on GeneChip arrays using 75ng genomic DNA. *Y.K. Wang, M. Moorhead, S. Lin, C. Chen, T.D. Willis, G. Karlin-Neumann, M. Faham* Affy Lab, Affymetrix, South San Francisco, CA.

Copy number polymorphism (CNP) has been identified in recent years as one of the many sequence variations occurring to the human genome. CNP could underlie mechanisms of segmental duplication, fragile sites and regulation of gene expression. Copy number change is also a hallmark of tumor cells. Distinguished patterns of amplification / deletion are associated with various tumor types at specific stages of tumorgenesis. Many chip based technologies, including Bacterial Artificial Chromosome (BAC) CGH, cDNA CGH, and oligonucleotide arrays have been used to address the issues. We are developing technology for copy number analysis based on Molecular Inversion Probes (MIPs). Advantages of this technology include: 1. Sensitivity: Detection of single copy changes. Our performance evaluation is based on false positive and false negative rates using 1X & 3X samples as known single copy changes evaluated against known diploid samples. 2. Customization: Ability to focus on the most relevant genomic segments. 3. Preservation of allele information: loss-of-heterozygosity (LOH) and other disturbances in the allele ratio can be detected. 4. Ability to work with FFPE (formalin fixed paraffin embedded) samples: Given the small probe footprint, degraded samples can work successfully with MIP. We have further developed the protocol in order to use a small amount of DNA (75ng total) and to have 60,000 plex reactions in a single tube. Algorithms have also been developed to 1) correctly assess the data quality of every sample that has been assayed, including highly disintegrated tumor genomes; 2) correct for non-linear effects in signal response (such as saturation at very high copy numbers) so that the inferred copy number measurements are accurate over a wide dynamic range. Examples will be given in the presentation to highlight all the above.

Linkage and association of the CHD7 gene with susceptibility to adolescent idiopathic scoliosis. *C. Wise[1], X. Gao[1], D. Gordon[2], J. Gillum[1], R. Browne[1], C. Helms[3], D. Zhang[1], S. Shoemaker[4], M. Lovett[3], A. Bowcock[3], J. Herring[4]* 1) Seay Center for Musculoskeletal Research, Scottish Rite Hospital, Dallas, TX; 2) Department of Genetics, Rutgers, The State University of New Jersey; 3) Department of Genetics, Washington University School of Medicine, St. Louis, MO; 4) Dept. Orthopaedics, Texas Scottish Rite Hospital, Dallas, TX.

Idiopathic scoliosis (IS) (OMIM 181800) is the most common spinal deformity in children, with a prevalence of 1-2%. The underlying etiology of IS is unknown, although inheritance is complex and linkages to chromosomes 6p, 8q, 9q, 10q, 15q, 16q, 17p, 18q, 19p, and Xq have been described by us and others. A family harboring a co-segregating pericentric inversion of chromosome 8 (inv(8)(p23q11.21) has also been described, where one of the breakpoints resides near a previously detected linkage peak. When a new cohort of 52 IS families was evaluated for linkage to candidate regions as part of a linkage follow-up study, strongest evidence for linkage was obtained with 8q loci (multipoint LOD = 2.77; NPL P = 0.001). Two linked loci also displayed association with IS susceptibility (TDTae < .02). The gene encoding the chromodomain helicase DNA binding protein 7 (CHD7) lies within the interval defined by these loci and approximately 11 Mb distal to the previously reported 8q break. Coding and splice site mutations in CHD7 have been described in the CHARGE syndrome of multiple developmental anomalies. Moreover, a high prevalence (>60%) of later-onset scoliosis has been reported in surviving CHARGE syndrome patients. Linkage and association studies of SNPs within CHD7 in the 52 families revealed overlapping peaks of linkage (two-point ASP LOD = 2.08, P = 9.8x10-4) and association with TDTae P < .005 for multiple loci in a 57 kb block of relatively high linkage disequilibrium encompassing exons 2-4 within CHD7. Our results identify CHD7 as the first candidate gene for IS susceptibility mapped by linkage, and provide insight into its underlying pathogenesis. These findings also suggest possible etiological overlap between more rare, early-onset (CHARGE) and common, later-onset (IS) developmental diseases.

The NCBI RefSeq Project: maintaining annotated genomes. *K. Pruitt* National Center for Biotechnology Information, DHHS/NIH/NLM, Bethesda, MD.

Deriving benefits from genomic sequencing, especially in the area of health care applications, depends on the availability of high-quality sequence and annotation. The National Center for Biotechnology Information (NCBI) supports the maintenance of, effective use of, and access to annotated genomes in several ways including: (a) calculating periodic updates to the annotation; (b) supporting updates of the assembled genome sequence (human and mouse); (c) providing numerous cross-linked resources that are critical to integrating sequence data with biological knowledge; (d) providing the RefSeq collection.

The Reference Sequence (RefSeq) project provides curated sequence standards for proteins, transcripts, and genomes and is used internationally as a reagent for annotating several genomes. Since sequence annotation is provided by more than public resource using different methods, the resulting information is not always identical; the inconsistency in both annotation results and the data presentation style among public annotation resources hampers use of the human genome data and may confound interpretation. The Consensus CDS (CCDS) project was established as a collaboration to identify validated protein coding sequences that have been annotated identically on the human genome by both major providers of annotation data: (a) NCBI (as displayed in the Map Viewer), and (b) WTSI and EBI (as displayed in the Ensembl and UCSC genome browsers). The CCDS collaboration includes ongoing curation of protein coding genes by the collaboration members (NCBI, EBI, WTSI, UCSC). The RefSeq collection is an integral component of the CCDS project; CCDS curation decisions are first reflected in updates to the RefSeq collection, and then in updates to the displayed genome annotation.

The presentation will include the current status of the RefSeq collection, with a focus on the human collection, and including aspects of: a) quality assessment; b) curation; and c) their use in maintaining and updating genome annotation including identification of consistent annotations via the CCDS project.

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Coexistence in the same family of both focal and diffuse forms of hyperinsulinism. *F. Sauvat[1], V. Valayannopoulos[2], M. Vaxillaire[3], F. Jaubert[4], J. Rahier[5], M. Ribeiro[6], M. Polak[7], C. Nihoul-Fekété[1], P. de Lonlay[2]* 1) Pediatric Surgery; 2) Metabolic Pediatrics, Necker-Enfants Malades Hosp, Paris, France; 3) CNRS UMR 8090, Lille, France; 4) Pathology Department, Necker-Enfants Malades Hosp, Paris, France; 5) Pathology Department, St-Luc Hosp, Brussels, Belgium; 6) CEA, Orsay, France; 7) Ped Endocrinology.

Neonatal hyperinsulinism (HI) is the most important cause of hypoglycemia in early infancy. The inappropriate oversecretion of insulin is responsible for profound hypoglycemia. The histopathological lesions associated with neonatal HI are diffuse or focal. Focal islet cell hyperplasia is sporadic and associated to a loss of maternal alleles from the 11p15 region in the hyperplasic lesion associated to a somatic reduction to hemi- or homozygosity of a paternally inherited mutation of the sulfonylurea-receptor (SUR1) gene. Diffuse HI may be familial and involves SUR1 or the inward-rectifying K+ channel (Kir6.2) in recessively inherited HI. We present here the first case of coexistence of both focal and diffuse neonatal HI in the same consanguineous family. The first child presented at birth with severe neonatal HI. A transhepatic selective pancreatic catheterization revealed a local insulin hypersecretion in the body of the pancreas and a limited pancreatectomy permitted to fully cure the child. A paternally inherited heterozygous mutation of the SUR1 gene has been identified along with a loss of 11p15 heterozygosity (LOH) of maternal origin in the lesion. The second child, a girl, suffered from severe neonatal HI. An 18F-fluoro-L-DOPA PET scan revealed a diffuse uptake of the radiotracer compatible with a diffuse form of HI. Molecular analysis of the SUR1 gene displayed a homozygous SUR1 mutation identical to the one found in the first child at the heterozygous form. Both parents are heterozygous for this mutation. A subtotal pancratectomy has been performed. In conclusion, genetic counseling for couples that had a child with a focal form of HI should be prudent in the case of a consanguineous couple as a heterozygous mutation can be carried by both parents that can lead to a diffuse form of HI in the following pregnancies.

Association of LRRK2 gene tSNPs with early onset Parkinsons disease. *B. Patra[1], A.J. Parsian[1], B. Racette[2], J. Zhao[3], J.S. Perlmutter[2], A. Parsian[1]* 1) University of Arkansas for Medical Sciences, Little Rock,AR; 2) Department of Neurology, Washington University School of Medicine, St. Louis, MO; 3) MRC Epidemiology Unit, Strangeways Research Laboratory, Cambridge, UK.

Pathogenic mutations within the leucine-rich repeat kinase 2 (LRRK2) gene is reported to be responsible for autosomal dominant and late-onset Parkinson disease (PD) along with other six genes that have been identified in PD families (SNCA, PRKN, PINK1, DJ-1, MAPT and UCH-L1). LRRK2 has been described recently as the most common candidate gene for familial and sporadic form of PD (SPD). Mutations in LRRK2 gene have been reported from the PD patients of all ages. Six tagging SNPs (tSNP, Skipper et al., 2005) have been found in LRRK2 that capture the variations within the gene and associated with sporadic PD. In present investigation we screened a sample of 225 familial PD (FPD), 349 SPD, and 187 matched normal controls with the six tSNPs. For tSNP rs10784486, the genotype frequency for CC was significantly (2 = 6.32, p = 0.043 0.0016 SE) higher in SPD than controls. Allele frequency of tSNP rs1388598 C was significantly higher in SPD than FPD (2 =5.48, p=0.0220.0011) and controls (2 =4.48, p=0.0340.0014 SE). The PD affected individuals were categorized into two groups based on age of onset (AON) of 50 and 51 years. Early onset PD (AON <50) showed higher significant association with tSNPs rs10784486 and rs1388598. For tSNP rs10784486 the genotypes CC and AC frequencies were significantly higher in SPD than FPD (AON < 50, 2 = 7.55, p = 0.024 0.0012 SE) and controls (2 = 6.86, p = 0.03 0.0014 SE). Similarly, the rs1388598 CC and CT genotypes frequencies were significantly higher in SPD individuals than FPD (AON < 50, 2 = 6.65, p = 0.026 0.0012 SE) and controls (2 = 9.54, p = 0.008 0.0007 SE). The allele frequency of rs1388598 C allele was significantly higher in SPD (AON <50 years) than controls (2 = 9.60, p = 0.003 0.0004 SE) and FPD (AON < 50, 2 =7.15, p=0.010.0008 SE). We can conclude from our study that variations within LRRK2 gene have strong association with SPD than familial form and may also contribute to onset of SPD.

Investigation of variants within dopamine pathway genes on risk of Parkinsons disease. *J.M. van der Walt, W.K. Scott, G. Mayhew, M.A. Hauser, Y.J. Li, K. Fujiwara, J.M. Vance, E.R. Martin* Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC.

Dopamine is a vital neurotransmitter responsible for controlling both mental and physical health. Dopaminergic neurons degenerate in Parkinson's disease (PD) leading to motor defects observed in patients. We examined polymorphisms within the dopamine beta-hydroxylase (DBH) gene and the aldehyde dehydrogenase 1 family member A1 (ALDH1A1) gene for association with PD risk. These genes were chosen based upon their biological function and location in an area of linkage on chromosome 9q. DBH catalyzes the conversion of dopamine to noradrenaline and has been previously implicated as a protective genetic factor for PD. ALDH1A1 acts in the detoxification of aldehydes that result from the metabolism of dopamine by monoamine oxidase. We investigated the effect of the protective polymorphism located within the promoter region of DBH plus six SNPs including both tagging and supplementary SNPs located throughout the gene for association with PD in a large family-based study (N=760). No significant association was observed between PD and any SNP by the pedigree disequilibrium tests (PDT) or genotype-PDT tests in our overall dataset. We also analyzed 11 SNPs throughout ALDH1A1 and demonstrated significant association in the early onset PD families (age at onset 40 years; N=110) with four markers: rs918836 (5 UTR;p=0.01), rs595958 (intronic; p=0.02), rs8187915 (exon 3, synonymous; p=0.006) and rs2017362 (intron; p=0.01). All associated SNPs are in moderate linkage disequilibrium (r2 range=0.4 - 0.6). Since ALDH1A1 also contributes to the metabolism of ethanol, we examined the interaction of SNPs with alcohol consumption of PD patients using GEE models. A significant interaction was detected with exonic SNP rs81879125 and ever consuming of beer, wine, or liquor (p=0.019). Our findings do not confirm the protective effect of the previously associated polymorphism in DBH; however, these results suggest that variants within ALDH1A1 might influence risk of early onset PD. Defects in ALDH1A1 may lead to impairment in dopamine turnover and cause subsequent oxidative damage within neurons.

Examination of chromosome 1q43 in multiple sclerosis. *N. Schnetz-Boutaud[1], J.L. McCauley[1], Y. Bradford[1], S.G. Gregory[2], D.M. Mortlock[1], S. Schmidt[2], J.R. Oksenberg[3], S.L. Hauser[3], L.F. Barcellos[4], M.A. Pericak-Vance[2], J.L. Haines[1]* 1) Center for Human Genetics Research and Dept of Molecular Physiology and Biophysics, Vanderbilt University Medical Center, Nashville, TN; 2) Center for Human Genetics and Dept of Medicine, Duke University Medical Center, Durham, NC; 3) University of California at San Francisco, San Francisco, CA; 4) University of California at Berkeley, Berkeley, CA.

Although the genetic influence in MS is overwhelming, studies have failed to consistently identify genes specific to MS susceptibility outside of the major histocompatibility complex (MHC). Genomic screens have identified numerous regions of interest for MS loci but, with the exception of the MHC, these studies have largely failed to replicate significant results. We previously performed a genome-wide linkage screen using a panel of 390 microsatellite markers and detected significant evidence of linkage to a region spanning approximately 7Mb within chr1q43 in a dataset of 245 multiplex families. This region is also supported by results from other MS linkage screens and linkage seen in additional autoimmune disorders such as rheumatoid arthritis and systemic lupus erythematosus.

For our follow-up of this region, we selected and tested association to MS for 768 SNPs prioritized based on multi-species conservation. This approach sought to increase the likelihood of successfully identifying an associated SNP given the hypothesis that nucleotides strongly conserved across species are of greater biological importance than those which are less conserved. Association analyses of these follow-up markers revealed multiple (>60) nominally significant (p 0.05) results in an expanded dataset of multiplex and singleton MS families. While none of these markers alone demonstrate overwhelming significance, several of the significant SNPs are clustered within small genomic regions. Furthermore, multiple markers within the *RYR2*, *FMN2*, and *RGS7* genes were significant. Thus these genes are the strongest candidates for involvement in the etiology of MS and are being examined in further detail.

Analysis of nine autosomic STR loci on recent human phylogenies of southeast of Brazil. *R. Silva[1], A. Debes-Bravo[2], L. Morganti[2], S. Bydlowski[2,3], R. Moura-Neto[4]* 1) Instituto de Biofisica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Brazil; 2) Divisão de Pesquisa e Biologia Molecular, Fundação Pró-Sangue Hemocentro de São Paulo, Brazil; 3) Departamento de Hematologia, LIM-31, Faculdade de Medicina da Universidade de São Paulo,Brazil; 4) Instituto de Biologia, Universidade Federal do Rio de Janeiro,Brazil.

We have investigated the use of nine STR loci (CSF1PO, TPOX, TH01, vWA, F13A01, FESFPS, F13B, D16S539, D7S820, and D13S317), routinely applied in forensic analysis, for delineating phylogenetic relationships among 5 different geographic groups in Southeast of Brazil, comprising the three major ethnic groups (European-, African-, and Asian-descents). The resulting tree topology was consistent with the ethno-history and ethno-partitioning, corroborating data obtained using classical genetic polymorphisms. The results showed a low coancestry coefficient value across our population. The highest value obtained was 0.0159, taking the São Paulo Asian-descendants and Rio de Janeiro European-descendants. The Brazilian sample data corroborate the notion of human phylogenies in well-defined clusters of ethno geographic origins: African, Caucasian, Greater Asians and Amerindians. It seems reasonable to assume the absence of any population substructure in the São Paulo and Rio de Janeiro databases. Our data on coancestry coefficient (= 0.0045) do not support any calculations that may infer ethnic background and the UPGMA tree analysis has shown no significant genetic distance among the studied groups. Therefore, the profiles of nine STRs on both analyses, PCA and Genetic Distance Analysis were essentially similar and agreed to those data described for other genetic systems already described. Supported by: CNPq, FAPERJ,

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MAPK Pathway Mutations in CFC Syndrome: Delineation of Molecular Heterogeneity and Genotype-phenotype Correlation. *K.A. Rauen, P. Rodriguez-Viciana, A.L. Estep, W.E. Tidyman* University of California, San Francisco, CA.

Cardio-facio-cutaneous (CFC) syndrome is a multiple congenital anomaly disorder in which individuals have characteristic dysmorphic craniofacial features, cardiac defects, ectodermal anomalies, developmental delay and hypotonia. We recently reported that CFC is caused by alteration of activity through the mitogen-activated protein kinase (MAPK) pathway due to heterogeneous de novo mutations in dual specificity protein kinases B-Raf, MEK1 and MEK2 (Rodriguez, et al., Science. 2006. 311:1287-90.). In this study, we sequenced 13 additional individuals with the clinical phenotype of CFC including two HRAS-mutation negative individuals who phenotypically meet criteria for the clinical diagnosis of Costello syndrome (Estep, et al., Am J Med Genet A 140:8-16.2006). Novel missense BRAF mutations in exons 13 and 16 (the protein kinase domain) were identified that have not been previously described in CFC individuals (Rauen, Am J Med Genet. In Press. 2006). In addition, heterogeneous missense BRAF mutations in exon 11 and 12, novel small in-frame deletions in BRAF exon 11, and a novel MEK2 missense mutation in exon 3 were identified. As more heterogeneous mutations are identified, a genotype-phenotype correlation is emerging. CFC individuals with Q257R B-Raf mutations have many phenotypic features in common including characteristic facies, cardiac defects, short stature, failure to thrive, abnormal brain imaging, musculoskeletal and ocular abnormalities and relatively mild developmental delay. In contrast, individuals with the kinase impaired G596V B-Raf substitution have a milder phenotype as indicated by normal growth and development, and no cardiac, GI or brain abnormalities. Individuals with missense amino acid substitutions reported altered in cancer appear to have more severe phenotypes. CFC syndrome is genetically heterogeneous with several genes within a defined pathway being causative including BRAF, MEK1 and MEK2. As larger cohorts of patients are studied, genotype-phenotype correlations are emerging. In addition, molecular testing will assist in clarifying the correct diagnosis and subsequently will lead to the appropriate management for these patients.

Identifying candidate genes associated with Autism by whole genome tilepath microarray analysis. *J. Virgadamo[1], J.J. Connelly[1], R. Abramson[2], M. Cuccaro[1], J.P. Hussman[3], J.M. Vance[1], M.A. Pericak-Vance[1], S.G. Gregory[1]* 1) Duke Center for Human Genetics, DUMC, Durham, NC; 2) Dept of Neuropsychiatry, SOM-USC, Columbia, SC; 3) Hussman Foundation, Ellicott City, MD.

Autistic disorder (AutD) is a neurodevelopmental disorder characterized by disturbances in social, communicative, and behavioral functioning. Several linkage screens and numerous association studies have been carried out, however a consensus gene implicated in the etiology of AutD has yet to be identified. It has been established that at least 5% of individuals with idiopathic autism contain chromosomal rearrangements, suggesting that genomic loss or gain could underlie the development of AutD. Here we describe use of whole genome BAC tilepath microarrays, at 100kb resolution, to identify regions of chromosomal rearrangement within 121 probands from our unique multiplex AutD families. We have identified 85 regions of large genomic gain or loss (average size 0.8 Mb) contained within at least one or more of the 121 individuals within our dataset. Rearranged regions were defined as those containing a minimum of 3 BAC clones with the same trend of loss or gain within a sliding window of 6 clones. Twenty-eight of these regions contain known copy number polymorphisms (CNPs); 10 contain known segmental duplications; 1 was a novel region of CNP from our control experiments using 54 phenotypically normal individuals; and 46 contained no known duplication or CNP. A total of 15 regions localized to previous linkage data, while 12 of the regions did not contain any known genes. We also identified small regions of loss or gain (individual BAC clones, ~0.1Mb) within at least 10 of the 121 probands in our analysis. Clones needed to show loss or gain across multiple print runs and control individuals, not contain known CNPs and needed to be correctly localized by BAC end sequence. Twenty-seven clones showed genomic loss or gain, including 17 which contained genes and four which localized to known regions of genetic linkage. We are currently following-up these regions by dye-swap arrayCGH experiments and real-time quantitative PCR verification.

Common SNPs in *TCF7L2* are reproducibly associated with type 2 diabetes and reduce the insulin response to glucose in non-diabetic individuals. *R. Saxena[1,2], L. Gianniny[2], N. Burtt[2], V. Lyssenko[3], C. Giuducci[2], M. Sjögren[3], J. Florez[1,2], P. Almgren[3], B. Isomaa[4], M. Orho-Melander[3], U. Lindblad[3,5], M. Daly[1,2], T. Tuomi[4], J.N. Hirschhorn[2,6,7], K. Ardlie[2,8], L. Groop[4], D. Altshuler[1,2,7]* 1) Massachusetts General Hospital, Boston, MA; 2) Broad Institute of Harvard and MIT, Cambridge, MA; 3) Lund University, Malmö, Sweden; 4) University of Helsinki, Helsinki, Finland; 5) Skaraborg Institute, Skövde, Sweden; 6) Childrens Hospital, Boston, MA; 7) Harvard Medical School, Boston, MA; 8) Genomics Collaborative Inc., Cambridge, MA.

Recently, common non-coding variants in the *TCF7L2* gene were strongly associated with increased risk of type 2 diabetes in samples from Iceland, Denmark and the US. We genotyped 13 SNPs across *TCF7L2* in 8,310 individuals in family based and case-control designs from Scandinavia, Poland and the US. We convincingly confirmed the previous association of *TCF7L2* SNPs with risk of type 2 diabetes (rs7903146T OR 1.40 (95% CI 1.30 - 1.50); $P = 6.74 \times 10^{-20}$). In non-diabetic individuals, the risk genotypes were associated with a substantial reduction in the insulinogenic index derived from an oral glucose tolerance test (risk allele homozygotes have half the insulin response to glucose of non-carriers; $P = 0.003$), but not with increased insulin resistance. These results suggest that *TCF7L2* variants may act through insulin secretion to increase risk of type 2 diabetes.

Case Report: short rib-polydactyly and macrocephaly, a novel syndrome? *H.Y.C. Wanderley, L.O. Dewes, O.A.P. Artigalas, C. Deutschendorf, R. Giugliani, J.C.L. Leite* Medical Genetics Service, Hospital de Clínicas de Porto Alegre, Porto Alegre, Rio Grande do Sul, Brazil.

The proband is the third child of his parents. He has 2 older sibs. Maternal age at the time of his birth was 31 years old and the paternal age was 37 years old. There was history of a previous miscarriage. Family history was negative for malformations or consanguinity. During pregnancy was detected rib shortening and polydactyly and he was delivered at 38 weeks gestation. Birth weight was 3185 gr. (p25), length 46,5 cm (p5), OFC: 41 cm (p97) . The Apgar scores were 1/4/8. At the first day of life he developed respiratory distress and was transferred for the Neonatal Intensive Unit Care, needing mechanical ventilation. On clinical examination he has a macrocephaly, wide anterior fontanels, small nose, epicantus, lobulated gums, multiple oral frenula, short and narrow thorax, polydactily in hands and feet. X-rays showed narrow thorax with short ribs, shortening of long bones, hands and feet with postaxial polydactily, hypoplasic iliac bones and acetabular dysplasia hook-like downward protusion. These findings suggest Ellis-van Creveld Syndrome (polydactyly, narrow thorax, oral frenula) but macrocephaly is not found in the syndrome. The groups of the short rib-polydactyly syndromes is formed by many entities with little variation among them. The one closest to our case is the Beemer-Langer Type, with findings like craniofacial anomalies, hydrocephaly, small thorax, short limbs, polydactyly, oral frenula, flat face, hipertelorism. The clinical-radiographic features do not allow a definite distinction between the two syndromes, neither others short-ribs syndromes, leading to an overlaping phenotype or a novel syndrome.

Microarray expression profiling identifies genes with altered expression in the developing brain of an AGTR2-deficient mouse. *T. Pawlowski[1,2], S. Heringer-Walther[3], C-H. Cheng[2], J. Archie[1], C-F. Chen[2], T. Walther[3], A.K. Srivastava[1,2]* 1) Greenwood Genetic Center, Greenwood,SC; 2) Department of Genetics & Biochemistry, Clemson University, Clemson, SC; 3) Department of Cardiology, Charité,Campus Benjamin Franklin, Berlin, Germany.

Mental retardation (MR) is the most common developmental disability, affecting cognitive function in about 1-3% of the human population. Studies in humans and mice indicated a possible role for angiotensin II type 2 receptor (AGTR2) in learning, memory, and behavior. Mutant mice lacking AGTR2 ($Agtr2^{-/y}$) were found to be significantly impaired in their learning performance and exhibited abnormal dendritic spine morphology (T. Walther, unpublished), a feature consistently found to be associated with MR. However, AGTR2 gene function in brain and the molecular mechanism by which AGTR2 exerts its physiological action remains elusive. In order to identify transcripts in the developing brain that depend on AGTR2 function, we analyzed RNA isolated from brains of $Agtr2^{-/y}$ and control mice at developmental stage E15 and at birth using Agilent whole mouse genome 44K arrays. Gene expression profiles of the $Agtr2^{-/y}$ brain samples were compared to profiles of the control brains. A Q-test followed by a t-test (FDR0.1) produced a list of 39 differentially expressed genes at E15 in $Agtr2^{-/y}$ mouse brain. Data analysis using GeneSpring GX (FDR0.3 and p.001) produced a list of 133 genes (88 up-regulated, 45 down-regulated) differentially expressed at E15. A similar analysis (FDR 0.3 and p.005) in newborn brains identified a list of 397 differentially expressed genes (270 up-regulated, 127 down-regulated) in $Agtr2^{-/y}$ mouse brain. Validity of expression differences at E15 was verified by real time RT-PCR quantitation for 7 genes: Ahcy, Ube2m, Bmp2k, Syn2, Prosc, Prdm16, and Nfia. These differentially expressed genes encode molecules that are involved in multiple cellular functions. Further analysis might reveal if some of the genes influenced by AGTR2 may also contribute to the pathophysiology of mental retardation.

Detection of a de novo interstitial 2q microdeletion by CGH microarray analysis in a patient with limb malformations, microcephaly and mental retardation. *A.M. Svensson[1, 2], C.J. Curry[4], S.T. South[1], H. Whitby[1], J. Fisher[4], A.J. Brothman[1,3]* 1) Department of Pediatrics, Univ of Utah, Salt Lake City, UT; 2) Department of Pathology, Univ of Utah, Salt Lake City, UT; 3) Department of Human Genetics, Univ of Utah, Salt Lake City, UT; 4) Genetic Medicine Central California/UCSF, Fresno, CA.

We report on a 16 year old Laotian-American female, who first presented at age 2 ½ with microcephaly, developmental retardation, and skeletal abnormalities of the upper limb including mild, simple, incomplete syndactyly involving the web spaces between the second and third and the third and fourth fingers, short middle phalanges and significant clinodactyly of the fifth digit at the distal IP joint on both hands, as well as symphalangism involving the metacarpal joints of the second and fifth digits bilaterally and mildly anomalous palmar dermatoglyphics. Her lower limbs displayed symphalangism of the phalangeal-metatarsal junction of the second, third and fourth digits on both feet, with fusion of the middle and distal phalanges of the second and fifth digits and hallux valgus bilaterally. The patient is the daughter of healthy, non-consanguineous parents and has a 21-year old sister in good health. Family history is unremarkable. A G-banded chromosomal study performed at age 4 showed normal results. Following re-examination at age 15 ½, a comparative genomic hybridization analysis was performed. Genomic microarray analysis by the Spectral Genomics Constitutional Chip did not reveal any abnormalities. However, analysis using the Spectral Genomics 1 Mb Hu BAC array platform indicated a microdeletion involving two BAC clones mapping to chromosome 2 at band q31.1, suggesting partial monosomy for this region. FISH using a probe corresponding to one of the deleted BAC clones confirmed deletion of this region in the patient, but showed no deletion in either parent. The minimal size of the deletion is 1.207 Mb and the maximal size is 3.334 Mb. This small deletion is likely responsible for the patient's phenotype. The candidate genes involved in limb development in the implicated region will be discussed.

Nearly identical haploid karyotype in two tumors, masked by the larger pseudo-diploid subclone. *D.L. Van Dyke[1], S. Wei[2], K.G. Monaghan[2], P. Blunden[3], P. Mazzara[3], R. Raghavan[4], A.M. Oliveira[1], A.E. Wiktor[1], G. Keeney[1], R.P. Ketterling[1]* 1) Mayo Clinic, Rochester, MN; 2) Henry Ford Hospital, Detroit, MI; 3) St John Medical Center, Detroit, MI; 4) Loma Linda University Medical Center, CA.

Near-haploid karyotypes in cancer are very uncommon, except in acute lymphoblastic leukemia. They are observed sporadically in sarcoma and carcinoma, but are recurrent findings in inflammatory leiomyosarcoma. We describe the karyotype and genotype of two distinct tumors with strikingly similar nearly identical near-haploid karyotypes - a peritoneal mesothelioma and a retroperitoneal malignant peripheral nerve sheath tumor. The findings suggest that they share some of the same mechanisms of clonal evolution despite their distinct biology. Tumor diagnoses were confirmed by histopathology and immunohistochemistry. Cell cultures were established from tumor adjacent to the ovary (Case 1) or pelvic mass (Case 2). The karyotype of Case 1 (biphasic mesothelioma) was 27,XX,i(5)(p10),+7,der(15)t(8;15)(q24.1;p11.2),+dic(1;20)(p13;p13)[2]/54,idemx2[90]/101-108,idemx4[19]. The chromosome result of Case 2 (malignant peripheral nerve sheath tumor) was 26,X,+i(5)(p10),+7,der(15)t(1;15)(q12;p12),+20[5]/52,idemx2[20]. In both cases, reverse transcriptase polymerase chain reaction analyses were negative for the synovial sarcoma fusion genes. The karyotypes were near-haploid but diploid for 1q, 5p, 7, and 20. Chromosome 7 remains diploid in most near-haploid tumors, suggesting that monosomy or partial loss of chromosome 7 is lethal to these cells. A potential effect of near-haploidy is loss of tumor suppressor gene expression, with the remaining gene inactivated by another mechanism. For mesothelioma, other than p16, p14 and NF2, the importance of tumor suppressor genes is unknown. These cases illustrate an under-appreciated mechanism of global loss of heterozygosity by extensive chromosome loss from a diploid cell population. Near-haploid tumors may be more common than generally recognized because most exhibit a predominant near-diploid subclone masking its near-haploid origin (only two near haploid metaphase cells were found in Case 1).

Performance and data quality comparison for two genome wide association products: Illumina Sentrix Human-1 Genotyping Beadchip and Affymetrix GeneChip Human Mapping 100K Set using AREDS study samples. *Y. Tsai[1], C. Bark[1], K. Hetrick[1], H. Hobbs[1], J. Strand[1], E. Kwasnik[1], M. Barnhart[1], J. Gearhart[1], H. Chin[2], P. Dudley[2], K. Doheny[1], E. Pugh[1]* 1) CIDR, IGM, JHUSOM, Baltimore, MD; 2) NEI, NIH, Bethesda, MD.

A total of 400 case and 200 control Age Related Eye Diseases samples were analyzed on both products. Affymetrix sample/data integrity was assured via custom informatics tools integrated with Affymetrix SDKs and PE Multiprobe II worklisting. Samples were injected into GeneChips using the Multiprobe and all sample/experiment information was uploaded into the GCOS database. Illumina's automation programs were used on a Tecan robot as well as custom applications developed for the Biomek 2000 and PE Multiprobe II. Illumina sample/data integrity was assured using a custom LIMS application. The DM and BRLMM algorithms were used to analyze Affymetrix data. BeadStudio software and standard cluster file was used to analyze Illumina data. There was no manual data editing. The Affymetrix BRLMM and Illumina datasets excluded samples with call rates less than 97.5% (7 Illumina, 87 Affymetrix). The reproducibility rates for Affymetrix DM, BRLMM, and Illumina were 99.82%, 99.85% and 99.995%. The Mendelian consistency rates were 99.83%, 99.54% and 99.995%. Concordance with HapMap data for control samples was 99.75%, 99.75% and 99.64%. Overall data quality of BRLMM analyzed data was verified through comparison of 4,242 shared SNPs and 533 shared samples between the two 100K platforms. The concordance rate was 99.90%. For final release, we excluded SNPs with replicate or inheritance errors or call rates in the lowest one percentile (1,693 Illumina, 3,728 Affymetrix BRLMM). There were 116,190, 112,467 and 107,672 called SNPs for Affymetrix DM, BLRMM and Illumina, respectively. Likewise the respective call rates were 97.7%, 99.5% and 99.8%. After recalling all Affymetrix data using BRLMM, the call rate and other data quality measures are similar between the two products. However, the redo rate for Affymetrix remains higher than that of Illumina (14% versus 5%) and there is substantial more labor required to produce the Affymetrix dataset.

New Communities Added to the International HapMap Project Resource: Analysis of Microsatellite Frequencies.
L.H. Toji, C.M. Beiswanger, B.A. Frederick, D.L. Coppock Coriell Institute for Medical Research, Camden, NJ.

In Phase I and II of the International HapMap Project, four communities were selected for inclusion, the CEPH samples from Utah (of European ancestry), the Yoruba from Ibadan, Nigeria, Han Chinese in Beijing, China and Japanese in Tokyo, Japan. In collaboration with the NHGRI, the NIGMS Human Genetic Cell Repository has prepared lymphoblastoid cell lines and DNA from each of the submitted samples. Extensive analysis of the first set of samples from these communities yielded over 3,900,000 SNPs for each cell line. A second set of samples from each of these communities is being distributed for additional study. Cell lines and DNA are being prepared from samples from seven additional communities. Samples from the Denver (Colorado) Metropolitan Han Chinese Community, Luhya from Webuye, Kenya, People of Mexican Origin in Los Angeles, California, and People with African Ancestry in the Southwestern United States are available. The samples from the Tuscan Community from Prato, Italy and the Gujarati Community from Houston, TX are currently being collected. As part of the routine quality control procedure to distinguish individual samples in the Repository, the identity of each DNA sample in these panels has been confirmed using a set of six highly polymorphic microsatellite markers. The cumulative profiles for each marker for the HapMap panels will be compared to each other and to previously collected profiles from the NIGMS Repository; the similarities and differences between all of the panels will be discussed. Additional information about the availability of these samples can be found at: http://ccr.coriell.org/nigms/ products/hapmap.html.

Polyglutamine-expanded peptides produce neurodegeneration through a JNK-dependent, Bax-dependent pathway of apoptotic activation. *J.E. Young[1], J.P. Taylor[3], R. Martinez[1], A.C. Smith[1], K.H. Fischbeck[4], G.A. Garden[2], A.R. La Spada[1]* 1) Lab Medicine, Univ. of Washington, Seattle, WA; 2) Neurology, Univ. of Washington, Seattle, WA; 3) Neurology, Univ. of Pennsylvania, Philadelphia, PA; 4) NINDS, NIH, Bethesda, MD.

Nine neurodegenerative diseases are caused by polyglutamine (polyQ) tract expansions in unrelated proteins. Studies suggest that the pathology in these diseases stems from the production of a misfolded polyQ-expanded protein which disrupts normal cellular pathways through a gain-of-function effect. Many polyQ proteins are cleaved, yielding a polyQ-containing peptide with enhanced toxicity. To understand the toxicity of truncated polyQ-expanded peptides, we expressed N-terminal fragments of polyQ-expanded androgen receptor (AR), huntingtin (htt), and ataxin-7 (atx7) in neuronal cell lines and primary neurons. Expression of these N-terminal peptides induced caspase-dependent apoptosis in primary cortical or cerebellar granule neurons. Using neurons derived from transgenic mice or mice deficient in apoptosis-related genes, we determined that apoptosis induced by polyQ-expanded AR, htt, and atx7 is dependent on the pro-apoptotic gene Bax and can be inhibited by the anti-apoptotic protein Bcl-2 ($p < .05$ by ANOVA). Because several pro-apoptotic BH3-only proteins are transcriptionally regulated by Jun NH2-terminal kinase (JNK), we tested the role of JNK in the apoptotic activation pathway. We observed that phosphorylation of c-Jun was an early event by immunostaining primary neurons for c-Jun-P shortly after expressing polyQ-expanded proteins ($p < .05$) and the presence of a JNK inhibitor prevented cell death and degeneration in these cells ($p < 05$). Interestingly, cytosolic localization of the truncated polyQ-expanded proteins was required for toxicity and cell death. Therefore, our studies suggest that truncated polyQ-expanded peptides in the cytosol result in JNK-mediated cell stress, culminating in Bax-dependent activation of caspases in primary neurons. These findings provide impetus for the development of therapeutic agents aimed at the inhibition of proteolytic cleavage of polyQ proteins.

Symptomatic monoallelic dimorphic mosaicism resulting from early embryonic mitotic mutations. *U. Schwarze*[1], *D.L. Riegert-Johnson*[2], *H.C. Dietz*[2], *P.H. Byers*[1] 1) University of Washington, Seattle, WA; 2) Johns Hopkins University, Baltimore, MD.

We recently identified two individuals who were mosaic for two reciprocal mutations such that they had cells with one normal allele and one of each of two mutant alleles, but no cells with only normal alleles. The first individual is a 3 year old boy with bilateral congenital hip dislocation and significant joint hypermobility. Cultured fibroblasts made some normal and some abnormal type I procollagen molecules with the abnormal molecules resembling those seen in Ehlers Danlos syndrome (EDS) type VIIB, but with less than the expected amount of the abnormal pro2(I) chain. The cDNA contained normal *COL1A2* transcripts and two abnormal transcriptsone in which exon 6 was missing and another that lacked exons 4-10. The first mutation deleted the 3end of exon 6, and a portion of intron 6. The second mutation had breakpoints in introns 3 and 10 with the ends joined by a reverse complementary portion of intron 6. Single cells contained one normal allele and either mutant allele. The normal allele was the same in all cells and the two mutations had each occurred on the other chromatid. No cells had only normal alleles. The second individual had symptomatic vascular EDS and was mosaic for two mutations in the *COL3A1* gene that were in equal abundance and, combined, were equal in amount to the normal allele. Both mutations deleted 27bp from exon 48 of the gene. That exon has a repeat element of 14nt separated by a 13nt spacer; the repeat differs by one nucleotide. The deletions removed either the first repeat and the 13nt spacer or the spacer and the second 14nt repeat. The mutations occurred by mitotic rearrangement during division of the first cell that gave rise to the lineage from which the embryo developed. Other individuals in whom deletions or duplications are present could also have reciprocal or related mutations that have not been identified. Finally, such findings could explain the unexpected birth of children with different but related phenotypes to individuals with an identified mutation who is unknown to be mosaic for different mutations in the same gene.

A zebrafish model of cobalamin C deficiency displays development defects of the central nervous system. *J. Sloan[1], T. Blake[2], R.J. Chandler[1], M.S. Tsai[1], B.P. Brooks[3], C.P. Venditti[1]* 1) GDRB/NHGRI/NIH, Bethesda, MD; 2) Zebrafish Core Facility/NHGRI/NIH, Bethesda, MD; 3) OSD/NEI/NIH,Bethesda, MD.

Cobalamin C deficiency (cblC) features a combined impairment of the cobalamin dependent enzymes, methionine synthase and methylmalonyl-CoA mutase. It is caused by mutations in MMACHC, a gene of unknown function that is suspected to participate in intracellular cobalamin trafficking. The clinical spectrum of cblC is wide and can feature prenatal manifestations, such as congenital microcephaly and intrauterine growth retardation (IUGR). To examine the phenotype caused by loss of function of this gene, we have created and studied a zebrafish model of cobalamin C deficiency. The zebrafish MMACHC gene was identified using informatics and cDNA cloning. FITC-tagged morpholinos targeting the cognate ATG and an exonic junction were designed and used to knock-down the zebrafish homologue. Both morpholinos produced a similar phenotype and displayed a dose-dependent response. Injected fish began to display defects at 24 hours, including brain necrosis and diminished movement when compared to injected controls. The morphants had delayed hatching and by 48 hours, were significantly smaller in size, had less blood, smaller heads and eyes, and grossly abnormal swimming and behavior compared to controls. By 96 hours, some animals displayed pericardial edema. Additionally, histologic examination revealed a fatty liver. RT-PCR studies using total RNA from the exonic morphants harvested on day 6 showed a knock-down of the cognate message. Metabolic analysis using extracts derived from the morphants showed increased homocysteine, methylmalonic acid and cystathionine, a pattern similar to that seen in affected patients. The model presented here is the first animal model of cblC and faithfully replicates some of the more severe findings seen in humans. Furthermore, it demonstrates the utility of zebrafish to easily examine aspects of metabolic diseases that will be difficult to study in other organisms, such as embryonic manifestations, and should facilitate the testing of new therapies for cobalamin C as well as allow developmental mechanisms affected in this disorder to be explored.

Association of VEGFR-1, 2, and 3 htSNPs with susceptibility to preeclampsia. *S.M. Zeng[1], J. Yankowitz[1], J. Murray[2], D. Merrill[3]* 1) Dept OB/GYN, Univ Iowa Col Med, Iowa City, IA; 2) Dept Ped, Univ Iowa Col Med, Iowa City, IA; 3) Dept OB/GYN, Wake Forest College of Medicine, Winston-Salem, NC.

Preeclampsia (PE) is a multiorgan, pregnancy-specific disorder, characterized by hypertension and proteinuria. PE is a major cause of maternal and neonatal mortality and morbidity worldwide. The etiology of PE remains unclear. Placental angiogenesis-related factors such as sFLT-1 (soluble fms-like tyrosine kinase 1), vascular endothelial growth factor (VEGF), placental growth factor (PlGF) and their receptors including VEGFR-1 (FLT-1), VEGFR-2 (Flk or KDR) and VEGFR-3 (Flt-4) may be involved in PE pathogenesis. We previously performed a haplotype-based association study on VEGFR-1 and showed one SNP at intron 17 linked to PE risk. We hypothesize that some SNPs of VEGFR genes contribute to PE susceptibility.We investigated the association of VEGFR-1, -2 and -3 htSNPs with PE susceptibility. A haplotype-based association study was carried on in a Caucasian population including 564 patients with PE and 564 normal pregnant women. Diagnosis of PE was according to the standard criteria. The htSNPs were selected from HapMap (www.hapmap.org). We genotyped 3 htSNPs for upstream and 3 for downstream of VEGFR-1, 2 neighboring the htSNP previously linked to PE-risk, 6 htSNPs for VEGFR-2 and 4 htSNPs for VEGFR-3. Genotyping was performed using TaqMan-PCR assay. Over 400 patients and over 400 controls yielded clear genotype data for each htSNP.

One htSNP upstream to VEGFR-1 and 1 in VEGFR-2 had a significant difference in genotype and allele frequencies between patients and controls. Both were in Hardy-Weinberg equilibrium (P0.05). The other 16 htSNPs had no significant difference in their allele and genotype frequencies between controls and patients (P0.05). The upstream htSNP is between VEGFR-1 and C13orf12, 27 kb upstream from VEGFR-1. The frequency of its minor allele C was significantly higher in the patients (472/890=0.530) than in the normal women (443/930=0.476) (P0.025). The VEGFR-2 htSNP is in intron 12. Its minor allele T had a significantly lower frequency in patients (270/826=0.327) than in controls (354/930=0.381) (P0.025).

Mechanisms and clinical outcomes associated with placental mesenchymal dysplasia. *W. Robinson[1], J.L. Lauzon[2], A.M. Innes[2], J. Slee[3], K. Lim[1], S. Arsovska[1], N. Smith[4], A. Murch[4], D.E. McFadden[1]* 1) U. British Columbia, Vancouver, BC, Canada; 2) U. of Calgary, Alberta Children's Hosp., Calgary, AB, Canada; 3) Genetic Services of West. Australia, Princess Margaret Hosp. for Children, Perth, Australia; 4) King Edward Memorial Hospital Subiaco, Australia.

Placentae with mesenchymal dysplasia (PMD) are typically larger than average and show cystic areas on ultrasound. Diagnosis is confirmed by the observation of enlarged hydropic villi, abnormal placental blood vessels, and absence of trophoblast hyperplasia on pathological examination. Fetal outcomes are variable and are often associated with growth restriction. However, enigmatically, some fetuses show features of Beckwith-Wiedemann syndrome (BWS). PMD has recently been associated with androgenetic (complete paternal uniparental disomy) mosaicism in the placenta. To understand the origin and outcomes of PMD, we preformed detailed molecular testing of placental, fetal and parental samples from three new cases of PMD. Including two previous cases (Kaiser-Rogers et al. J Med Genet 43:187), four of five PMD cases showed a placental karyotype of 46,XX but had a mixed population of androgenetic and normal cells that appeared to be derived from fertilization of a single egg with either one or two sperm. All four cases showed some fetal growth restriction and two presented with liver cysts and/or liver hemangiomas. Three of these four cases survived to term and showed normal post-natal development, but all three had multiple capillary skin hemangiomas. In contrast, the fifth case was a male fetus showing overgrowth with an enlarged heart, marked fetal ascites and intrauterine fetal death at 34 weeks, but no other BWS features. Mosaicism for an unbalanced translocation leading to deletion of the maternal copy of the BWS region on 11p15.5 was observed in placental, but not fetal samples. Thus, PMD can be caused by mosaic loss of maternally expressed genes in 11p15.5 alone, though is more often associated with whole-genome androgenetic mosaicism or chimerism. Variation in fetal outcomes appear to result from differences in both the underlying mechanism and the level and distribution of abnormal cells.

Heterozygosity of A91V-PRF1 in patients with Familial Hemophagocytic Lymphohistiocytosis. *K. Zhang[1], J.A. Johnson[1], J. Biroschak[1], J. Villanueva[2], S.M. Lee[2], J. Bleesing[2], R.J. Wenstrup[1], A.H. Filipovich[2]* 1) Division of Human Genetics, Cinncinnati Children's Hosp, Cincinnati, OH; 2) Division of Hematology/Oncology, Cinncinnati Children's Hosp,.

Familial hemophagocytic lymphohistiocytosis (FHL) is a genetically heterogeneous disorder of immune regulation characterized by defects in cell-mediated cytotoxicity that results in fever, hepatosplenomegaly and cytopenias, typically in early childhood. FHL is inherited in an autosomal recessive manner. Mutations in either PRF1 or Munc13-4 are identified in about 50% of North American patients with FHL. The A91V-PRF1 genotype has been variously reported as a nonpathogenic variant, a genetic modifier, and as a disease-causing mutation. To further illustrate the relationship of A91V-PRF1 with FHL, we investigated the A91V-PRF1 allele in 150 unrelated patients with the clinical diagnosis of FHL in North America. The heterozygous A91V-PRF1 genotype was observed in 24 unrelated FHL families, significantly more frequent than observed in our race-matched control population (16% vs. 3%, $P<0.01$). This suggests that A91V-PRF1 is an important genetic susceptibility factor for developing of FHL. Four of the A91V-PRF1 patients had other bi-allelic PRF1 mutations. Mutational analysis of Munc13-4 was performed in 14 of the remaining A91V-PRF patients. Three patients with heterozygous A91V-PRF1 had bi-allelic Munc13-4 mutations, three were heterozygous for Munc13-4 mutations and eight did not have identified mutations in Munc13-4. The finding of A91V-PRF1 in combination with homozygous and heterozygous mutations in Munc13-4 and PRF1 suggests that A91V-PRF1 acts as a genetic modifier in individuals with underlying disease-causing mutations in PRF1 or Munc13-4 and may influence disease-expression. Additional studies need to be done to illustrate the exact functional effect of A91V-PRF1 in the cytotoxic pathway. In summary, A91V-PRF1 appears to be an important genetic susceptibility factor, but is not disease-causing per se. Mutational analysis of Munc13-4 and other HLH causing genes is recommended for HLH patients with A91V-PRF1 genotype.

Natural History of MPS II: Clinical Aspects. *L.L.C Pinto, I.V.D Schwartz, M.V.R Muñoz, T. A. Vieira, R. Giugliani*
Medical Genetics Service, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil.

Purpose: MPS II is an X linked disorder caused by a deficiency of iduronate sulphatase. The incidence of MPS II is 1:400.000 live births. Clinical findings are very heterogeneous. Phenotypes of MPS II can be classified as mild or severe due to absence/presence of neurological impairment. This is the first study performed in Brazilian MPS II patients about the natural history of this disease. The aim of this study was to characterize the natural history of this disease. Methods: An observational study was conducted in 20 Brazilian MPS II male patients. W e collected the data including asked to present age clinical course, age of onset, symptoms, history of neurological regression. A physical examination of all patients was performed. Results: 1) Regional distribution in Brazil: Southeast (10/20), Northeast (6/20) and South (4/20; 2) current age ranging between 3 and 26 yrs (average: 9.4 yrs); 3) Average age at symptoms onset: 2.3 yrs; 4) Average age at biochemical diagnosis: 5.9 yrs; 5) Major initial symptoms: increased abdominal volume and upper airway infection; 6) Sixteen (16/20) patients underwent surgical procedures, mainly hernioraphy; 7) Six (6/20) patients present mild MPS II; 8) Ten patients (10/20) presented neurological regression (5.1 yrs average); 9).Six (6/20) patients had history of epilepsy; 10).Two (2/20) patients were whealchair dependent; 11) Twelve (12/20) patients reported respiratory symptoms; 12) Seven (7/20) reported ear tubes usage and three (3/20) were using hearing aids; 13) The heigth was below 2 SD in 10/19 patients; 14) Macrocephaly was present in 18/20 patients; 15) Weight was above 2 SD in 7/20 patients; 16).Plebby ivory skin was observed in 3/20 patients. Conclusion: These data may contribute to a better understanding of the progressive nature of this disease, which should be taken into account on the assessment of efficacy of treatment protocols.

Association of vitamin D receptor haplotypes with early onset type 1 diabetes in Newfoundland. *K.S. Wang[1], M. Liu[1], B. Bharaj[1], H.T. Chen[1], J.A. Curtis[2], L.A. Newhook[2], A.D. Paterson[1,3]* 1) Program in Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, Canada; 2) Department of Pediatrics, Memorial University of Newfoundland, St. John's, Canada; 3) Department of Public Health Sciences, University of Toronto, Toronto, Canada.

Background: Type 1 diabetes (T1D) is a T-cell mediated autoimmune disease. Several epidemiological studies in humans provide evidence that vitamin D supplementation can decrease the risk of T1D while in animal models vitamin D can prevent or suppress the development of various autoimmune diseases. Vitamin D compounds are known to suppress T-cell activation by binding to the vitamin D receptor (VDR) on chromosome 12q12-14. Some VDR polymorphisms have been implicated in susceptibility to T1D, but reports have been inconsistent. Therefore, it is crucial to investigate the association of more VDR polymorphisms and their haplotypes with T1D and age at onset (AAO). **Methods:**Six SNPs in the VDR gene, one commonly studied SNP rs10735810 (FokI) and five newly identified SNPs covering three haplotype blocks (A, B and C), were genotyped in 512 families from a relatively isolated population in Newfoundland (NF) which has a high incidence of T1D. PBAT-GEE was used to asses the association. For haplotype analysis, we used a sliding window of 3 adjacent SNPs. We also stratified T1D according to their AAO (16 years) (420 early and 92 late onset probands) to reduce the possible heterogeneity. **Results:** An association was observed between rs2408876 and T1D in the early onset families (p=0.027). Particularly, a three-SNP haplotpye defined by rs10735810-rs2408876-rs6823 showed the most significant association with T1D (p=0.000035) in the early onset families. Furthermore, the same three-SNP haplotype revealed the most significant association with AAO in the early onset families (p=0.00026). **Conclusions:**Our findings strongly support a role of VDR haplotypes in the etiology of early onset T1D in NF population but the causative variant remains to be determined. In future research it is necessary to identify more variants and perform haplotype analysis combined with functional studies in order to elucidate the role of the VDR gene in T1D.

Generalized Genomic Distance-Based Regression Methodology for Multilocus Association Analysis. *J. Wessel*[1, 2, 3], *N.J. Schork*[1, 2] 1) Dept Psychiatry, Univ California, San Diego, La Jolla, CA; 2) Dept Family and Preventive Medicine, Univ California, San Diego, La Jolla, CA; 3) Graduate School of Public Health, San Diego State University, San Diego, CA.

Large-scale, multilocus genetic association studies that either focus on candidate genes or the whole genome require powerful and appropriate statistical analysis tools that are designed to relate genotype and haplotype information to phenotypes of interest. Many analysis approaches consider relating allelic, haplotypic, or genotypic information to a trait using extensions of traditional analysis techniques such as contingency table analysis, regression methods, and analysis-of-variance techniques. We consider a complementary approach that involves the characterization and measurement of the similarity of the allelic composition of a set of individuals diploid genomes at multiple loci in the regions of interest or across the entire genome. We describe a regression method that can be used to relate variation in the measure of genomic dissimilarity (or distance) among a set of individuals to variation in their trait values. Weighting factors associated with functional or evolutionary conservation information of the loci can be used in the assessment of dissimilarity. The proposed method is very flexible and easily extended to complex multilocus analysis settings involving covariates. In addition, the proposed method actually encompasses both single locus and haplotype-phylogeny analysis methods, which are two of the most widely used approaches to genetic association analysis. We showcase the method with data described in the literature taking both a candidate gene approach and whole genome association. Ultimately, our method is appropriate for high-dimensional genomic data and anticipates an era when cost-effective exhaustive DNA sequence data can be obtained on a large number of individuals over-and-above genotype information focused on a few well-chosen loci.

Low expression of *GJB2* and *GJB6* segregates with DFNB1 deafness and with a distant 131 kb deletion in an extended Michigan family. *E. Wilch[1], M. Zhu[2,3], K.B. Burkhart[3,4], M. Regier[5], J.L. Elfenbein[6], R.A. Fisher[7], K.H. Friderici[1,2,3,7]* 1) Genetics Program, Michigan State Univ, East Lansing, MI; 2) Cell & Molecular Biology Program, Michigan State Univ, East Lansing, MI; 3) Department of Microbiology & Molecular Genetics, Michigan State Univ, East Lansing, MI; 4) Life Sciences Institute, Univ of Michigan, Ann Arbor, MI; 5) Department of Epidemiology, Michigan State Univ, East Lansing, MI; 6) Department of Communicative Science & Disorders, Michigan State Univ, East Lansing, MI; 7) Department of Pediatrics & Human Development, Michigan State Univ, East Lansing, MI.

In a large kindred of German descent, we have identified a novel allele that segregates with profound deafness when present in *trans* with the 35delG allele of *GJB2*. Qualitative PCR-based allele-specific expression assays show that expression of both *GJB2* and *GJB6* from the novel allele is dramatically reduced. Individuals carrying this allele also have on the same chromosome a 131 kb deletion whose proximal breakpoint is about 148 kb upstream of the transcriptional start site of *GJB6*. This deletion overlaps two previously identified deletions of 309 kb and 232 kb that truncate *GJB6* and that segregate as recessive DFNB1 mutations. The three deletions share a common interval of 96 kb. Taken together, these data suggest that disruption of a relatively distant regulatory element of *GJB2* and *GJB6*, such as a locus control region, explains the low-expression phenotype that we have observed, and is responsible for the deafness in individuals bearing these deletions in *trans* with identified *GJB2* mutations. Characterization of *GJB2/GJB6* regulatory elements may provide the means to identify many more *GJB2* mutations that are believed to exist, but that have so far remained elusive.

Array CGH is superior to other methodologies at detecting somatic mosaicism. *V.R. Sutton, C. Shaw, D.A. Scott, A. Patel, S. Trilochan, A. Pursley, J. Li, P. Stankiewicz, A.C. Chinault, J.R. Lupski, A.L. Beaudet, S.W. Cheung* Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.

We have developed a targeted aCGH platform known as chromosome microarray analysis (CMA) for clinical use in our diagnostic laboratory. This platform contains bacterial artificial chromosome (BAC) clones that encompass telomeric and pericentromeric regions as well as regions of the genome involved in common microdeletion/microduplication (genomic) disorders. Therefore in a single assay, hundreds of genomic regions can be interrogated for gains or losses in copy number. We have performed CMA using the latest version of our array on 2585 samples and identified 12 cases in which the results were indicative of mosaicism. Ten had an aneuploidy, one had a ring chromosome and one had segmental aneusomy. In 11/12 cases, prior routine chromosome analysis had been normal. We therefore hypothesized that CMA may be a more sensitive method for detecting mosaicism. The presence of mosaicism in all cases was confirmed by one or more of the following methodologies: G banded chromosome analysis of stimulated T-cells or B-cells from peripheral blood or cultured skin fibroblasts; FISH analysis on stimulated T-cells or whole blood smear. Estimates of the level of mosaicism were higher for CMA compared with methodologies that use T-cells in all cases. We believe that this is due to the selection bias against aneuploid T-cells both *in vivo* during VDJ recombination and cellular division, and *in vitro* during T-cell stimulation for routine chromosome analysis and FISH. The estimates of the level of mosaicism were also higher for CMA compared with FISH of unstimulated nucleated cells from whole blood in almost all cases, likely because FISH requires that the cells are alive and aneuploid cells likely die at a faster rate than euploid cells. Thus, we assert that CMA is superior to other standard methodologies in detecting mosaicism and estimating the level of mosaicsm for both aneuploidies and other cytogenetic aberrations.

Gene-Gene Interaction in Nicotinic Receptor Genes: Association Study of Smoking in Adult ADHD. *S. Voineskos, V. De Luca, J. Umesh, J. Kennedy* Dept Psychiatry, CAMH, Clarke, Toronto, ON, Canada.

A number of studies have suggested that the alpha-7-nicotinic receptor subunit gene D15S1360 polymorphism is associated with schizophrenia, and a deficiency in the normal inhibition of the P50 auditory-evoked response. Schizophrenia patients and some patients with ADHD show a failure of inhibition in their 50 ms response to the second of a pair of tones. Furthermore, high-dose nicotine transiently normalizes the abnormality in P50 inhibition in schizophrenia patients and in their relatives. Psychiatric patients are heavy smokers. This rate is higher than the general population. In this study we hypothesized that the D15S1360 marker and other nicotinic receptor genes are associated with increased smoking in patients with Adult ADHD. Our sample consisted of 200 DSM-IV patients affected by Adult ADHD from the Toronto area. Current smoking status was assessed by medical history questionnaire, and there were 56% of smokers and 44% non-smokers. There was no difference in age or ethnicity between the two groups. We found no association between the D15S1360 alleles and smoking risk (chi-sq=4.45, 3df, p=0.21). Although these findings are negative, further study into the relationship between smoking and nicotine system genes is warranted in psychiatric population.

Replication of the association between NOS1AP (CAPON) and cardiac repolarization in the Old Order Amish.

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The electrocardiographic QT interval is a measure of cardiac repolarization that predicts risk for cardiovascular events in the general population. Through a genome-wide association study in the German KORA Study, we recently reported variation in QT interval to be associated with a SNP in NOS1AP (rs10494366), the gene encoding the PDZ ligand of neuronal nitric oxide synthase. These results were subsequently replicated in two other independent Caucasian populations from Germany and the U.S. Since genetic heterogeneity between populations may exist, we assessed the association between NOS1AP and the QT interval in the Old Order Amish using single SNP and haplotype analyses. Based on fine mapping in the KORA study, 4 SNPs in the 5 end of NOS1AP were selected using the Tagger tool in Haploview, that captured all major haplotypes (>1% frequency) in the region of interest (~120 kb segment). Genotyping in 439 subjects from the Amish Heredity and Phenotype Intervention (HAPI) Heart Study was performed using TaqMan. Association analyses were performed using a variance components methodology implemented in SOLAR which accounts for the relatedness of individuals. Variation in QT interval was modeled as a function of age, gender, and heart rate, additive genetic effects (or heritability) and a residual error component. Adjusted QT interval heritability was 0.42 (p= 0.003). The SNPs were highly correlated (D 0.97- 0.99) and common (MAF 34- 45%). Genotype frequencies satisfied Hardy Weinberg equilibrium. There were significant associations between 2 of the 4 SNPs (rs1415262, p=0.03 and rs10494366, p=0.009) and the adjusted QT interval. SNP rs10494366 explains 1.6% of the variability in adjusted QT interval. One of the 2 major haplotypes, comprising all the major alleles (frequency 53%), was associated with shorter QT interval (P=0.01). In summary, we have demonstrated association between NOS1AP and QT interval in the Amish, consistent with findings across other Caucasian populations studied to date.

Outcome analysis of pregnancies screening positive for Smith-Lemli-Optiz syndrome. *M. Steinraths[1], A. Mattman[2], S. Langlois[1,2]* 1) Dept of Medical Genetics; 2) Dept of Pathology, University of British Columbia, Vancouver, BC, Canada.

Smith-Lemli-Opitz syndrome (SLOS) is characterized by growth retardation, developmental delay, and minor/major malformations. Due to low fetal cholesterol, SLOS presents with low maternal serum unconjugated estriol (uE3). Other conditions presenting with low uE3 on triple marker screen (TMS) include trisomy 18, triploidy, anencephaly, steroid sulfatase (STS) deficiency, fetal adrenal abnormalities and intrauterine fetal demise (IUFD). Our study aims to define the risk for an abnormal outcome in pregnancies screening positive for SLOS when all known causes have been excluded. We applied a SLOS screening algorithm to 100,000 TMS collected between 1995-2005, identifying 260 as screen-positive. Outcome information was obtained for screen-positive pregnancies through chart review and physician questionnaires. To date, outcome is known for 204/260 (78%) pregnancies. Outcomes include IUFD in 87/204 (43%), aneuploidy or triploidy in 45/204 (22%), anencephaly in 12/204 (6%), STS deficiency in 11/204 (5%) and adrenal hypoplasia in 1/204 (0.4%). Maternal steroid treatment was noted in 3/204 (1%). There have been no pregnancies with SLOS identified to date. 45/204 (22%) pregnancies had no recognized cause of low uE3. Outcomes were obtained for these (group 1) and 69 age-matched control pregnancies with normal uE3 (group 2). In group 1, pregnancy and long-term outcome was normal in 25/45 (56%). Intrauterine growth restriction (IUGR) was seen in 6/45 (13%) and pregnancy-induced hypertension (PIH) in 4/45 (9%). Multiple congenital anomalies were present in 10/45 (22%). In group 2, outcome was normal in 63/69 (91%), IUGR was seen in 1/69 (1%) and PIH in 3/69 (4%). Developmental delay and congenital anomalies were seen in 2/69 (3%). Proposed etiologies for an increased rate of abnormal outcome in pregnancies with low uE3 on TMS include multiple congenital anomaly syndromes affecting the pituitary/adrenal axis or cholesterol biosynthesis disorders other than SLOS. This outcome information will allow comprehensive genetic counseling of pregnancies screening positive for SLOS and improve assessment of newborns with low uE3 identified prenatally.

Genotype to Biochemical Phenotype correlation in Tay-Sach's disease. *R. Zimmer[1], L. Mays[1], S. Bhatt[1], S. Marenberg[2]* 1) Genzyme Genetics, Orange, CA; 2) Genzyme genetics, Santa Fe, NM.

Tay-Sachs disease is an autosomal-recessive, lysosomal storage disorder caused by mutations of the HEX A gene that codes for enzyme beta-hexasaminidase A. The disease has variable age of onset, and variable clinical manifestations, with three main subtypes of infantile-onset, juvenile-onset, and adult-onset. Tay-Sachs disease has been reported in all ethnic groups, but it is most prevalent among Ashkenazi Jewish, Cajun, and French-Canadians individuals. Although carrier screening is often performed via an assay of total and percent HEX A enzyme activity, direct mutation improves Tay-Sachs disease detection, aids in identification of associated mutations, and helps differentiate between disease causing, and non-disease causing mutations. Certain mutations are more common within specific ethnic groups, and may be associated with a particular subtype of Tay-Sachs disease. We reviewed our data of over 700 Tay-Sachs carriers, to evaluate the relationship of the biochemical phenotype in terms of HEX A percentage, with a specific Tay-Sachs DNA mutation. The results showed that the HEX A percentage varied with the type of mutation. The HEX A percentage was observed to be relatively higher with an average value of 47.5 % (non carrier reference range more than 55%), for the pseudodeficiency alleles (R247W and R249W) and also for the late onset mutation (G269S). Whereas, the average values of HEX A percentage was 44.2% for 1421 +1 G C mutation, 42.6 % for 7.6 kb deletion mutation, 44.5 % for +TATC1278 mutation, and 45.6 % for IVS 7 +1 G A and IVS 9 +1 G A mutations. Our study indicates that the HEX A percentage varies with the type of mutation. The HEX A percentage is relatively higher with the less severe mutations or with non-disease causing mutation as compared to the infantile onset mutations.

Pharmacokinetic gene variants do not influence response or tolerance to citalopram in a STAR*D sample. *E. Peters[1], S. Slager[2], J. Kraft[1], G. Jenkins[2], M. Reinalda[2], P. McGrath[3], S. Hamilton[1]* 1) Psychiatry/Biopharm Sci, Univ of California, San Francisco, San Francisco, CA; 2) Division of Biostatistics, Mayo Clinic College of Medicine, Rochester, MN; 3) New York State Psychiatric Institute and Columbia University.

Clinical outcome of antidepressant treatment is variable, in terms of both efficacy and side effect burden. This study investigated the potential role of pharmacokinetic gene polymorphisms in determining patient response and tolerance to the selective serotonin reuptake inhibitor (SSRI) citalopram. Using 1,914 depressed subjects from the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study, we examined several known and putatively non-functional alleles in the CYP2D6 (*3-*9) and ABCB1 genes, as well as the CYP2C19*2, CYP3A4*1B, and CYP3A5*3C polymorphisms for association to citalopram response and tolerance. In order to limit type I error, we used a split-sample study design, and required a p-value of less than 0.05 in both the discovery and validation sample sets. None of the variants met this criteria for study-wide significance. Our most significant association in the discovery set was between the medication tolerance phenotype and the CYP2C19*2 variant ($p<0.01$; recessive odds ratio, 95% CI: 0.25, 0.06 - 0.96). This association was not replicated in our validation sample set ($p=0.57$), which had adequate power to detect an effect of this magnitude. CYP2D6 and CYP2C19 poor metabolizer (PM) status, inferred from genotype data, was also not significantly associated with response or medication tolerance. Furthermore, we investigated pairwise interactions using all variants genotyped and found no significant results. Thus, for pharmacological treatment with citalopram, routine screening of common pharmacokinetic DNA variants may be of limited clinical utility.

An automated method for extracting normalized mentions of human genes and proteins in biomedical text. *P.S. White[1,2], K. Murphy[2], R. O'Hara[2], M. D'arcy[2], S. Carroll[2], Y. Jin[2], H-R. Fang[2], J. Kim[2], M. Mandel[3], M. Liberman[3,4,5], R. McDonald[5], F. Pereira[5]* 1) Division of Oncology, Children's Hospital of Philadelphia; 2) Department of Pediatrics, University of Pennsylvania; 3) Linguistic Data Consortium, University of Pennsylvania; 4) Department of Linguistics, University of Pennsylvania; 5) Department of Computer and Information Science, University of Pennsylvania.

We developed an automated text mining process to identify and normalize references to human genes in biomedical text. Gene named entity recognition (NER) was performed using a machine-learning algorithm that considers semantic and syntactic features in text. Identified mentions were then normalized to standard gene names using vocabulary and approximate string matching. The combined gene NER and normalization process performed document retrieval with 95.7% precision and 85.7% recall at the document level. When applied to MEDLINE, the process identified 36,953,389 gene mentions, 17,897,933 of which normalized to 14,501 human genes. We built a web interface (FABLE) to retrieve MEDLINE articles mentioning human genes, and to compile lists of keyword-defined concepts and articles. FABLE supports searches for gene names and aliases and returns MEDLINE articles in which the query genes are mentioned, regardless of which gene alias(es) were used in the article. Results can be sorted in various ways, including by query relevance and journal impact factor. FABLE demonstrated 93.9% accuracy when comparing its ten most relevant articles with PubMed for 50 random genes. FABLE identified on average 33% more articles than PubMed. FABLE also allows users to generate lists of MEDLINE-mentioned genes implicated in any keyword-defined concept (e.g., schizophrenia NOT bipolar). A query of FABLE with a set of keywords results in a list of genes that co-occur in an article with the input keyword(s). Lists consist of normalized gene symbols, the number of articles in which each gene is mentioned, and the implicating articles. FABLE-generated gene list evaluation indicated comparable precision and higher recall than manually-established lists. Access FABLE at http://fable.chop.edu.

Variation in Liver Enzymes Share Genetic Factors with the Metabolic Syndrome. *D. Wang[1], X. Guo[1], H. Yang[1], M. Quiñones[2], W.A. Hsueh[2], J.I. Rotter[1]* 1) Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA; 2) Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA.

Aspartate transaminase (AST or SGOT), alanine transaminase (ALT or SGPT), and alkaline phosphatase (ALP) are commonly used biomarkers in detecting liver damage. Clinical and epidemiological evidences suggest that components of the metabolic syndrome, such as obesity, diabetes, and elevated triglycerides, may be associated with nonalcoholic fatty liver (NAFL), which would manifest as elevated liver enzymes. To investigate genetic contributions to liver enzymes and their interrelationship with the metabolic syndrome, we estimated the heritability of liver enzymes and their co-heritability with components of metabolic syndrome in 101 two-generation Mexican-American families ascertained via a parent diagnosed with CAD (the MACAD study). Liver blood enzymes (AST, ALT, and ALP) and features of the metabolic syndrome (body mass index (BMI), systolic and diastolic blood pressures (SBP and DBP), high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC), triglyceride, fasting glucose and fasting insulin) were measured in 274 apparently healthy adult offspring. Heritability and co-heritability ($_G$) were estimated using the variance component methods implemented in SOLAR. The estimated heritability was 0.64 ($p=1x10^{-7}$) for AST, 0.44 (p=0.0002) for ALT, and 0.75 ($p=2x10^{-11}$) for ALP. AST shared significant genetic contributions with fasting glucose ($_G$=0.57, standard error (se) =0.19) and fasting insulin ($_G$=0.50, se=0.23). ALT shared significant genetic contributions with SBP ($_G$=0.38, se=0.17) and fasting insulin ($_G$=0.66, se=0.21). ALP shared significant genetic contributions with BMI ($_G$=0.50, se=0.14), SBP ($_G$=0.61, se=0.13), DBP ($_G$=0.59, se=0.15), and fasting insulin ($_G$=0.41, se=0.19). Our data suggest that a significant portion of the variability in liver enzymes is explained by genetic factors in healthy (but at risk) Mexican Americans and that variation in liver enzymes shares substantial genetic contributions with components of metabolic syndrome in this population.

Signatures of recent natural selection in drug-metabolism genes. *M. Yeager-Jeffery[1], F. Hyland[2], K. Lazaruk[2], K. Haque[1], R.A. Welch[1], F.M. De La Vega[2]* 1) SAIC-Frederick, Inc., CGF, DCEG, NCI-Frederick, MD 21702; 2) Biosystems, Foster City, CA.

Drug metabolizing enzymes (DME) participate in the biotransformation of various endogenous and exogenous compounds, including therapeutic drugs. Functional polymorphisms within DME genes have been shown to alter drug responses and their frequencies often vary between populations. Since the human xenobiotic environment varies with diet and lifestyle, these genes are prime candidates for finding signatures of recent natural selection. Previous studies have identified patterns of variation suggestive of selective pressure in members of the CYP3A gene family and a few other DME genes. We genotyped 2,394 non-synonymous SNPs in 217 DME genes (TaqMan Drug Metabolism Genotyping Assays) on the HapMap Project samples. We investigated if we could identify evidence of natural selection across these genes independently and in combination with the HapMap genotypes. We analyzed the distribution of population differentiation statistics (Fst, Pexcess, and d) and extended homozygosity for core gene haplotypes. Preliminary results have identified a number of genes as possibly having very different evolutionary histories in different human populations, including NAT2, ALDH2, ADH1B, and members of the ABC-transporter and cytochrome P450 gene families, some of which are consistent with previous reports. Genome-wide empirical scans for selective sweeps have been recently performed with the HapMap data. However, DME genes and their functional variants are underrepresented in such studies due to the difficulty in obtaining working genotyping assays across these highly homologous gene families; e.g. fewer than 30% of the SNPs in our collection were successfully typed by the HapMap. Therefore, combining our data with that of the HapMap allows for the first time comprehensive scan for signatures of natural selection across DME genes, important mediators of adaptation of human populations to their environments. Funded by NCI Contract N01-CO-12400.

MOLECULAR ANALYSIS OF A NOVEL FLN1 GENE MUTATION. *S. S. Tsuneda[1], F.R. Torres[1], M.A. Montenegro[2], M. Guerreiro[2], F. Cendes[2], I. Lopes-Cendes[1]* 1) Medical Genetics Department, Faculdade de Ciencias Medicas UNICAMP, Campinas, SP, Brazil; 2) Neurology Department, Faculdade de Ciencias Medicas, UNICAMP, Campinas, SP, Brazil.

Recent studies have demonstrated that mutations in FLN1 gene are responsible for bilateral periventricular nodular heterotopia (BPNH). We identified a novel mutation (1159G>C), in a family segregating BPNH. However, the exact molecular mechanism by which this mutation lead to abnormal FLN1 protein was unclear. The purpose of this study was to investigate the molecular mechanism of this new mutation. Total RNA was obtained from peripheral blood samples, from 2 BPNH patients (mother and daughter) and control individuals. RT-PCR was performed by standard techniques and the cDNA was amplified using specific primers spanning the region containing exon 6 and exon7. Amplicons were cloned into pGEM-T vector, gel-purified and subsequently sequenced, using SP6 and T7 primers. Analysis of the cDNA amplicon demonstrated a different pattern of electrophoretic migration between patients and controls. The sequencing of these fragments showed that amplicons from individuals with BPNH kept the intronic sequence between exons 6 and 7. Our data clearly showed that the molecular mechanism of the mutation 1159G>C is the abolishment of the exon 6 donor splicing, resulting in an alternative stop codon and, possibly, in a truncated protein. Thus an aminoacid substitution, as suggested previously, is not the mechanism involved in the etiology of BPNH sydrome in the patients analysed.

Self-collected buccal cell samples: DNA yield and participation rates. *V.J. Smith[1], M.M. Jenkins[2], C. Sturchio[2], M. Gallagher[3], C.A. Hobbs[1]* 1) University of Arkansas, Little Rock; 2) Battelle contractor to CDC, Columbus, OH; 3) Centers for Disease Control and Prevention (CDC), Atlanta, GA.

The National Birth Defects Prevention Study (NBDPS) is a population-based case-control study of environmental and genetic risk factors. Following a maternal interview, participants are instructed to collect buccal cells from mother, father, and child using 2 cytobrushes each and return them by mail to the study site. One brush per participant is shipped to CDC and the other is retained by the local site. Due to concerns regarding the amount of DNA extracted from 1 cytobrush, a pilot study was conducted to determine if doubling the number of brushes per participant would increase the quantity of DNA while maintaining the participation rate. 554 Arkansas (AR) families representing 404 case- and 150 control-parental triads were sent 4 cytobrushes per individual during a 16 month period. Two brushes per individual were shipped to CDC and two retained at the study site. DNA was extracted from the 2 pooled cytobrushes sent to CDC and quantified using RT-PCR. 341 case and 112 control families returned kits, resulting in an overall participation rate of 81.8%. Participation rates during the pilot study did not vary significantly from rates when AR participants were asked to collect 2 cytobrushes per individual. DNA yields for 2 pooled brushes from AR families were compared with yields for 1 brush from the other 8 NBDPS study sites for the same 16 month period. DNA extracted from 1 brush resulted in a mean DNA yield of 1.8 g, 1.5 g, and 1.6 g from mothers, fathers, and children, respectively. DNA extracted from 2 cytobrushes resulted in respective mean DNA yields of 2.3 g, 1.9 g, and 2.1 g. Thus, the respective percent increases in DNA were 27.6%, 25.6%, and 32.1%. The results of the pilot study did not warrant the additional expense needed to request four cytobrushes from each NBDPS participant and Arkansas reverted back to collecting two cytobrushes per participant. The effects of increasing the number of cytobrushes sent per NBDPS participant may be of value to others involved in large genetic epidemiology studies.

Analysis of the 3 UTR of the *MECP2* gene in patients with clinical diagnosis of Rett syndrome and mental retardation. *M. Santos[1,2], A.M. Coutinho[3], J. Yan[4], C. Yang[4], J. Feng[4], A. Vicente[3], T. Temudo[5], S. Sommer[4], P. Maciel[1]* 1) ICVS, School of Health Sciences, Univ. Minho, Braga, Portugal; 2) ICBAS, Univ. Porto, Portugal; 3) IGC, Oeiras, Portugal; 4) Centre for Molecular Diagnosis, City of Hope Medical Centre, Duarte, California, USA; 5) HGSA, Porto, Portugal.

Mutations in the methyl-CpG binding protein 2 (*MECP2*) gene are associated with Rett syndrome (RTT), a developmental disorder affecting mainly females with neurological manifestations including mental retardation and autistic features. Mutations in the coding region of *MECP2* are identified in around 80% of classic RTT patients and 30% of variant forms of the disease. It has been proposed that mutations in non-coding regions of the gene or in other genes may be the unidentified cause of the disorder in these cases. The role of the 3 untranslated region (UTR) of a gene might be in regulating the stability of the mRNA, its translatability or sub-cellular localization. Mutations in the 3 UTR of genes have been identified as the genetic cause of a number of neurological diseases. In this work we explored the role of the 3UTR of the *MECP2* in patients with clinical diagnosis of RTT and mental retardation who had no mutations in the coding region of the *MECP2* gene: focusing on regions of the 3UTR with almost 100% conservation at the nucleotide level among mouse and human, searching for pathogenic variants. A total of 67 affected females were studied,and compared to 143 caucasian blood donors of Portuguese origin. The 3UTR was scanned for mutations using DOVAM-S and the variants identified were automatically sequenced. Two 3UTR variants in the *MECP2* were found (2763GA and 10158CG) in our group of patients. However, the 10158CG variant was also present in the unaffected father of the patient, whereas variant 2763GA was identified in the control population. Our data indicate that mutations in this region must be rare and do not account for a significant proportion of classical RTT without genetic explanation. Nevertheless, in order to clarify this point a larger number of patients should be screened for mutations in these regions of high conservation.

Genetic Models for Li-Fraumeni Syndrome in the Presence of Germline p53 Mutations. *C.C. Wu[1], S. Shete[1], C.I. Amos[1], L.C. Strong[2]* 1) Dept Epidemiology, Univ Texas MD Anderson CA Ctr, Houston, TX; 2) Clinical Cancer Genetics, Dept Molecular Genetics, Univ Texas MD Anderson CA Ctr, Houston, TX.

Germline p53 mutations have been identified in some families with Li-Fraumeni syndrome (LFS). 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. In the presence of a stable germline p53 mutation segregating in a family, the age of cancer onset and multiplicity of tumors within a family is highly variable, suggesting the presence of additional genetic factor(s). Given these observations, we proposed to investigate the residual genetic effects accounting for the observed genetic and phenotypic heterogeneity. We analyzed 6 pedigrees with hereditary germline p53 mutations, which is a subset of 159 extended LFS pedigrees ascertained through 3-year survivors of childhood soft tissue sarcoma who were treated at M. D. Anderson Cancer Center from 1944 to 1975. In those kindreds, a total of 62 germline p53 mutation carriers have been identified. To determine the hypothetical genetic model for the unobserved non-p53 gene(s) or modifier(s) of cancer risk in the presence of germline p53 mutations, we included confirmed invasive cancers excluding nonmelanoma skin cancer and in situ carcinoma as a single combined phenotype. We applied a complex segregation analysis that allowed us to associate the simultaneous effects of germline p53 mutations and unobserved gene(s) underlying LFS with cancer incidence in these families, possibly accounting for genetic modification of cancer risk among or within these families. Our finding showed modest evidence of an interaction between germline p53 mutations and the unobserved gene, suggesting the presence of p53 modifier(s). However, the effects of the modifier were very small, and the genotype-dependent effects of p53 in terms of age-of-onset changes were all within 2 years.

Identification of novel small RNAs in adult neural stem cells. *K. Szulwach[1], X. Zhou[2], P. Jin[1]* 1) Department Human Genetics and Program in Genetics and Molecular Biology, Emory Univ, Atlanta, GA; 2) Department of Neuroscience, University of New Mexico, Albuquerque, NM.

Small RNAs (18-28nt), including microRNAs (miRNAs), have been increasingly recognized to play critical roles in many forms of gene regulation and diverse cellular pathways, including stem cell maintenance and differentiation. Defining the molecular events underlying stem cell self-renewal and differentiation are essential steps towards understanding stem cell biology. It has been hypothesized that small RNAs are key mediators of stem cell fate decisions and act in concert with transcriptional regulators to control adult neural stem cell (aNSC) self-renewal and differentiation. aNSCs continuously generate new neurons that are functionally integrated into neural circuits and so are thought to hold therapeutic potential in neurodevelopmental and neurodegenerative disease. In an effort to gain an understanding of how small RNAs may contribute to the maintenance and differentiation of aNSCs, we have created a cDNA library of 18-28nt small RNAs from mouse primary aNSC. Initial high-throughput sequencing of this library has produced 338,144 reads including 4,243 unique sequences that occurred a total of 13,138 times. While 178 known miRNAs were observed 7,925 times, an additional 2,889 unique sequences were seen a total of 5,876 times and represent potentially novel small RNAs specific to aNSC. The ~16-fold-increased observation of unique sequences compared to known miRNA sequences in primary aNSCs indicates the potentially large numbers of undiscovered small RNAs with important functions in aNSC. Interestingly, many of the potentially novel small RNAs have correlated with previously identified ESTs found specifically in stem cells and have mapped to evolutionarily conserved genomic loci. Some of the identified RNAs, along with flanking sequences, are predicted to form energetically stable stem-loop hairpins capable of processing by components of the RNAi pathway. Identification of this large group of small RNAs will lead to the discovery of adult neural stem cell specific small RNAs important for proper maintenance and differentiation of aNSCs.

CAG repeat instability and motor deficit in a transgenic mouse model of Machado-Joseph disease. *A. Silva-Fernandes[1], M.C. Costa[1], R. Franco-Duarte[1], P. Oliveira[1,2], P. Maciel[1]* 1) ICVS, School of Health Sciences, Univ. Minho, Braga, Portugal; 2) Dep. Production and Systems Engineering, School of Engineering, Univ. Minho, Braga, Portugal.

Machado-Joseph disease (MJD) is a late-onset autosomal dominant neurodegenerative disorder caused by a CAG repeat expansion in the *ATXN3* gene, encoding ataxin-3. Its main neurological features are progressive ataxia, ophthalmoplegia, pyramidal and/or extrapyramidal signs. We have generated a transgenic mouse model that expresses a human cDNA variant of the *ATXN3* gene under the regulation of a general expression promoter (PCMV). We have obtained two founders: founder A (94 CAGs, two transgene copies), and founder B (83 CAGs, ten transgene copies). Human ataxin-3 carrying an expanded polyQ tract was detected in both peripheral tissues and in the central nervous system (CNS) in both transgenic lineages. In order to study the intergenerational instability of the CAG repeat, we analyzed a total of 46 transmissions of lineage A and 26 transmissions of lineage B, for both maternal and paternal meioses. We observed intergenerational instability in both lineages. Additionally, somatic instability was observed in lineages A and B, in both peripheral tissues and in the CNS. In order to assess motor coordination, we have performed the Rotarod test using six constant speeds (5, 8, 15, 20, 24, 31 rpm) and an accelerating rod task (4-40 rpm). Until now, we tested the animals at 16, 24 and 36 weeks of age. Both heterozygous and homozygous transgenic mice of lineage A, presented a significant decrease in the latency to fall in both paradigms since 16 weeks of age. For lineage B no motor impairment was yet found, suggesting that repeat length may be more relevant than gene dosage for disease manifestation. In summary, this transgenic mouse model presents a progressive motor coordination impairment phenotype as well as intergenerational and somatic instability of the CAG repeat, as the human MJD patients, suggesting that it could be useful both in the study of MJD pathogenesis and therapy development, as for the analysis of the molecular basis of the CAG repeat instability in this disorder.

Uncovering the complex nature of an Apparently Balanced Translocation by High Density SNP Oligonucleotide Microarray Analysis (SOMA) and High Resolution Comparative Genomic Hybridization. *A. Sobrino[1], O. Nahum[2], V. Jobanputra[1,3], K. Anyane-Yeboa[3], Y. Sun[2], E. Bottinger[2], W. Zhang[2], D. Warburton[1,3], B. Levy[1,3]* 1) Genetics Lab, New York Presbyterian Hosp, New York, NY; 2) Depts Medicine & Pediatrics, Mount Sinai School of Medicine, New York, NY; 3) Depts Genetics and Development, Pathology & Pediatrics, Columbia University Medical Center.

We report the work-up of a patient with clinical abnormalities and an apparently balanced translocation. The patient, a 1 yr-old male, presented with mild developmental delays and dysmorphic features which included hypertelorism, large cupped ears, micrognathia, wide-spaced nipples, left simian crease, and hypotonia. His OFC was 47 cm (50%), weight 10.45kg (50%) and height 80cm (90%). His birth weight was 3900g. Cytogenetic analysis indicated a rearrangement between chromosomes 8 and 13 such that the entire long arm of chromosome 13 appeared to be translocated to the terminal region of the short arm of chromosome 8. The karyotype was initially performed by another lab and designated 45,XY,t(8;13). FISH using a subtelomeric 8p probe revealed a single signal on the normal chromosome 8 but no signal on the derivative chromosome 8, indicating a terminal deletion. In addition, FISH using a 13/21 alpha satellite probe showed no signal on the translocated chromosome 13. High resolution CGH indicated a loss of the most distal region of the short arm of chromosome 8 (8p23.3-8pter) and the most proximal region of the long arm of chromosome 13 (13q12.1-13q12.2). Interestingly, CGH also detected an interstitial gain on the short arm of chromosome 8 (8p12-8p23.1). Further characterization of these regions was achieved by means of high-density SNP oligonucleotide microarray analysis (SOMA) where copy number gains and losses were determined by the Circular Binary Segmentation method. SOMA confirmed the CGH results and improved the resolution of the size of the imbalances. The deletions on 8p and 13q are estimated to be approximately 6.7 and 2.0 Mb respectively, while the duplication on 8p21.2 is about 12.1 Mb. This case illustrates the utility of SOMA as well as conventional CGH to define more accurately the nature of an abnormal derivative chromosome.

CGH analysis of a rare case of squamous cell carcinoma of the urachus of the bladder. *W. Thelmo[1], M.J. Macera[2,3], J. Breshin[2,3], P. Chandra[3], A. Babu[2,3]* 1) Dept Pathology; 2) Div Molec Medicine & Genetics; 3) Dept Medicine, Wyckoff Heights Med Ctr, Brooklyn, NY.

A seventy two year old female was admitted for gross hematuria. She has a history of hypertension, asthma and urinary reflux. She denied dysuria or increase of urinary frequency. She had no weight loss, fever, chills, nausea or vomiting or a history of passing out stones in the urine. She is a non-smoker. She underwent cystoscopy and urinary bladder biopsy which showed high grade urotherlial carcinoma with necrosis. Three weeks later, this was followed by radical cystectomy and ileal conduit. There was a tumor in the dome of the bladder with ulceration demonstrating squamous cell carcinoma with invasion of the urachal remnant. The rest of the bladder showed chronic cystitis, diffuse without squamous metaplasia or any evidence of parasites (Schistosoma). Right and left pelvic lymph nodes were all negative for tumor. Comparative genomic hybridization (CGH) analysis was done on DNA extracted from formalin fixed tumor and identified loss of a chromosome 17 as well as the entire 16p arm. The UroVysion probe was then applied to sections of the tumor. Three abnormal clones were detected: one with a single 17 centromere and a single p16 gene (located at 9p21) (27.0%), the second with a single 17 centromere and two p16 genes (17.0%), and the third with two 17 centromeres and a single p16 gene (25.0%). The remaining cells (31.0%) showed normal signal numbers for all probes. No loss of 9p was seen by CGH, likely due to a small deletion below the detection level of the test. Deletions of part of or loss of the entire chromosome 9 are the most common findings in all bladder cancers. Loss of chromosome 17 has been associated with progression in this disease. Only approximately 7% of non schistosome related bladder cancer is squamous cell, and aberrations of chromosome 17 and loss of 16p are more frequently observed in transitional cell carcinoma than squamous cell carcinoma. This case represents an unusual presentation and further study may help to elucidate this complex disease.

A Single Nucleotide Polymorphism in the *COX2* gene is significantly associated with prostate cancer in men of European Ancestry. *P. Pal[1], H. Xi[1], R. Kaushal[1], G. Sun[1], J. Mallik[1], B.K. Suarez[2], W.J. Catalona[3], R. Deka[1]* 1) Center Genome Information, Univ Cincinnati, Cincinnati, OH; 2) Department of Psychiatry and Genetics, Washington University, St. Louis, MO; 3) Department of Urology, Northwestern University Feinberg School of Medicine, Chicago, IL.

We have tested for association of variants in *COX1* (9q32-33.3) and *COX2* (1q25.2-25.3) genes with prostate cancer in men of European origin. COX1 and COX2 are two isoforms of cyclooxygenase that are involved in the inflammatory pathway and have been implicated in the etiology of prostate cancer (PC). Using the SNPlex platform (ABI), thirty single nucleotide polymorphisms (SNPs) in *COX1* (15 SNPs) and *COX2* (15 SNPs) were genotyped in 347 histologically confirmed PC cases from 275 multiplex sibships and 346 unrelated controls; all cases and controls are of European ancestry. We did not find association with *COX1* variants. We observed a highly significant association with one SNP (rs2206593, GA) in the 3UTR of *COX2* (Minor Allele Frequency: 8% in cases and 2% in controls, $p<0.0001$). A haplotype defined by the minor allele of this SNP was found to be significantly over-represented in cases (7% versus 2%; OR: 4.74, 95% CI 2.4-9.4, multiple adjusted $p<0.0001$). Previous studies have shown that COX2 is consistently over-expressed in PC tumors. Our results suggest a plausible role of *COX2* in PC tumorigensis.

Reconstruction of the ancestral allele of the 8p23 inversion polymorphism. *E. Slaten[1], P.N. Rao[2], R.A. Ophoff[1,3]* 1) Cntr for Neurobehavioral Genet, Univ of California, Los Angeles, Los Angeles, CA; 2) Dept of Pathology, Univ of California, Los Angeles, CA; 3) Dept. of Human Genetics, Univ. of California, Los Angeles, CA.

Recent studies have shown a widespread distribution of genomic variations in the human genome. For some of them a functional consequence is known but for the vast majority it is not clear whether these variations have beneficial or negative effects on phenotype. While a large number of duplications and deletions have been reported while only a limited number of inversion variants have been described. Our study focuses on a common inversion polymorphism at chromosome 8p23 spanning nearly 4 megabases and flanked by large blocks of segmental duplications. The 8p23 inversion region is reported to be associated with severe bipolar, panic disorder and schizophrenia. However, there is no evidence of direct involvement of this inversion in these diseases. The observation that different haplotypes are linked to the inversion suggests recurrent inversion events in human history, possibly mediated by the flanking segmental duplications. Comparative syntenic analysis between human and mouse suggests the inverted order to be ancestral. To test this hypothesis we examined inversion status in three different ethnic groups of >25 individuals each: African American (AA), European American (EA), and Hispanic American (HA). We observed different inversion allele frequencies ranging from 65% in AA to 53% in HA. A previous study had reported an allele frequency of 27% in an Asian population. We further examined inversion status of some non-human primates including two chimpanzees, gorilla, rhesus monkey and bonobo. In these primate species, not only was the inverted allele was the most common but we also observed heterozygosity in the chimpanzees. Our data suggests that in human history the inverted allele is most likely to be ancestral, and shows that this inversion polymorphism extends into the other branches of the phylogenetic tree of primates.

Combined use of Cytogenetics, FISH and Array-CGH in Detection of Interstitial 7q Deletion. *M.L. Wiggins, D.L. Pickering, D.H. Zaleski, I.S. Kanev, S.D. Nielsen, G.B. Schaefer, B.J. Dave, W.G. Sanger* Human Genetics, University of Nebraska Medical Center, Omaha, NE.

We present a case of an 11 year old female with an interstitial deletion of 7q diagnosed by conventional cytogenetic methods combined with high resolution array-CGH (aCGH). She presented with marked growth and developmental delays, microcephaly, moderate to severe scoliosis, absence of the distal sacrum and coccyx with residual ossification center and prominence of the posterior bony elements, right renal atrophy, status post repair of severe bilateral ureteral vesicle junction obstruction, syrinx, with concerns for her vision. Upon additional examination, her physician also noted a hypoplastic uvula, normal extremities and digits, normal genitalia and normal chest with early breast budding. Neurologically, she had an unusual gait with stereotypic repetitive hand movements. Cytogenetics, FISH, aCGH and MECP2 studies were requested on peripheral blood from this patient who was referred for possible Rett syndrome. Cytogenetic analysis, at a 750 band level, revealed a deletion within the 7q terminal region. Subsequent FISH studies using a telomere probe for 7q were normal as were aCGH studies using the Spectral Genomics Constitutional Array 400. We then used the Spectral Chip 2600 and the deletion of 7q was confirmed and further characterized as an interstitial deletion of 4 clones encompassing an approximate 6MB region from chromosomal region 7q36.2-7q36.3 as follows: arr cgh (RP11-43L9 - RP11-58F7)x1. The findings in the case demonstrate the importance of continuing to perform cytogenetic studies in addition to FISH and in depth aCGH in the diagnosis of congenital abnormalities. This combination of tests enhances accurate delineation of chromosomal regions and the DNA segments deleted or duplicated and assists with genotype/phenotype correlations.

A novel COL1A2 mutation leading to osteogenesis imperfecta type 1 in identical twins. *T.P. Ponnapakkam, D. Sledge, R. Gensure* Pediatrics, Ochsner Clinic Foundation, New Orleans, LA.

Osteogenesis imperfecta (OI) is an autosomal dominant disorder of bone collagen matrix with increased bone fragility and multiple fractures. It is further classified based on severity, with type 1 being the least severe. Most individuals with OI have mutations in one of two genes encoding type 1 collagen, COL1A1 or COL1A2 (>200 different mutations described). We now report on identical twins with multiple fractures who were found to have a novel COL1A2 mutation. Twin 1 suffered 5 fractures by age 16, including wrist fractures sustained while tripping and trying to break his fall. Twin 2 suffered 3 fractures in the same time period. A complete metabolic workup for each brother showed only increased bone turnover (elevated alkaline phosphatase and urinary crosslinked N-telopeptides). The spinal bone mineral density of twin 1 was Z=-2.6, and that of twin 2 was Z=-2.3 (2-3 standard deviations below the mean). A review of the family history revealed that the twins mother had sustained one fracture, a tibial fracture after falling on grass at age 13. She has been diagnosed with osteopenia (T-score =-1.5) and has been treated with residronate and alendronate. The patients father has not had any fractures. Sequence analysis for each twin revealed a C3495>G missense mutation in COL1A2. No other mutations were found in COL1A1 or COL1A2. Sequence analysis of the parents revealed the same C3495>G mutation in the mother but not in the father. This mutation is predicted to cause an D1165E change in the encoded protein, alpha-2(I) collagen. This substitution occurs in the C-propetide region of the protein, and the substitution of aspartic acid with glutamic acid is a conservative change. Alterations of this amino acid have not been previously described as associated with OI or as a known polymorphism. In fact, there are only a few known mutations in the C-propetide region of alpha-2(I) collagen associated with OI, and most of those are associated with the more severe types. We postulate that although the mutation occurs in an apparently critical region, the substitution is conservative and thus the bone fragility in this family is relatively mild.

Haplotyping Human Leukocyte Antigen (HLA) and Killer Immunoglobulin-like Receptor (KIR) Genes with Haplotype-Specific Extraction. *G.S. Scavello[1], C. Turino[1], D. Ferriola[2], M. Kunkel[2], J. Dapprich[2], N. Murphy[1]* 1) QIAGEN, West Chester, PA; 2) Generation Biotech Lawrenceville, NJ.

Haplotype-Specific Extraction (HSE) is a simple automated technique that allows haplotype identification from genomic DNA, without familial knowledge, by physically separating a diploid sample into haploid components. Determining haplotypes, gene order and dosage are current foci for researchers in KIR and HLA genetics, which may be of particular interest when evaluating graft-versus-host-disease (GVHD) in well-matched transplants. Since each KIR haplotype comprises 7-14 genes (A haplotypes include 7 genes, B haplotypes comprise many combinations of 7 to 14 genes), haplotyping an individual with two B haplotypes is virtually impossible without familial studies. Samples were haplo-extracted from genomic DNA at the HLA and KIR loci. Generic PCR primers were designed to amplify from intron 8 to intron 1 of the KIR genes including all intragenic and intergenic regions. Amplicons were sequenced to verify gene presence and orientation. Heterozygous polymorphisms were then used to tile the extractions and form a contiguous KIR haplotype spanning 150kb. HLA-B, HLA-Cw, HLA-DRB1, and HLA-DQB1 extractions were amplified at the extraction points to verify haploid state. The extractions were genotyped by real-time PCR at SNPs flanking and intermediate to the targeted genes to form a contiguous 128kb haplotype encompassing HLA-B to HLA-Cw and an 89kb haplotype spanning HLA-DQB1 to HLA-DRB1. Two complete KIR and two abbreviated HLA haplotypes were defined using a combination of HSE, real-time PCR and DNA sequencing. The samples were genotyped and allele level typing was performed for all genes in each haplotype. Haplotype construction with HSE should prove a useful method for validating statistically derived haplotypes from population studies, for directly evaluating gene dosage (copy number) without long-range PCR and for assigning KIR haplotypes when familial studies are not possible.

Impact of Long Term Storage and Purification of Whole Genome Amplified DNA on High Throughput Genotyping Technologies. *R. Tewhey[1], C. Guiducci[1], A. Rachupka[1], L. Gianniny[1], L. Ziaugra[1], S. Gabriel[1], L. Groop[2], N.P. Burtt[1], D. Altshuler[1,3,4,5,6,7]* 1) Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, MA; 2) Department of Endocrinology, University Hospital MAS, Lund University, Malmö, Sweden; 3) Center for Human Genome Research, Massachusetts General Hospital, Boston, MA; 4) Department of Molecular Biology, Massachusetts General Hospital, Boston, MA; 5) Diabetes Unit, Massachusetts General Hospital, Boston, MA; 6) Department of Medicine, Harvard Medical School, Boston, MA; 7) Department of Genetics, Harvard Medical School, Boston, MA.

DNA integrity is central to the success of any genotyping technology. Whole genome amplification (WGA) offers a robust means of extending limited DNA supplies for future use. The fidelity of this method has been assessed for several technologies, yet the impact of long term storage and components of the WGA reaction on various genomic assays has not been formally investigated. We evaluated genotyping success rates and accuracy for 980 DNA samples isolated from whole blood, where each sample was whole genome amplified, and stored at -80C for 10 months. Using the Sequenom MassARRAY hME and iPLEX platforms, we genotyped 30 polymorphic SNPs and observed call rates of 95.1% (hME), 79.2% (iPLEX) for stored wgaDNA, with an accuracy of 95.3% (hME), 97.2% (iPLEX). As a control, we genotyped the 980 native genomic DNA counterparts with an average call rate of 99.2% and an accuracy of 99.8%. Concerned that either poor amplification or DNA degradation was the origin of such a decrease in performance, we purified the 980 WGA samples, by ultrafiltration. Surprisingly, both call rate and accuracy increased to levels comparable to genomic DNA (98.3% and 99.7%, respectively). This observation is key to our growing understanding of the behavior of wgaDNA. Presently, we are assessing the role different storage concentrationS and buffers may play in maintaining the integrity of wgaDNA. Additionally, we are testing freshly amplified wgaDNA product for genotyping success. Finally, we plan to demonstrate the impact of purification and stored wgaDNA on both the Illumina GoldenGate Assay and the Affymetrix 500K Array Set.

Females with X-linked Fabry disease frequently have significant organ involvement. *W.R. Wilcox[1], D.P. Germain[2] for the Female Working Group of the North American and European Fabry Registry Board of Advisors* 1) Cedars-Sinai Medical Ctr, Los Angeles, CA; 2) Hôpital Européen Georges Pompidou Paris, France.

Fabry disease (FD, OMIM 301500) is an X-linked lysosomal storage disease caused by deficiency of alpha-galactosidase A activity. Until recently, females with FD were thought to be infrequently clinically affected. The Fabry Registry (www.fabryregistry.com) is a global program to collect longitudinal clinical data on FD patients, regardless of whether or not they are receiving enzyme replacement therapy (ERT). Although registries tend to preferentially enroll more symptomatic patients, there has been a special effort to enroll females regardless of symptomatology. 809 females have been enrolled with a mean age at enrollment of 39.617.0 years. 82.6% had symptoms and signs of FD, but only 33% have ever received ERT compared to 77% of males. The mean age of symptom onset was 19.115.3 years but the diagnosis was not made until 16.415.4 years later. 81% reported a family history of FD. The most common presenting symptom in symptomatic patients was acroparesthesia pain in 41.4% beginning at an age of 13.811.6 years, but 10.7% presented with renal disease. 20.5% have had a major cerebrovascular, cardiac, or renal event at an average age of 45.114.1 years. 17.7% had stage 3 or worse chronic kidney disease (CKD). An even greater percentage, 61.2%, had a GFR < 90 ml/min. Proteinuria greater than 300 mg/24 hours, a major risk factor for renal disease progression, was present in 36.6%. 19.7% had more than 1 g of proteinuria per day. 16.9% had left ventricular hypertrophy and 6.8% had a cardiac arrhythmia. Quality of life measured by the SF-36 survey declined significantly with age. We conclude that females with FD have a significant risk for major organ involvement and decreased quality of life. Females with Fabry disease should be regularly monitored and ERT initiated, when indicated, before there is irreversible end-organ damage. Guidelines for FD monitoring and management are available (Eng et al. Genet Med, in press).

Deficiency of alpha-aminoadipic semialdehyde dehydrogenase in lysine metabolism; another cause for vitamin B-6 responsive seizures in the newborn. *G. Scharer[1,3], S. von Kaenel[3], A. White[2], E. Spector[3], G. Creadon-Swindell[3], J. van Hove[1]* 1) Div. of Clinical Genetics and Metabolism, Children's Hospital/UCDHSC, Denver, CO; 2) Div. of Neurology, Children's Hospital/UCDHSC, Denver, CO; 3) DNA Diagnostics Laboratory, Dept. of Pediatrics, UCDHSC, Denver, CO.

While rare, pyridoxine-dependent seizures (PDS) are a serious complication in the newborn period if not recognized and treated immediately. Psychomotor retardation and death can result. Mills et al. (Nature Medicine, 2006) have shown that defects of the alpha-aminoadipic semialdehyde dehydrogenase (-AASA) in the lysine degradation pathway lead to accumulation of a metabolite, which inactivates pyridoxal 5-phosphate. Subsequent depletion of active vitamin B-6 in the brain results in seizures typically within hours of birth and resistant to conventional anticonvulsants. Mutations in the *ALDH7A1* gene coding for -AASA are responsible for this type of PDS. We report a case of PDS in a now 4-year old female, who presented at 7 days of life with intractable seizures unresponsive to standard antiepileptic medications. A trial of IV pyridoxine resulted in cessation of seizures and normalization of the EEG. Shortly after weaning from pyridoxine seizures returned and stopped only after reintroducing the vitamin. The patient has been seizure free on 100mg of pyridoxine daily. Serum pipecolic acid level was measured in the twice normal range suggesting -AASA deficiency as the likely cause for this patients phenotype. Direct sequencing of all exons and exon/intron boundaries of *ALDH7A1* revealed two new base changes that are thought to be disease-causing mutations including a novel splice-site mutation. A heterozygous G477R mutation in exon-17, a heterozygous IVS 16(5) A>G mutation in intron-16 and two likely polymorphisms were identified. While classic PDS does present in the neonatal period, genotypic variations have to be considered resulting in atypical phenotypes, and therefore patients may present with milder symptoms and later in life. PDS should be considered in a patient with intractable seizures and no other recognizable etiology. Molecular analysis will allow for diagnostic confirmation and prenatal diagnosis.

Fast computation of large numbers of LOD scores for genetic linkage analysis via a novel ''polynomial'' implementation. *H. Wang[1], A. Segre[1,2], Y. Huang[1], J. O'Connell[3,1], V. Vieland[1]* 1) Center for Statistical Genetics Research, The University of Iowa, Iowa City, IA; 2) Department of Computer Science, The University of Iowa, Iowa City, IA; 3) Department of Medicine, University of Maryland, Baltimore, MD.

LOD scores are usually computed assuming specific values for parameters of the trait model. However, for complex disorders the specified parameter values are almost certainly incorrect. Under some circumstances, maximizing over the trait parameters is a statistically correct way to redress this difficulty, basing inference on the MOD (Clerget-Darpoux et al. 1986). A second way to handle the unknown trait model is via integration, e.g., with inference based on the PPL (Vieland 1998, Logue et al. 2003). Both approaches generally rely on use of an exhaustive grid search (or average) over several trait parameters, calculating the LOD for each parameter vector in turn, and this can entail very large numbers of calculations. For instance, a typical genome scan utilizing a simple trait model allowing for heterogeneity can easily entail calculating millions of LOD scores; and recent extensions to incorporate multipoint analysis, trait-marker linkage disequilibrium, and quantitative trait models have exacerbated this difficulty. Here, we propose an alternative computational approach, replacing the usual LOD calculation based on a fixed parameter value by an algebraic expression, essentially "compiling" the calculation over a pedigree into a symbolic form and then evaluating the compiled expression with different parameter values. That is, rather than processing each pedigree (including pedigree peeling) one time for each parameter vector, the pedigree is processed one time to construct a polynomial expression, which can then be rapidly evaluated an arbitrary number of times. Here we present initial results showing that this approach can speed up genetic linkage calculations by a factor of between 10 and 1000, when LODs are evaluated for large numbers of parameter vectors .

CGH-based microarray analysis in a Caucasian newborn with NSD1 deletion and Sotos syndrome: Identification of chromosome 5q35.2 microdeletion. *J.D. Ranells[1], A. Patel[2], P. Eng[2], K. Pham[2], J.R. Lupski[2], S.W. Cheung[2]* 1) Dept Peds/Div Genetics, University of South Florida Tampa, FL; 2) Dept Molec and Human Genetics, Baylor College of Medicine, Houston, TX.

Sotos syndrome is an autosomal dominant condition characterized by pre- and postnatal overgrowth, facial dysmorphology including macrodolichocephaly, hypertelorism and down-slanting palpebral fissures, and intellectual impairment. A point mutation or genomic deletion of the NSD1 gene has been identified in the majority of affected individuals. An NSD1 deletion, detected by FISH analysis, has been reported in approximately 50% of patients of Japanese heritage, but in only 10% of non-Japanese. Rarely, a cytogenetic abnormality involving the 5q35 locus may be present.

We describe a newborn Caucasian male with multiple congenital anomalies and features of Sotos syndrome in whom deletion of NSD1 was detected by FISH analysis. Initial chromosome analysis on cultured amniocytes was normal. Birth weight at term was 3385g (80th centile), length 49.7cm (70th centile) and OFC 38 cm (90th centile). In addition to facial dysmorphia, ASD, VSD, PDA and hydronephrosis consistent with Sotos syndrome, the infant had imperforate anus, excess nuchal folds, preauricular pit, simian creases and minor digital anomalies. Chromosomal microdeletion involving the Sotos syndrome locus at 5q35 was suspected.

High resolution chromosome analysis revealed a 46,XY,del(5)(q35.2q35.3) chromosome pattern. A CGH-based microarray designed to interrogate clinically relevant genomic regions revealed a loss of copy number in the subtelomeric region on the distal long arm of chromosome 5, detected by 6 clones encompassing approximately 5 Mb. This large deletion involving the breakpoint region upstsream of the Sotos critical region may explain the unusual clinical findings. Detailed genotype and phenotype correlation will be presented.

Concordance between the definitive diagnostic status and reference diagnoses of birth defects and genetic diseases: The Harris County experience. *F. Suarez[1], L. Potocki[2]* 1) Inst de Genetica Humana, Univ Javeriana, Bogota, Colombia; 2) Department of Molecular and Human Genetics, Baylor College of Medicine. Ben Taub Genetics Clinic.

To describe the concordance between the reference diagnostic of birth defects and genetic diseases and the final diagnosis in a birth defects clinic. All the active clinical histories of the clinic of congenital defects of the Hospital of Ben Taub were reviewed; we analyzed, registry of definitive diagnose specified in the clinical chart, and the diagnostic by which the proband was sent to the clinic. To demonstrate differences between groups a chi square was used, to compare the concordance between groups we use the Kappa index. The total number of reviewed clinical histories was of 542. A definitive diagnose was reached in 302 cases (55.7%) .Of the 240 undiagnosed cases, 91 cases (37.9 %) consisted of patients with mental retardation or development delay with one or more minor anomalies. A total of 131 patients (24.1 %) were referred with a precise diagnosis, disease name or the eponym of the suspected genetic syndrome, from this group 80 cases (61.7 %) correspond to Down syndrome and 6 patients (4,6 %) of Turner syndrome, 77 Down syndrome cases were confirmed in the Hospital and the other two cases were considered to be healthy patients, 8 patients (6.1 %) had a different diagnostic from the initially referred. 5 cases (3.8 %) did not have a final diagnose. Between the referred cases with a specific diagnostic compared with the cases only referred for physical features anomaly, there was a more precise diagnostic in the first group (p=0.0). When comparing the concordance between the reference diagnostic and the definitive diagnostic after the evaluation at the clinic we found agreement of 0,402 (95% Confidence Interval: 0.042-0.12. Kappa: 0,081). Very low concordance were found between reference diagnostic from the non specialist and definitive final diagnostic at the birth defects clinics, it affirms the necessity of medical genetic evaluation in difficult cases related to dysmorphology, developmental delay and birth defects, in order to reach a conclusive and definitive diagnostic.

Genomic Evolution in Myelodysplastic Syndrome (MDS). *C. Szych, R. Felgar, J. O'malley, J. DeFeo, M.A. Iqbal, N. Wang* Pathology, University of Rochester Medical Center, Rochester.

To define the genomic aberrations associated with the genesis and progression of MDS, 366 cases with an indication of myelodysplastic syndrome were analyzed using G-banding. Chromosomal aberrations were detected in 101 cases. Of these, single genomic aberrations were detected in 66 cases; 9.09% with del(5q), 7.58% with del(20q), 7.58% with +21, 4.55% with -7, 4.55% with +8 and 28.79% with -Y. Multiple genomic aberrations were detected in 35 cases; 54.28% with del(5q)/-5, 34.28% with del(7q)/-7, 22.86% with del(20q)/-20, 22.86% with -18, 20.00% with -13, 17.14% with +8, 8.57% with +21 and 17.14% with -Y. The incidence of -5/5q- and -7/7q- is high in both cases with single aberrations and multiple aberrations. This suggests that these abnormalities are associated with genomic instability which leads to multiple chromosomal aberrations. In contrast, the aberrations of +8, +21, -13, del(20q) and -Y are more prominent in the cases with a single aberration than those in multiple aberrations, which suggests that these abnormalities are primary aberrations with less genomic instability toward chromosomal evolution. The aberration of -Y was identified only in cases over the age of 55 indicating that aging contributes a great deal to the loss of the Y chromosome. Statistical analysis is being conducted to verify the concordance and discordance of the various chromosomal aberrations identified in MDS.

***CNR1* variation modulates risk for drug and alcohol dependence.** *L. Zuo[1,2], J. Covault[3], X. Luo[1,2], H.R. Kranzler[3], J. Gelernter[1,2]* 1) Dept Psychiatry, Yale Univ Sch Medicine, West Haven, CT; 2) VA CT Healthcare System, West Haven, CT; 3) Dept Psychiatry, Univ CT Sch Med, Farmington, CT.

Human cannabinoid receptor 1 (CB1) may play an important role in the development of drug dependence (DD) and alcohol dependence (AD), potentially via interaction with dopaminergic signaling in brain reward circuits. Following initial reports of the association of the *CNR1* gene (which encodes CB1) with DD or AD, we examined the association in a large case-control sample. Ten *CNR1* markers and 38 ancestry-informative markers were genotyped in 451 healthy controls and 550 substance dependent (SD; i.e. AD and/or DD) patients [including European-Americans (EAs) and African-Americans (AAs)]. The ancestry proportions of each subject were derived using the program STRUCTURE. Haplotype and diplotype probabilities for each subject were estimated using the program PHASE. The common confounding effects on association analysis from admixture, age, and sex, were controlled for using logistic regression analysis, Haplotype Trend Regression analysis or Diplotype Trend Regression analysis. The disease risk and protective alleles were fine-mapped using a linkage disequilibrium measure (). In EAs, risk for each SD subtype significantly increased with the number of G alleles at rs6454674 (SNP1). The frequencies of T alleles at rs806368 (SNP2) in the cases with DD or comorbid DD and AD were suggestively higher than in controls. SNP1^G+ (the genotypes containing a G allele) and SNP2^T/T genotypes had significant interaction effects on risk for each SD subtype (p=0.0003 for comorbid DD and AD, 0.0002 for DD, and 0.007 for AD). The peak values among all the markers were seen for SNP1 and SNP2. The magnitude of the odds ratios and positive likelihood ratios for SNP1 and SNP1×SNP2 in different SD subtypes was in the following order: comorbid DD and AD > DD > AD. In AAs, SNP1 was suggestively associated with each SD subtype (nominal p<0.05). These results suggest that *CNR1* variation and interactions play important roles in risk for both DD and AD and that the putative disease-influencing loci for SD are close to SNP1 or SNP2.

Homozygosity mapping in 13 families with autosomal recessive primary microcephaly: Linkage of three families to MCPH3 locus on Chr. 9q34. *A.J. Parsian[1], M.A. Karim[1], M. Cleves[1], A. Jankhah[2], S.M. Elsayed[3], E. Elsobky[3], A. Parsian[1]* 1) Dept Pediatrics, Univ Arkansas Medical Sci, Little Rock, AR; 2) Shiraz Medical Genetic Counseling Center, Shiraz, Iran; 3) Medical Genetic Center, Cairo, Egypt.

Autosomal recessive primary microcephaly (MCPH) is a neurodevelopmental disorder characterized by global reduction in brain size affecting mostly the cerebral cortex. The individuals with MCPH suffer from mental retardation of variable degrees. MCPH is a heterogeneous disorder and six loci (MCPH1-6) have been identified so far. MCPH5 that is caused by the mutations in ASPM that encodes the human homologue of fly abnormal spindle gene (asp) is the most common. We did homozygosity mapping and linkage analysis by using 47 microsattelite markers across the six loci in the affected individuals from 13 consanguineous families. We mapped the MCPH3 locus to a 1.55 cM region of chromosome 9q34 in three independent MCPH families. The marker D9S1881 produced a LOD score of 3.33 at the recombination fraction of zero using the sub-program Mlink in the software package Linkage 5.1 (Lathrop and Lalouel, 1984). The gene for this locus is CDK5RAP2. We excluded any mutation in the exon 4 and exon 27 including the splice sites (S81X and E385fsX4, Bond et al. 2005) of this gene by sequencing genomics DNA from three affected patients from these three linked families. Currently, we are in the process of sequencing the other translated exons of CDK5RAP2 gene. The remaining ten families did not linked to any of the MCPH reported loci. Our data strongly suggests MCPH locus heterogeneity and the existence of additional novel MCPH loci elsewhere in the human genome.

Dissection of the roles of collybistin, a neuron-enriched GDP-GTP exchange factor, in synapse formation and function.*ARHGEF9*. *A. Sertie, G. Alencastro, R. Passos Bueno* CEGH, Sao Paulo, Brazil.

Inhibitory glycine and many $GABA_A$ receptors subtypes are clustered at axon terminal contacts by the scaffold protein gephyrin, which in turn is targeted to the cell membrane by collybistin. Collybistin is a neuron-enriched member of the guanine nucleotide exchange factor (GEF) family for Rho GTPases. Mutations in *ARHGEF9* gene (Xq22.1), encoding human collybistin, were identified in a patient with hyperekplexia (exaggerate startle reflex and neonatal hypertonia), epilepsy and mental retardation and in patients with mental retardation (2 isolated cases and 1 familial). Although these findings reveal that collybistin is an important determinant of inhibitory synaptogenesis and neuron plasticity, our understanding about collybistin functions and regulation is far from complete. In order to gain further insights into collybistin function, we sought to identify novel collybistin-binding proteins by yeast two-hybrid screening. Screening a human fetal brain cDNA library with human collybistin as bait, we identified eigth different positive cDNA clones fused in frame with the Gal4 activation domain. After retesting each protein interaction in yeast, only three interesting cDNA clones were defined truly positives. We are currently performing coimunoprecipitation, immunocytochemistry and GST-pull down experiments to substantiate these interactions biochemically. In additon, in order to investigate the involvement of *ARHGEF9* gene in the etiology of hyperekplexia and epilepsy associated with mental retardation in Brazilian patients, affected individuals are being examined for mutations in this gene by dHPLC and sequence analysis. We have analysed a total of 6 hyperekplexia patients (4 males and 2 females) and 8 males with epilepsy and mental retardation and no pathogenic mutations were identified. We are currently attempting to collect DNA samples from other patients. This study may contribute to the understanding of collybistin roles in neurons, its regulation, the spectrum of clinical variability associated with mutations in *ARHGEF9* gene and how these mutations affect neuronal development and function. CEPID / FAPESP; CNPq; asertie@hotmail.com.

Functional and physical interaction between ZIC3 mutants and GLI3 *in vitro*. *L. Zhu, S. Poole, J.W. Belmont*
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Mutations in *ZIC3* (OMIM 306995), a zinc finger transcription factor, cause X-linked Heterotaxy (HTX1), a congenital disorder resulting from failure to establish normal left-right asymmetry during embryonic development. A targeted deletion of the murine *Zic3* locus has been created and the phenotype of Zic3 null mice correctly models the defects found in human HTX1. Multiple abnormalities in the central nervous system (CNS) and axial skeleton were observed in Zic3 null mice and the patients with the majority of *ZIC3* mutations. Previous studies indicate that Gli proteins are involved in multiple aspects of neural and skeletal development. In this study we provide evidence that human ZIC3 protein can bind to the GLI consensus binding site (GLIBS) and physically interact with GLI3 protein. Investigation of the ZIC3 mutants identified in HTX1 patients revealed that all five intact zinc finger domains were necessary for the binding of ZIC3 to GLIBS. Although the role of GLIBS in the activation of TK promoter by ZIC3 is modest, ZIC3 can synergistically activate TK promoter with GLI3 in the presence of GLIBS. All the nonsense and frameshift ZIC3 mutants lacking one or more of the zinc finger domains could not physically interact with GLI3; however, the missense mutant K405E, located in the fifth zinc finger domain, retains its binding ability to GLI3, as well as the missense mutant P217A located in the N-terminal region before the zinc finger domains. Luciferase reporter assay indicated that both P217A and K405E mutants could still co-activate the reporter gene expression with GLI3, while all the GLI3-nonbinding ZIC3 mutants were unable, suggesting that physical interaction between ZIC3 and GLI3 is required for their functional interaction. Interestingly, no CNS or skeleton abnormalities were observed in the human patients associated with P217A or K405E mutants.

Approaches to whole genome association analysis: estimation of global and local genomic sharing using genome-wide SNP data. *S.M. Purcell[1], B.N. Neale[2], M.J. Daly[1,3], P.C. Sham[2,4]* 1) CHGR, Massachusetts General Hospital, Boston, MA, USA; 2) SGDP Centre, Institute of Psychiatry, Kings College London, UK; 3) The Broad Institute of Harvard & MIT, Cambridge, MA, USA; 4) Genome Research Centre, University of Hong Kong, Hong Kong.

Recent technological developments have made possible the efficient genotyping of hundreds of thousands of SNPs in individual DNA samples. Genetic data of this quantity and detail will provide information on the ancestry of individuals and on the relationship between pairs of distantly-related individuals. We have developed a simple identity-by-descent method to estimate the global genomic sharing between any two individuals from the same population. This method was used to detect previously unknown relationships among individuals in the HapMap population samples. We are now developing a Hidden Markov Model (HMM) to obtain estimates of local genomic sharing between pairs of individuals, given the genotype data and the estimated global genomic sharing. The methodology will take some account of complications such as genotyping errors and linkage disequilibrium between SNPs, and results in estimated probabilities of sharing none, one or both haplotypes by descent, at any section of the genome. The method is be implemented in the PLINK software (http://pngu.mgh.harvard.edu/purcell/plink/) and applied to the HapMap Phase 2 data. PLINK is a whole genome association analysis toolset, offering both computationally efficient implementations of existing methodologies (data management, summary statistics, sample matching based on inferred ancestry and a variety of association statistics) as well as novel approaches such as IBD estimation. Finally, we will discuss the potential use of this methodology to gene-mapping studies of complex diseases and quantitative traits.

Assay development and quantitative high through-put screening of -glucocerebrosidase: the search for pharmacological chaperones. *D. Urban[1], W. Zheng[2], A. Simeonov[2], E. Goldin[1], J. Padia[2], A. Jadhav[2], O. Goker-Alpan[1], E. Sidransky[1]* 1) MGB/NHGRI, NIH, Bethesda, MD; 2) NCGC/NHGRI, NIH, Bethesda, MD.

Gaucher disease is the inherited deficiency of the lysosomal enzyme -glucocerebrosidase (GC). The most common alterations found in Gaucher disease are missense mutations, which can lead to conformational changes in the protein structure. It has been reported that chemical chaperones might correct misfolding of the mutant enzyme and restore normal function. We have developed a fluorogenic enzyme assay to screen libraries of compounds to identify potential chaperones. Two profluorophore-labeled synthetic GC substrates, one yielding a blue fluorescent product and the other a red fluorescent product, were developed for these screens. The use of two different substrates helps to eliminate false positives from the compound screening. Approximately 62,000 compounds were screened in a quantitative high throughput screening (qHTS). We identified 299 inhibitors and 56 activators with a hit rate of 0.57 percent. Selected compounds were then tested in a cell-based assay using a lysosomotropic substrate to examine their potential to act as chaperones by restoring -GC activity in the mutant cells. This strategy may ultimately identify a set of novel small molecules that can be tested as therapeutic agents for the treatment of Gaucher disease.

A Rare Form of Dextrojuxtaposition of the Left Atrial Appendage. *M. Thompson[1], S. Keating[1], T. Cavalle-Garrido[2], D. Chitayat[3,4]* 1) Dept Pathology & Lab Medicine, Mount Sinai Hospital, Toronto, ON, Canada; 2) Div of Cardiology, Dept of Pediatrics, Hospital for Sick Children, Toronto; 3) The Prenatal Diagnosis Medical Genetics Programme, Mount Sinai Hospital, Toronto; 4) Div of Clinical and Metabolic Genetics, Hospital for Sick Children, Toronto.

Juxtaposition of the atrial appendages is a rare congenital cardiac anomaly with a previously reported incidence of 0.28% of patients referred for echocardiogram to a tertiary medical centre. Dextrojuxtaposition of the left atrial appendage is 6 to 8 times less common than levojuxtaposition of the right atrial appendage.

We report a rare case of dextrojuxtaposition of the left atrial appendage (JLAA) found at autopsy. The karyotype was that of a normal female, and no deletion was detected at 22q11.2. Both atrial appendages were placed to the right of the great arteries, the left atrial appendage superior to the right. There was atrial situs solitus and atrioventricular discordance. The left atrium emptied into a superoanteriorly positioned hypoplastic right ventricle and tricuspid valve. The mitral valve and a large left ventricle with right hand topology were connected to the right atrium. Atrial and ventricular septal defects were noted. An overriding, left ventricle-dominant aortic trunk provided a single outlet and the pulmonary artery, with an atretic valve, originated posteriorly at the aortic root. As in other reported cases, no pattern of associated non-cardiac malformations was apparent while a JLAA syndrome of cardiac anomalies has been described.

This form of juxtaposition is a rare finding and likely occurs as a result of abnormal looping of the embryologic tubular heart resulting in both atrial appendages situated to the right of the great arteries.

Using a murine model to identify genes important in pulmonary arterial hypertension (PAH). *M.W. Pauciulo[1], L. Liu[2], W.A. Tuchfarber[1], P.T. Hale[1], T. Foroud[2], W.C. Nichols[1]* 1) Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 2) Department of Medical and Molecular Genetics, Indiana University Medical Center, Indianapolis, IN.

PAH can be an autosomal dominant disorder characterized by occlusion and remodeling of the pulmonary arteries which leads to sustained elevation of pulmonary vascular resistance and progressive right heart failure. We previously performed a survey of 11 inbred mouse strains and identified substantial strain specific differences with respect to the development of hypoxia-induced pulmonary hypertension (PH). The CAST/EiJ strain displayed the highest susceptibility to hypoxia-induced PH as measured by the % increase (50%) in the size of the right ventricle relative to the left ventricle and the septum (RV/LV+S). Muscularization of CAST/EiJ distal pulmonary arteries confirmed PH. Relative to normoxic controls, hypoxic CAST/EiJ mice also demonstrated increases in right ventricle relative to body weight (RV/BW)(150%), left ventricle and septum relative to body weight (LV+S/BW)(64%), and hematocrit (70.5 vs. 48.3). To map loci contributing to PH in CAST/EiJ mice, F1 and F2 animals were generated by matings with FVB/nJ mice, which demonstrated a 2.5 fold lower increase in RV/LV+S after chronic hypoxia as compared to CAST/EiJ mice. RV/LV+S, RV/BW, LV+S/BW, and hematocrit were determined for 316 F2 animals after chronic hypoxia exposure. A genome screen was performed using 93 microsatellite markers spaced 20 cM, and data were analyzed with Mapmaker/QTL. For RV/LV+S as the quantitative trait, chromosome 9 exhibited a LOD score of 3.76 while chromosomes 5, 8 and 11 each showed a LOD score >2. Chromosome 11 showed a LOD score of 3.64 for RV/BW. Using LV+S/BW, chromosome 17 showed a LOD score of 4.07. For hematocrit, a high LOD score of 4.70 was detected on chromosome 9. Additional LOD scores >2 were seen across the genome for the latter 3 phenotypes. In conclusion, we have presented evidence that multiple loci may be contributing to murine hypoxia-induced PH. Identification of the specific genes at these murine loci may further our understanding of genetic factors important in human pulmonary hypertension.

Identification of *EFHC2* as a quantitative trait locus for fear recognition in Turner Syndrome. *L.A. Weiss[1,2], S. Purcell[1,2], S. Waggoner[2], K. Lawrence[3], D. Spektor[3], M.J. Daly[2,4], D. Skuse[3], P. Sklar[1,2]* 1) Psychiatric and Neurodevelopmental Genetics Unit, Center for Human Genetic Research, Massachusetts General Hospital, Harvard Medical School, Boston, MA; 2) Medical and Population Genetics Group, Broad Institute of MIT and Harvard, Cambridge, MA; 3) Behavioural and Brain Sciences Unit, Institute of Child Health, Univ. College London, London, UK; 4) Department of Medicine, Center for Human Genetic Research, Massachusetts General Hospital, Harvard Medical School, Boston, MA.

Interpretation of facial expressions is critical for functioning in society. This can be seen clearly in autism; someone with average IQ can be profoundly disabled by social impairment. A third of women with Turner syndrome (45,X) have social difficulty similar to autism. Deletion mapping of the X-chromosome implicated a region of Xp11 critical for recognizing fearful faces. Our aim was to identify a gene influencing fear recognition by dense mapping of the 5 Mb locus. 93 (45,X) females were assessed by Schedules for the Assessment of Social Intelligence. Regression-based association mapping in the critical region was performed by the program *whap* using genotype data generated by mass spectrometry at 252 single nucleotide polymorphisms (SNPs). Initial quantitative analyses identified three regions of interest with association evidence ($P < 0.01$). To increase the SNP density in associated areas, 52 additional SNPs were genotyped in the three regions of interest. After analysis including the additional data, the third region contained four SNPs associated with decreased fear recognition ($P < 0.003$). Replication was performed in another 77 (45,X) females genotyped for 77 SNPs in the three regions of interest. Only the third region showed association of the same variation with fear recognition (rs7055196, rs7887763; $P = 0.022$ each). The associated SNPs span 40 kb within a novel gene, EF-hand domain containing 2 (*EFHC2*). The most strongly associated allele ($P = 0.00007$) has a frequency of 8.8% and accounts for 13% of the variance in fear recognition in the combined sample. *EFHC2* is now an excellent candidate to influence variation in social skills in the general population and in autism.

Investigation of the CCND1 gene in a variant t(9;22;11)(q34;q11.2;q13) chronic myeloid leukemia. *I. Yudelman[1], M.J. Macera[2,3], R. Zeng[2,3], J. Breshin[2,3], P. Chandra[3], A. Babu[2,3]* 1) Dept Medicine, Lenox Hill Hospital, New York, NY; 2) Div Molec Medicine & Genetics; 3) Dept Medicine, Wyckoff Heights Medical Ctr, Brooklyn, NY.

Five to ten percent of all Ph positive cases have a variant translocation and involves at least one additional chromosome in the rearrangement. There have been a number of studies done to determine prognostic value of the variant' translocation in response to Gleevec therapy. There appears to be no prognostic difference in the two groups. Recently, it has been described that some CML patients have a deletion in the 5 ABL1 gene and/or the 3 BCR gene. It has been reported that such deletions may have a survival disadvantage. Approximately 10% of the typical t(9;22) cases were shown to have a deletion in ABL and/or BCR; deletions are more frequent in the variant cases (up to 40%), some with deletions in the additional chromosome involved. A variant translocation, 46,XY,t(9;22;11)(q34;q11.2;q11), was seen in a patient referred for CML. FISH with a dual color-dual fusion BCR/ABL probe showed three signals of ABL1 (ABL1 x3) and three signals of BCR (BCR x3) with a single fusion of BCR/ABL (ABL1 con BCR x1). A second fusion was not seen as the 3BCR translocated to 11q13. The BCR/ABL1 probe showed no loss of signal and, therefore, no detectable deletion. The chromosome region 11q13 is the second most frequent in variant translocations. Oncogene cyclin D1 (CCND1/BCL1) is located in 11q13. To determine the role, if any, and location of the cyclin D1 oncogene and detect any loss of chromosome material, the CCND1/MYEOV break-apart probe was applied. The entire CCND1/MYEOV probe was translocated to the der(9), suggesting that the breakpoint is proximal to CCND1. No deletions at the breakpoints of this translocation were detected by this probe. The patient was treated with a standard Gleevec therapy and has shown a complete hematological response and a partial cytogenetic response. It is becoming clear that although there appears to be a higher percentage of variant translocations with deletions at the breakpoints, additional data will help to delineate clinical response in these patients.

MtDNA mutations in Leigh syndrome expression: recurrent incidence of the ND5 genetic defect. *S. Zhadanov[1,2], E. Grechanina[3,4], Yu. Grechanina[4], V. Gusar[3], N. Fedoseeva[3], S. Lebon[5], A. Munnich[5], T. Schurr[1]* 1) Department Anthropology, University of Pennsylvania, Philadelphia, PA; 2) Institute Cytology and Genetics SB RAS, Russia; 3) Kharkov Medical Genetics Center, Kharkov, Ukraine; 4) Department of Medical Genetics, State Medical University, Kharkov, Ukraine; 5) Department of Genetics, Hôspital Necker-Enfants Malades, Paris, France.

A variety of nuclear gene and mtDNA mutations has been reported to cause Leigh syndrome (LS) - a severe early childhood disorder characterized by bilateral necrotic lesions in the brainstem and basal ganglia. In our investigation of this disease, we conducted a genetic analysis of several LS cases carrying the novel 12706C mtDNA mutation that was earlier suspected to play a role in disease expression. Whole mtDNA genome sequencing revealed that each of these cases belonged to different Western Eurasian haplogroups, thereby pointing to the independent and recurrent occurrence of the 12706C variant. These findings also confirmed that this mutation was associated with LS regardless of the mtDNA background in which it occurred, or the presence of any additional changes in the mtDNA that would promote the expression of the LS phenotype. Thus, we conclude that this mutation plays a substantial deleterious role in LS development. The phylogenetic comparison of ND5 secondary structure in distant species further revealed that this subunit exhibits the highest sequence similarity to Na+/H+ antiporters. These results suggest a possible mechanism for ND5 dysfunction, and provide a further link between the pathogenesis of mtDNA defects and the LS phenotype.

Genetic analysis of 100 loci for coronary artery disease and associated phenotypes in a founder population. *G. Pare[1,2], D. Serre[1,2], D. Brisson[3], A. Montpetit[1], T.J. Hudson[1,2], D. Gaudet[3], J.C. Engert[2]* 1) Centre dInnovation Génome Québec, Montreal, QC; 2) McGill University, Montreal, QC; 3) Centre de médecine génique communautaire de lUniversité de Montréal, Chicoutimi, QC.

Coronary artery disease (CAD) is a major health concern for both developed and developing countries. With a heritability estimated at around 50%, there is a strong rational to better define the genetic contribution of CAD. This project involves the analysis of over 850 individuals from 142 families (with average sibships = 6.2) as well as a total of 558 cases and controls from the Saguenay Lac St-Jean region of Quebec using 1536 single nucleotide polymorphisms (SNPs) in 103 candidate genes for CAD. Candidate gene selection was based on published evidence of involvement in CAD risk as well as biochemical participation in pathways of interest (mainly lipoprotein metabolism). SNP selection was based on HapMap linkage disequilibrium data, common (MAF>5%) coding SNPs and previously published contributing SNPs. The gene and SNP panel was designed in collaboration with other laboratories involved in the Interheart project. Genotyping was done using the GoldenGate technology from Illumina and generated an overall call rate >99%. Since haplotypes of SNPs can be used as maximally informative markers, linkage analysis is possible with our dataset. Suggestive linkage for HDL cholesterol is seen on chromosome 1p36.22 (analysis done with MERLIN). Furthermore, both family-based and total association can be explored. Several Bonferroni-corrected significant associations are observed with lipoprotein-related traits as well as adiponectin plasma concentration (analysis done with QTDT). In particular, SNPs at the APOE locus are associated with apolipoprotein B levels and LDL cholesterol levels. APOA5 SNPs are associated with triglycerides levels. SNPs at the ADIPOQ (adiponectin precursor) locus are associated with adiponectin concentrations. Out of 13 different associations observed, 2 are new, whereas 11 are described in the current literature.

High frequency of sex differentiation impairment in males with 9p- : a series of ten patients. *L. Van Maldergem[1], C. Wetzburger[2], M. Francois[3], C. Heijmans[4], B. Candi[5], P. Mossay[6], J-P. Stalens[7], Y. Gillerot[8], C. Fourneau[9], D. Sartenaer[9], B. Parmentier[9], P. Deschamps[9], M. Fellous[10]* 1) Ctr Genetique Humaine, Université de Liège, Liège, Liège, Belgium; 2) Dept of Pediatrics,CHU, Charleroi; 3) Dept of Pediatrics,CHR du Val de Sambre, Auvelais; 4) Dept of Pediatrics,Hôpital de Jolimont, Haine St Paul; 5) Dept of Pediatrics, Clinique St Joseph, Lobbes; 6) Dept of Pediatrics, Clinique Ste Rosalie, Liège; 7) Dept of Pediatrics, Clinique Notre Dame Tournai; 8) Eurocat Hainaut-Namur; 9) Centre de génétique humaine, Institut de Pathologie et de génétique, Loverval; 10) Human Genetics Cochin Institute, Team 21 Pavillon Baudelocque Paris, France.

Deletion of the short arm of chromosome 9 is known to cause a characteristic 9p- syndrome associating mental retardation and typical dysmorphic features that include trigonocephaly, upward slanting of palpebral fissures, a long philtrum, a stunted tip of the nose, short neck, and dolichomesophalangy. Congenital heart disease, hernia and scoliosis may also be present. The critical region determining the typical phenotype is thought to be located in 9p22. Of interest is the fact that a number of male patients do present some degree of uncomplete sex differentiation. We report on ten males with a 9p- syndrome (pure in six cases, associated to duplication of another chromosomal segment in three cases and associated to Y chromosome inversion in one case) and discuss their sexual differentiation. All were mentally retarded. Seven of them (70%) had abnormalities of external genitalia, ranging from hypospadias to complete sex reversal. One of them (10%) developed a germ-cell tumour. These results confirm impaired male differentiation as a major clinical component of 9p- syndrome. Doublesex and mab3 related transcript (DMRT1) was identified as a candidate gene for human 9p24.3 associated sex reversal. In marsupials, DMRT1 protein was localized in the germ cells and the Sertoli cells of the testis, but in addition it was detected in the Leydig cells, peri-tubular myoid cells and within the acrosome of the sperm heads. However, its exact role in human remains to be elucidated.

Comprehensive Scanning of the mitochondrial genome in patients with kidney oncocytoma. *S.A.H. Zanssen[1], X. Hong[1], E. Kessel[1], B. Gunawan[2], L. Fuzesi[2], D. Warburton[3,4], E. Schon[1,4]* 1) Dept. of Neurology, Columbia University, New York, NY; 2) Dept. of Pathology, University of Goettingen, Goettingen, Germany; 3) Dept. of Pediatrics, Columbia University, New York, NY; 4) Dept. of Genetics and Development, Columbia University, New York, NY.

Mitochondrial defects have been associated with both severe neurodegenerative disorders and primary human cancers. Mutations of mtDNA have also been identified in various human cancers. Hereditary nuclear mutations in succinate dehydrogenase and fumarase genes cause a predisposition to paraganglioma and kidney cancers. Oncocytomas are mostly benign epithelial tumors and their predominant feature is a massive accumulation of mitochondria in the cytoplasm. Oncocytomas of the kidney are classified cytogenetically into 3 different groups characterized by loss of chromosome 1, by rearrangements involving chromosome 11q13, and by absence of cytogenetic aberrations but presence of mtDNA alterations. We performed a comprehensive scanning of the mitochondrial genome for point mutations in the latter group applying high resolution techniques such as SSCP and fluorescent sequencing. We detected new point mutations with a cluster in mitochondrial cytochrome c oxidase genes. We discuss these novel findings in the context of our previous findings in 11q13-aberrated oncocytomas with breakpoints near CCND1.

Rare Copy Number Changes Identified In Congenital Heart Disease Using ROMA. *D. Warburton[1], V. Jobanputra[1], K. Targoff[1], K. Anyane-Yeboa[1], M. Chen[2], J. Sebat[2], W. Chung[1], M. Wigler[2]* 1) Columbia Univ, New York, NY; 2) Cold Spring Harbor Laboratories, Cold Spring Harbor, NY.

Genomic microarray analysis has recently revealed small-scale changes in gene copy number to be a major form of human variation. Congenital heart disease (CHD), the most common congenital malformation, has a strong genetic component, yet most cases remain unexplained. Since CHD is a feature of most segmental aneuploidy syndromes, heart development must be sensitive to copy number changes (CNCs) in numerous parts of the genome. We hypothesize that small CNCs not detectable by standard cytogenetic analysis may be a significant cause of CHD.

We present preliminary data using ROMA (Representational Oligonucleotide Microarray Analysis) and an 85K NimbleGen chip with 35K resolution to search for CNCs on 24 patients with hypoplastic left heart syndrome and 13 patients with conotruncal defects. Reverse color CGH was performed using a normal control DNA. Segmentation analysis used a Hidden Markov Model to compute the most likely event. CNCs were considered rare and of interest if they were not observed in a comparison sample of 500 previously studied cases that include 175 normal individuals and 325 with other conditions not associated with CHD.

As expected, all CHD patients differed from the control in 7 to 14 regions of the genome. Most of these changes were observed frequently in the comparison sample and are presumably polymorphic normal variants. Using a cut-off probability of ?.70, there were 18 rare CNCs, including one of 3.2 Mb, which is very rare among normal variants. Nine of these CNCs contained known genes, and one contained a gene known to be involved in heart development. While intriguing, these studies are very preliminary. Verification by FISH and parental analyses to identify de novo changes are in progress. In addition, all confirmed de novo CNCs will be reanalyzed at a higher 6kb resolution using a 390K chip. While illustrating the significant problems involved in interpreting copy number variation, these data suggest that rare CNCs may contribute to the cause of CHD.

Linkage Analysis of Obesity Related Traits on Chromosomes 3, 7, 10 and 17 in a Yupik Eskimo Population. *R. Plaetke[1], Y. Wang[1], A. Goropashnaya[1], S. Hopkins[1], A.G. Comuzzie[2], G.V. Mohatt[1], B.B. Boyer[1]* 1) The Center for Alaska Native Health Research, University of Alaska, Fairbanks, AK; 2) Southwest Foundation for Biomedical Research, San Antonio, TX.

Prevalence of obesity and obesity-related chronic diseases are increasing among Alaska Native people. For example, in the CANHR sample of Yupik Eskimos, 64% of participants are overweight or obese; the prevalence of type 2 diabetes mellitus is approximately 3%. To investigate the genetic background of obesity-related phenotypes in a sample of extended pedigrees of Yupik Eskimos, we performed a 10 cM genome scan in 648 individuals 14-94 yrs of age using variance component analysis (VCA). Since family ascertainment is still on-going, we present preliminary results from the scan of the four chromosomes 3, 7, 10, and 17.

Markers from the ABI Prism Linkage Mapping Set vers. 2.5 were genotyped. Genotypic data and pedigree structure were checked by applying the programs infer, preswalk, and simwalk2. Phenotypic data were analyzed regarding normality; appropriate transformations were applied to reduce kurtosis and skewness. Narrow-sense heritabilities were determined for a variety of (transformed) phenotypes by including significant effects of covariates, including age, sex, and BMI (if appropriate). The following phenotypes with narrow-sense heritability 0.2 were selected for VCA: BMI, adiponectin, bodyfat %, fasting plasma glucose, and HDL-cholesterol. Two-point and multi-point VCA was performed with 56 extended pedigrees; 45% (25) of the pedigrees consisted of at least 6 individuals (range: 3-529; average: 22.5).

Multi-point analyses showed lodscores 1.4 in chromosomal regions covering known candidate genes for obesity related phenotypes: chrom 3q27: lod= 1.9, phenotype: adiponectin; 7q32: 2.3, glucose; 10p13-14: 1.5, BMI; 10q23-26: 1.4, adiponectin; 17p11: 1.6, bodyfat %. Including pleiotropy on BMI & HDL-cholesterol, multipoint analysis resulted in a lod=2.1 on 10p13-14.

[Grant support: P20 RR16430, R01MH59490].

CTCF regulates ataxin-7 gene expression through promotion of an anti-sense non-coding RNA: a novel system of transcriptional control linking CTCF with non-coding RNAs. *V.V. Pineda[1], R.T. Libby[1], G. Dunn[1], S. Baccam[1], A.C. Smith[1], S.J. Tapscott[2], G.N. Filippova[2], B.L. Sopher[1], A.R. La Spada[1]* 1) Lab Medicine, Univ. of Washington, Seattle, WA; 2) FHCRC, Seattle, WA.

Recent surveys of transcriptional units in the mouse and human genomes suggest the existence of >150,000 non-coding RNA (ncRNA) transcripts. A considerable fraction (~ 70% in the FANTOM survey) corresponds to anti-sense transcripts. CTCF is a multifunctional protein with a role in transcriptional regulation. Spinocerebellar ataxia type 7 (SCA7) is a polyglutamine disease characterized by retinal and cerebellar degeneration. The CAG repeat in ataxin-7 (Atx7) is flanked by two binding sites for CTCF. In order to understand the role of CTCF in regulating repeat instability and Atx7 transcription, we introduced Atx7 mini-genes into transgenic mice. Studies of these animals demonstrated the existence of an alternative Atx7 gene promoter whose activity is regulated by CTCF. We report the existence of an anti-sense non-coding RNA (AS ncRNA) at the Atx7 locus and have found that its expression is inversely correlated with the activity of the alternative promoter. Chromatin IP analysis indicated that CTCF occupancy correlated with expression of the AS ncRNA, suggesting that CTCF modulates Atx7 expression through the promotion of this AS ncRNA. To test this hypothesis, we have validated two different CTCF shRNAs and have derived a dual CTCF knock-down vector, using a lentiviral delivery system to transduce Y-79 retinoblastoma cells. Real-time RT-PCR analysis showed a significant reduction in the expression level of the Atx7 AS ncRNA message ($p < .001$ by t-test) concomitant with CTCF knockdown. We have surveyed human tissues and documented a reciprocal relationship between the levels of expression of the Atx7 sense transcript and Atx7 AS ncRNA. As Atx7 is a core component of the STAGA complex, our findings implicate CTCF in the regulation of the STAGA co-activator complex through an AS ncRNA whose expression modulates the activity of the Atx7 alternative promoter. Our findings support a model in which CTCF can regulate a transcriptional co-activator by promoting the expression of an AS ncRNA.

Cohesin association to chromatin is deregulated in Roberts syndrome. *H. Vega[1], O. Gualdron[1], M. Gordillo[1,2], E.W. Jabs[2]* 1) Universidad Nacional de Colombia, Bogota,Colombia; 2) Johns Hopkins University, Baltimore,MD.

Roberts Syndrome (RBS) is an autosomal recessive disorder characterized clinically by growth retardation, phocomelia, and craniofacial anomalies, and cytogenetically by the lack of cohesion at heterochromatic regions in the pericentromeric area of all chromosomes and the long arm of chromosome Y. Previously we reported that RBS is caused by mutations in ESCO2, one of the two human homologs of ECO1, a gene essential for establishment of cohesion in yeast. Sister chromatid cohesion is mediated by a multi-subunit complex called cohesin in a process that involves loading of cohesin onto chromatin, establishment during S phase, maintenance in G2, and dissolution in mitosis. To gain insight into the function of ESCO2, we investigated in RBS lymphoblastoid cell lines (LCLs) the association of cohesin subunits Rad21, SA1 and SA2 to chromatin during S and G2. By immunofluorescence we found that in control LCLs SA1 was distributed in a fine-granular nuclear pattern that was more intense in S phase than in G2. Despite a similar staining pattern, the intensity of the signal in RBS cells did not decrease at G2 and was even stronger when compared to control LCLs. Immunoblot of SA1, SA2 and Rad21 in cellular fractions enriched in chromatin-bound proteins confirmed the increase in the amount of chromatin associated cohesin complexes in RBS cells during G2/M. Although cohesin could not be detected in mitotic chromosomes, analysis of maintenance of arm cohesion in RBS cells treated with nocodazole for different periods of time showed that arm cohesion persists for up to twelve hours. This suggests that the normal dissociation of cohesin complexes from arm chromosomes in prometaphase is delayed or deregulated in RBS and explains the characteristic railroad track appearance of RBS chromosomes. Remarkably, inhibition of cohesin dissociation, persistence of arms cohesion and loss of centromeric constriction is observed in prometaphase arrested human cells lacking Plk1 or Aurora B activity. We propose that in addition to its role in establishment of sister chromatid cohesion during S phase, ESCO2 might regulate directly or indirectly the dissolution of cohesion in mitosis.

Ultra-high resolution oligonucleotide array CGH evaluation of a whole genome amplification protocol. *R.R. Selzer, T.A. Richmond, S.M. Rupp, J.M. Geoghegan, P.S. Eis* NimbleGen Systems Inc, Madison, WI.

Microarray-based comparative genomic hybridization (array CGH) methods have been widely used to investigate chromosomal abnormalities associated with cancer, developmental disorders, and population studies of normal copy number variation. Many of these studies are limited in the amount of sample collected, for example by microdissection of tumor samples or buccal swabs for which DNA quantities are limited. As higher resolution array CGH assays are becoming increasingly important for investigation of genomic rearrangements in both normal and disease populations, it is essential to validate that sample amplification does not introduce copy number bias into the results. We have used Qiagens Repli-G whole genome amplification service, which uses the Phi29 DNA polymerase multiple displacement amplification, on immortalized cell line DNA from Burkitts lymphoma, DNA from disease-free individuals, and DNA from primary tumors. From 100 ng of genomic DNA, 1000-fold amplification was obtained. The unamplified and the WGA samples were assayed on a two-color oligonucleotide-based array CGH platform, which contains 390K unique probes per array, to observe whether the WGA has introduced any bias into the data. Data from both a whole-genome, tiling-path design and a much higher resolution fine-tiling design will be presented. Initial analysis by the highest resolution array CGH available suggests this WGA method provides high fidelity amplification with virtually no introduction of noise or bias.

Mutations of a novel tight junction protein are associated with DFNB49 nonsyndromic hearing loss. *S. Riazuddin[1], Z.M. Ahmed[1], A.S. Fanning[2], K. Ramzan[3], A. Lagziel[1], R. S. Shaikh[3], P. Chattaraj[1], P.L. Friedman[4], J.M. Anderson[2], I.A. Belyantseva[1], A. Forge[5], S. Riazuddin[3], T.B. Friedman[1]* 1) SHG, LMG, NIDCD, NIH, Rockville, MD; 2) Dept of Cell and Mol Physiol, Univ of NC, Chapel Hill, NC; 3) CEMB, Univ of the Punjab, Lahore, Pakistan; 4) IMCS, CRC, NIH, Bethesda,MD; 5) CAR, UCL Ear Institute, London.

We mapped profound congenital deafness segregating in two families to chromosome 5q12.3-q14.1 (Ramzan et al 2005). The meiotic information from six additional families refined the disease linked haplotype to 2.4 megabases. One of the genes in the refined interval is *TRIC* which encodes tricellulin, a tetraspan integral-membrane tight junction (TJ) protein. Tricellulin is localized at the tricellular attachment points of most epithelial cells. Antibodies generated to the N-terminus of tricellulin show that tricellulin is concentrated at the tricellular TJs in epithelial cells of the inner ear including hair cells, their supporting cells, Reissners membrane and the stria vascularis. In the organ of Corti tricellulin is localized along the structurally complex junctions between supporting and neurosensory hair cells. Given the expression of *Tric* in a variety of tissues, the deafness phenotype caused by these mutations is surprising. In eight DFNB49 families we found four different mutations of *TRIC* including a nonsense and three splice site mutations. Exon trapping and RT-PCR data using RNA from lymphocytes of DFNB49 subjects show that the splice site mutations cause aberrantly spliced mRNA, resulting in frameshifts and premature truncations of the protein. These mutations remove a conserved domain of this protein which is predicted to bind the cytosolic scaffolding protein ZO-1. We provide data showing the affect of these mutations on the binding ability to ZO-1. There are some isoforms of tricellulin which remain unaffected by the mutant alleles and may play a role in maintaining the epithelial barrier of other tissues. Further studies of mouse models of mutant *Tric* will provide insight and give us an explanation as to why mutations in *TRIC* selectively affect inner ear function.

Novel mutations in the *OTOF* gene in Brazilian subjects with auditory neuropathy. *J. Romanos[1], M.L. Fávero[2], P.A. Otto[1], R.C. Mingroni-Netto[1]* 1) Depto de Genética e Biologia Evolutiva, Instituto de Biosciências, Univerisidade de São Paulo, São Paulo, Brazil; 2) Divisão de Educação e Reabilitação dos distúrbios da Comunicação, Pontificia Universidade Católica de São Paulo, São Paulo, Brazil.

Nonsyndromic prelingual inherited deafness is mainly autosomal recessive. The *OTOF* gene is one of the 23 identified genes responsible for autosomal recessive deafness. To date, there are 22 different pathogenic mutations from populations of variable origins. A Q829X mutation was found to be the most frequent in Spain. Some affected individuals are reported to present auditory neuropathy (AN), characterized by an absent or severely abnormal auditory brainstem response, with preservation of otoacoustic emissions and/or cochlear microphonics. We enrolled 64 Brazilian unrelated probands and family members with either autosomal recessive nonsyndromic deafness, or AN or both, for mutation screening and evidence of phenotype linkage to the *OTOF* gene. All families were genotyped for microsatellites linked to the *OTOF* gene, tested for the Q829X mutation and for the previously identified mutations. Families with microsatellite segregation patterns consistent with linkage and subjects with diagnosis of AN (7 and 11 subjects, respectively) were further screened for mutations in all 48 coding exons of the *OTOF* gene. To date, no causative mutations were found in the linkage group. Among the 11 cases of AN, we found four novel variants in three cases: c.1552-1567del16, c.3400C>T (R1134X) in homozygous form and c.1841G>A (G614E), and c.3239G>C (R1080P) together in a compound heterozygote. The c.1552-1567del16 and R1134X mutations are probably the cause of deafness in two patients. The two other mutations, G614E and R1080P, were considered potentially harmful based on amino acid changes; further studies are needed to verify if they are pathogenic. In the heterozygote for c.1552-1567del16, a further mutation remains to be identified. Further, homozygosity for the mutation R1134X probably results from consanguinity. While our study shows that mutations in the *OTOF* gene are frequent causes of auditory neuropathy in Brazil (3 of 11 families), we fail to confirm the high frequency of Q829X mutation found elsewhere.

Severe phenotypic expression of Beckwith-Wiedemann syndrome associated with high levels of paternal uniparental disomy for chromosome 11p15. *A. Smith[1,2], C. Shuman[3,4], L. Steele[5], P. Ray[5], R. Weksberg[1,2,3,4]* 1) Genetics & Genomic Biology, Hosp Sick Children, Toronto; 2) Inst of Medical Science, U of Toronto, Toronto; 3) Div of Clinical and Metabolic Genetics, Hosp for Sick Children, Toronto; 4) of Medical Genetics and Microbiology, U of Toronto, Toronto; 5) Dept of Ped and Lab Med, Hosp for Sick Children, Toronto, CANADA.

Beckwith-Wiedemann syndrome (BWS) is an overgrowth syndrome characterized by macrosomia, macroglossia, omphalocele, increased tumor risk and hemihyperplasia. BWS can be associated with genetic and/or epigenetic alterations that alter imprinted gene expression on chromosome 11p15.5. Somatic mosaicism for chromosome 11p15 paternal uniparental disomy (UPD), found in 20% of BWS cases, is associated with specific features of BWS including hemihyperplasia, Wilms tumor and hepatoblastoma. Despite the fact that tissue sampling may not always reflect the level of constitutional UPD we hypothesized that extremely high levels of UPD might drive severe phenotypic expression of BWS. We report two patients with severe presentations of BWS and high levels of UPD (>70%) in DNA from lymphocytes. Patient A was a male infant, delivered at 32 weeks gestation, with coarse facies, macrosomia and hypoglycemia. At 3 months of age, he was admitted for persistent hypoglycemia. At that time he had hemihyperplasia, hypertrophic cardiomyopathy with valvular pulmonary stenosis and respiratory compromise due to abdominal organomegaly. Abdominal imaging revealed enlarged dysplastic kidneys, hepatomegaly, hepatoblasoma, enlarged pancreas and spleen. Ventilatory support was reduced and he died at 5 months of age. Patient B was a female infant delivered at 35 weeks gestation. She was noted to have macrosomia, extreme macroglossia, umbilical hernia, hepatomegaly, hemihyperplasia and hypoglycemia. Abdominal imaging revealed hepatoblastoma and nephromegaly. She developed dramatic hyperplastic cardiomegaly, abdominal distention, upper airway congestion and died at 5 months. In order to determine whether the high levels of UPD are etiologically important for severe presentations of BWS it will be important to undertake analysis in further cases.

Analysis of the markedly increased incidence of cancer in individuals with Blooms Syndrome. *W. Shi[1], A. Zauber[1], M. Sanz[2,3], J. German[3]* 1) Dept. Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, NY; 2) Dept. Biology, Molloy College, Rockville Centre, NY; 3) Dept. Pediatrics, Weill Medical College of Cornell University, New York, NY.

Blooms syndrome (BS) is a rare recessively transmitted form of proportional dwarfism. A single locus is mutated*BLM* that encodes the nuclear protein BLM that when absent or non-functional also results in a mutator phenotypethe clinical consequence is cancer of a wide variety of cellular types and anatomical sites. We determined the standardized incidence ratio (SIR) of specific types of cancers diagnosed in 237 individuals with BS (BSI) ascertained since 1954 by the Blooms Syndrome Registry. The number of cancers expected to arise was calculated based on age, sex, and calendar interval-specific rates published by the Surveillance, Epidemiology, and End Results (SEER) program, NIH. 138 primary invasive cancers were diagnosed in 108 BSI during 4952 person years of follow-up. The mean age of diagnosis of the first primary cancer was 23 years (SD=12). The SIR (95% CI) by sex (M=128, F=109) is given below. An almost 100 fold increased risk of cancer existed for BSI as compared to the general population. A markedly increased risk of developing cancer exists for both males and females with BS. Early and continued surveillance for cancer is critical for affected individuals.

Type of cancer	No. of cancers	SIR for All BSI	SIR for Males	SIR for Females
All Sites	138	99 (83-117)	106 (84-131)	90 (68-117)
Lymphoma	32	166 (114-234)	141 (83-223)	215 (118-361)
Leukemia	29	152 (102-218)	191 (120-289)	92 (37-191)
Colorectal	20	521 (318-804)	607 (339-1001)	365 (118-852)

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12q14: A novel linkage region in extended autism families. *M.A. Pericak-Vance[1], D.Q. Ma[1], D.A. Skaar[1], A.L. Collins[1], I. Konidari[1], J. Crowley[1], J. Jaworski[1], R.K. Abramson[2], H.H. Wright[2], M.L. Cuccaro[1], J.R. Gilbert[1], J.L. Haines[3]* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) School of Medicine, University of South Carolina, Columbia, SC; 3) Center for Human Genetic Research, Vanderbilt University Medical Center, Nashville, TN.

Autism is a common neurodevelopmental disorder, with a significant genetic component and substantial locus heterogeneity. No consensus linkage region or associated candidate gene from any proposed region has emerged despite extensive studies. However, the primary substrate for the linkage studies has been small nuclear families. A contrasting and powerful alternative is the use of large, extended pedigrees to identify significant linkage results, as these families contain more potential linkage information than sibpair families. We collected 26 extended autistic families (65 affected, 184 total individuals), with each family having two to four affected individuals comprised of either avuncular or cousin pairs. We then performed a genome-wide linkage analysis using a high-density single-nucleotide-polymorphism (SNP), Affymetrix GeneChip Human Mapping 10K array and identified a genome-wide significant linkage signal on chromosome 12q14-15. Nine more microsatellites covering the interested region have now been typed on these families and confirm the linkage signal with the peak at D12S1686 [78.75 cM] (heterogeneity LOD [HLOD]: 3.49 [recessive model]). The linkage signal was enhanced in 17 male affected only families with a 4.56 HLOD under the recessive model. Using the power of the extended pedigrees, we constructed haplotypes and narrowed the minimum candidate region to a 4 cM segment from 75cM to 79 cM. This region contains 10 known genes, including interesting candidates AVPR1A; SRGAP1; WIF1; GRIP1 and TPH2. Analysis of these genes is underway.

Pharmacogenetic Study of 10 Dopamine Receptor DRD3 Gene Polymorphisms and Tardive Dyskinesia in Caucasians with Schizophrenia. *C. Zai[1], V. DeLuca[1], D.J. Muller[2], G. Remington[1], H.Y. Meltzer[3], J.A. Lieberman[4], S.G. Potkin[5], J.L. Kennedy[1]* 1) Dept Neurogenetics, CAMH, Toronto, ON, Canada; 2) Dept Psychiatry, Charite University Medicine Berlin, Campus Charite Mitte, Berlin, Germany; 3) Psychiatric Hospital at Vanderbilt University, Nashville, Tennessee, USA; 4) New York State Psychiatric Institute, Columbia University Medical Centre, New York City, New York, USA; 5) Brain Imaging Centre, Irvine Hall, University of California at Irvine, California, USA.

Tardive dyskinesia (TD) is a motor adverse effect of chronic exposure to antipsychotic medications. Its severity is measured by the Abnormal Involuntary Movement Scale (AIMS). The pathophysiology of TD has not been clarified, but changes in dopamine neurotransmission have been hypothesized to be involved. The role of the dopamine D3 receptor, coded by the DRD3 gene, in TD is supported by gene expression and pharmacological studies. The Ser9Gly polymorphism of DRD3 has been associated with TD in several (Basile et al, 1999; Lerer et al, 2002) but not all studies. Other DRD3 polymorphisms have not been assessed in TD. In addition, other genes, like Brain Derived Growth Factor (BDNF), may influence DRD3 expression and TD development. In the present study, we investigated 10 polymorphisms spanning DRD3 for association with TD. Our schizophrenia sample was restricted to European Caucasians (N=196). The rs905568 polymorphism in the promoter region of DRD3 was significantly associated with TD occurrence (p=0.004) and AIMS scores (p=0.003), while the other nine polymorphisms were mostly negative. We also tested if the DRD3 polymorphisms are associated with the diagnosis of schizophrenia; preliminary results with rs905568 did not reach statistical significance. Taken together, the present study suggests that DRD3 may contribute to the risk of TD development in European Caucasians, but replication studies are required. Preliminary data for five BDNF polymorphisms as well as genetic interaction analysis of BDNF and DRD3 in TD will also be presented.

Combined cytogenetic, aCGH and gene expression analyses of cisplatin resistant ovarian cancer cells. *A. Tsalenko[1], M. Prasad[2], J. Paderova[2], C.-H. Lee[2], P. Marrano[2], Z. Yakhini[1], A. Ben-Dor[1], J. Squire[2], M. Barrett[1]* 1) Agilent Laboratories, Santa Clara, CA; 2) Ontario Cancer Institute, Princess Margaret Hospital, University of Toronto, Toronto, Canada.

Chemotherapies are often restricted by the development of acquired resistance. We use a combination of array-based comparative genomic hybridization (aCGH), high resolution cytogenetics and gene expression (GE) to study the genomic basis of such phenotypes. To demonstrate the utility of combined analyses for studying drug resistance we compared the genomic profiles of A2780 ovarian cancer cells and a cisplatin-resistant derivative (A2780cis). Copy number aberrations were detected with Agilent prototype 95k 60mer oligonucleotide CGH arrays; GE was measured with Agilent 44k GE arrays. Aberrant intervals were identified using an efficient aberration detection algorithm ADM1 and integrated with spectral karyotyping (SKY) and chromosome-specific m-banding analyses. The majority of differential aberrations were deletions in A2780cis cells associated with down-regulation of GE relative to parental A2780 cells. These included deletions on 1p22.1-cen with a nested homozygous deletion targeting the SNX7 locus at 1p21.3, 2pter-p25.2, Xq12.2-qter, and loss of one copy of chromosome 13 but with focal (<1Mb) duplication at 13q12.12. In addition A2780cis cells had gain of a single copy of 8q11.22-qter. Derivative chromosomes in A2780cis cells, including der(8)t(1;8)(p22;p23) and der(X)t(X;1)(q12;q11) that were associated with differential copy number aberrations, were identified by SKY and mBAND and their breakpoints mapped to a single gene resolution. A significant enrichment of differentially expressed genes is observed in these regions including chromosomes 1p (p-value 3.5E-07), 13q (p-value 0.003) and 8q (p-value 0). Gene Ontology analysis revealed functional enrichment of down-regulated genes within the 1p interval in the hydrolase activity category (p-value 6.08E-07), with more specific terms of GTPase activity (p-value 5.85E-06) and ATPase activity (p-value 0.002). Mitosis (p-value 0.0002) and cell cycle (p-value 0.003) pathways are overrepresented in differentially down-regulated genes on chromosome 13.

Array-CGH increases detection rate of constitutional chromosome abnormalities. *D.L. Pickering, D.M. Golden, R.J. Stroebele, B.J. Dave, W.G. Sanger* Human Genetics Laboratories, Munroe Meyer Institute, Omaha, NE.

The identification of unbalanced chromosome abnormalities is an important aspect in the diagnosis and management of constitutional disorders however, many affected individuals have normal FISH and/or karyotype results. Array-CGH (aCGH) has emerged as an effective tool in the cytogenetics laboratory for the detection of chromosomal deletions and duplications that are associated with constitutional disorders because of its ability to rule out multiple genetic abnormalities in a single test. This is particularly beneficial in newborn studies and in older individuals lacking a unifying diagnosis following normal cytogenetic investigations. Our laboratory analyzed 445 individuals by aCGH utilizing a commercially available constitutional array platform covering genetic loci implicated in known diseases. Fifty-four of the cases were newborn patients less than 2 months old with dysmorphic features and the remaining cases were older individuals with clinical findings suggestive of a genetic disorder. All of the cases had a previous or concurrent normal karyotype and in some cases, normal FISH studies. Thirty-two out of 445 cases (7.2%) were abnormal by aCGH studies. Deletions were observed in 19/32 abnormal cases, duplications were identified in 11/32 abnormal cases and 2/32 unbalanced translocations were detected. Three of the abnormal cases represented newborn studies. The incorporation of aCGH in our laboratory for the study of constitutional disorders in which there was a normal cytogenetics study has increased the overall chromosomal abnormality rate by 7.2%. Additionally, aCGH has curtailed the effort, time, and expenses spent in performing multiple FISH tests in cases with nonspecific clinical features. Our findings demonstrate the usefulness of this technology in identifying chromosomal abnormalities, particularly in cases that remain a diagnostic enigma and in newborn studies where establishing a diagnosis upfront will improve patient management.

The gene encoding *ECT2*, a candidate substrate of the Angelman syndrome protein E6-AP, is associated with autism. *L.T. Reiter[1], R.J. Delahanty[2], J.S. Sutcliffe[3]* 1) Department of Neurology, University of Tennessee HSC, Memphis, TN; 2) Department of Molecular Physiology & Biophysics and; 3) Center for Molecular Neuroscience, Vanderbilt University, Nashville, TN.

Autism is a neurodevelopmental disorder affecting ~1 in 500 individuals with deficits in language, social reciprocity, and patterns of repetitive behaviors and restricted interests. Despite a recognized complex genetic etiology, locus heterogeneity has confounded efforts to identify loci broadly contributing to idiopathic disease. Maternal chromosomal duplications of 15q11-q13 are the most frequent cytogenetic abnormalities found in autism. Data suggest that dup(15) phenotypes result from over-expression of contiguous loci including the maternally-expressed E6-AP ubiquitin ligase (*UBE3A*) gene. Maternal deficiency of *UBE3A* causes the neurodevelopmental autism spectrum disorder Angelman syndrome (AS). The E6-AP protein targets other proteins through ubiquitinylation for subsequent ubiquitin-dependent degradation. Altered expression of *UBE3A* is therefore predicted to result in dysregulation of E6-AP substrates. We used a proteomics strategy in *Drosophila* to identify E6-AP targets and determine if they correlate with phenotypes potentially-relevant to AS and autism. In our first proteomic screen, we identified the Rho-GEF gene *pebble* as a neurologically-relevant target of Ube3a in *Drosophila* and mouse. We hypothesize that genetic variation at E6-AP target protein loci, like the *pebble* ortholog *ECT2*, contributes to autism susceptibility. An initial test of this hypothesis involved an association study in 546 combined multiplex and simplex autism families using tag SNPs representing common alleles at *ECT2*. Transmission data revealed significant association (P=0.02), predominantly in males (P=0.04), at a marker in intron 20. We are currently designing additional SNP association assays in and around the *ECT2* locus to extend these findings.

Has the consanguineous marriage any effect in infertility? *S.M. Seyedhassani[1,2], A. Aflatoonian[1], N. Tabibnejad[1], S.M. Kalantar[1], M. Houshmand[2]* 1) Medical Genetic Department, Research & Clinical Center for Infertility, Yazd, Yazd, Iran; 2) National Institute for 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. Infertility is most commonly defined as the absence of pregnancy for one year of unprotected intercourse. 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 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, infertility and related epidemiological findings . 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. Among these couples , 276 cases of infertility were encountered and the overall prevalence of infertility was about 5.52%(CI 95%from 4.9%to 6.14%) containing of primary 3.48% and secondary 2.04%. In infertile couples 135 had consanguineous marriage (48.9%)(p= 0.009 and Odds Ratio= 1.38,1.07<OR<1.77). Infertile males (56 cases) and females(114 cases) were born from consanguineous marriage in 42.9%(p=0.826) and 35.1%(p=0.175) respectively. Conclusion: In Iran, there was an incidence of consanguineous marriage near to neighboring countries such as Pakistan, Kuwait and Saudi Arabia. Consanguinity was related to in expression of infertility, but, there wasnt significant difference in primary and secondary infertility separately.These findings can implicate the probability of bilateral recessive genetic effects leading to preclinical abortion in encountered couples.

Gene mutations for NELF in 75 patients with Kallmann syndrome and cellular localization of NELF in GnRH neurons. *N. Xu[1,2], W.C. Xiong[2,3], R.S. Cameron[2,4,5], L.C. Layman[1,2]* 1) Dept. Obstetrics & Gynecology; 2) Developmental Neurobiology Program, IMMAG; 3) Dept Neurology; 4) Dept Cellular Biology and Anatomy; 5) Dept Medicine, Medical College of Georgia, Augusta, GA, United States, 30912.

Kallmann syndrome (KS) is a developmental disorder with the combination of idiopathic hypogonadotropic hypogonadism (IHH) and anosmia or hyposmia. Nasal embryonic luteinizing hormone-releasing hormone factor (NELF) is a candidate gene for KS, and has been proposed to affect the GnRH neuron migration pathway from the olfactory placode region to the hypothalamus. The human NELF cDNA was cloned previously, and it is hypothesized that mutations in NELF are present in patients with KS but not in controls. In addition, we wanted to determine the precise cellular localization of Nelf in immortalized GnRH neurons. Primers were designed to amplify 16 exons of NELF and splicing junctions. 75 patients with KS were analyzed for NELF mutations by direct DNA sequencing, and three putative mutations were found in introns near splice junctions: IVS8-21G>A, IVS11-13C>T, and IVS10-49G>T. None of these was reported in the SNP database, or identified in controls. Nelf mRNA was expressed in the GnRH neuronal cell lines (NLT, GN11 and GT1-7). To determine the localization of Nelf in GnRH neurons, endogenous Nelf was predominantly detected in the nuclei by western blot and immunofluorescence using anti-Nelf antibodies. These findings were consistent with the program PSORT II, which predicted two putative nuclear localization signals (NLS) in the Nelf protein. By site direct mutagenesis, two mutants changing either of two putative NLS (RRKR->AAKA and RK->AA), and one reported missense mutant in a KS patient (T480A) were constructed, and all three mutated constructs were overexpressed in GnRH neurons. One NLS mutant (AAKA) reduced the nuclear to cytoplasmic ratio by more than 5-fold. In summary, we found 1) putative NELF mutations around the splicing junctions of NELF in patients with KS, and 2) the protein Nelf resides predominantly in the nuclei of GnRH neurons and a putative NLS was identified. These results suggest that NELF plays an important role in the process of the GnRH migration.

Inter-individual Epigenetic Variation in Healthy Human Subjects. *M. Shinawi, E. Montz, P. Fang, A.L. Beaudet*
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Epigenetic regulation refers to stable changes in gene expression that are not mediated by a change in DNA sequence. The biochemical basis of epigenetic regulation includes DNA methylation, histone modifications, and the action of nonhistone proteins in chromatin. DNA methylation is crucial for the embryo development and has an essential role in X chromosome inactivation, genomic imprinting, and chromatin modifications. In addition, early nutrition at critical ontogenic stages affects DNA methylation and changes the expression of specific subsets of genes. Although a major effort is being made to elucidate the genetic variations (e.g.SNPs and the Haplotype Map) in the human genome, little has been done to assess the epigenetic variability. Principal challenges for the future will be to assess the variation of DNA methylation and whether environmental conditions influence these factors. We are testing the hypothesis that epigenetic variations are common and might contribute to the phenotypic differences between healthy individuals and to disease, particularly complex disease traits. The aim of this study is to perform methylation analysis at different genomic regions to detect epigenetic variations between healthy individuals. The methylation status is being analyzed in 50 females and 50 males by using gel-based radioactive bisulfite sequencing in the CpG islands of the following genes and regions: *SNRPN*, *MEG3*, DMR1 of *IGF2* (three imprinted genes), *28S rDNA*, *Satellite 2* (repetitive DNA), and *MAOB* (X-linked gene subject to X inactivation). Our preliminary analysis on 30 individuals showed minor variations in the methylation of the imprinted genes *SNRPN*and *MEG3*, and of the X-linked *MAOB* gene. For 7% of individuals, there was significant hypermethylation of the DMR1 of *IGF2* compared to the majority. However, the most remarkable degree of variation was detected in the *28S rDNA* repetitive sequence. Additional studies are underway to test more samples and to statistically evaluate the DNA methylation differences. Our data provides evidence for inter-individual epigenetic variability in human which might have a role in human variation and disease.

A new case with Clavicular Hypoplasia, Zygomatic Arch Hypoplasia and Micrognathia. *L.E. Wong-Ley[1], J.E. Garcia-Ortiz[1], A. Serralde[2], L.E. Figuera[1]* 1) GENETIC, CIBO-IMSS, Guadalajara, Jalisco, Mexico; 2) Unidad de Medicina Familiar No.54, IMSS, Tlaquepaque, Jalisco, Mexico.

In 2000, Ponzio and Cunningham reported a patient with an unique phenotype characterized by cleidocranial hypoplasia, mild frontonasal malformation (FNM), micrognathia and hypoplastic zygomatic arches; this phenotype was later named with the acronym CHZAM (clavicular hypoplasia, zygomatic arch and mandibular hypoplasia, OMIM 605040) and it was proposed to be part of a continuum spectrum of CCHA and mandibulofacial dysostosis presentations and mutations. Here, we describe a 30 year-old female with multiple anomalies including the leading features of CHZAM in addition to short neck, pterigium colli, bilateral Sprengel anomaly, Tanner II-III breast development and external genitalia; upper extremities with bilateral camptodactyly and clinodactyly of 5th digit, ungueal streaks in 1st, 3rd and 4th digits of right hand and 4th digit of left hand; lower limbs with bilateral clinodactyly of 3rd and 4th digits and postaxial polydactyly of left foot. X-ray examination showed cranial digital impressions and dysplastic changes in the basiocciput, hypoplastic sinuses, verticalization of hypoplastic clavicles, cervical fusion C2-C3, hypoplastic scapulae, delayed ossification of pubic bone, hypoplastic iliac wings, large femoral neck, large epiphyses, short middle phalanges and metacarpals/tarsals III-V, hypoplastic distal phalanges, accessory epiphyses especially of 2nd metacarpal, long 2nd metacarpal, cone shaped epiphyses and supernumerary teeth. A psychological evaluation showed mental retardation. Karyotype (GTG banding technique at 550-650 band resolution) was normal. Our patient fits all the diagnostic criteria for CHZAM is, in the best of our knowledge, the second one reported in the literature and the first reported female and adult leading us to further delineate the clinical spectrum of this rare condition previously reported in a 6-year-old boy; both cases have been sporadic but Mendelian inheritance can not be ruled out.

Demonstration of Genetic Interactions between TRIM32 and other Bardet-Biedl Syndrome Genes. *M.K. Tayeh[1,2,3], H-J. Yen[1,2,3], R.F. Mullins[4], A.P. Chiang[1,2,3], J.S. Beck[1,3], C.C. Searby[1,3], T.A. Westfall[5], H. Griesback[5], E.M. Stone[3,4], D.C. Slusarski[5], V.C. Sheffield[1,3]* 1) Dept Pediatrics, Univ Iowa, Iowa City, IA; 2) Genetics Ph.D. program, Univ Iowa, Iowa City, IA; 3) Howard Hughes Medical Institute; 4) Dept Ophthalmology, Univ Iowa, Iowa City, IA; 5) Dept Biology, Univ Iowa, Iowa City, IA.

Bardet Biedl syndrome (BBS) is a pleiotropic, genetically heterogeneous, autosomal recessive disorder with the primary clinical features of obesity, retinopathy, polydactyly, learning disabilities, renal abnormalities and hypogenitalism. To date, 11 BBS genes have been identified and experiments suggest that BBS proteins play a role in the function of cilia and intracellular transport. We have used antisense oligonucleotides (morpholinos) for knock down of BBS gene expression in zebrafish to demonstrate a role for BBS genes in cilia maintenance and retrograde intracellular transport, as well as to evaluate novel BBS candidate genes. In addition, we have used the zebrafish model system to investigate genetic interactions between paired combinations of BBS genes. Interactions in the zebrafish were analyzed by double knock down of BBS genes using pair wise co-injection of low dose BBS morpholinos. Injected embryos were analyzed for cilia and intracellular transport defects, as well as other abnormalities. Of the 28 possible paired combinations of eight BBS genes (BBS1-BBS8), we detected only two combinations of BBS gene double knockdowns that resulted in evidence of genetic interaction in the zebrafish model system. We recently identified TRIM32, an E3 ubiquitin ligase gene, as BBS11. We investigated genetic interactions between the zebrafish orthologue of TRIM32 and other BBS genes. Our data demonstrate interactions between zebrafish trim32 and multiple other BBS genes. These data suggest a likely role for TRIM32 in the ubiquitination of BBS gene products and indicate a possible role for TRIM32 in the modification of BBS phenotypes in human patients.

A familial cases of LEOPARD syndrome associated with childhood onset behavioral disorder and Asperger syndrome. *Y. Watanabe[1], S. Yano[2], M. Yoshino[1], T. Matsuishi[1]* 1) Dept Pediatrics, Kurume Univ, Kurume, Japan; 2) Pediatrics/Genetics Div, 1G24, Univ SC, Women & Child Hosp, Los Angeles, CA.

LEOPARD syndrome is a rare autosomal dominant disorder characterized by Lentigines, Electrocardiogram abnormalities, Ocular hypertelorism, Pulmonic valvular stenosis, Abnormalities of genitalia, Retardation of growth, and Deafness. This syndrome is caused by germ line missense mutations in the PTP catalytic domain of PTPN11. The mutations induce catalytically impaired defective protein-tyrosine phosphatase SHP2 by dominant negative effect (Kontaridis, 2006). Approximately 50 % of Noonan syndrome patients are due to PTPN11 mutations that are scattered over the entire SHP2 regions including the catalytic domain. The mutations resulting in Noonan phenotype are the gain of function mutations and they exhibit substantially increased catalytic ability (Kontaridis, 2006). It has been known that SHP2 positively controls the activation of the RAS/MAPK. Recently, SHP2 negative Noonan syndrome patients have been identified to have KRAS mutations (Schubbert, 2006). SHP2 is widely expressed in both embryonic and adult tissues. Mutations in PTPN11 result in abnormal axial and paraxial mesodermal structures. Mutations in PTPN11 are also known to cause rapid apoptosis in trophoblast stem cells (Yang, 2006). We report a family with three affected individuals (father and two affected sons) with typical LEOPARD syndrome. The father has developed severe behavioral problems over the past 10 years with aggressive behavior. His sons have childhood onset behavioral problems and learning difficulties and diagnoses was made with ADHD in one and Asperger syndrome in the other. Although it is well known that patients with LEOPARD syndrome are often associated with developmental delay/mental retardation, there have been no reports about cases of LEOPARD syndrome who satisfied a criteria for ADHD or Asperger syndrome. Although considerable progress has been made in molecular pathology in Noonan syndrome and LEPARD syndrome, understanding the mechanisms how these molecular changes affect central nervous system function remains unknown.

Systematic analysis of tumor genetic alterations on Cancer Genome WorkBench (CGWB). *J. Zhang, R. Finney, W. Rowe, M. Edmonson, T. Dracheva, J. Jen, J. Struewing, K. Buetow* Lab Population Genetics, NCI, Bethesda, MD.

Systematic investigations of genetic changes in tumor such as The Cancer Genome Atlas (TCGA) project are expected to lead to molecular approaches to the diagnosis and the treatment of cancer. To facilitate such studies, we have developed Cancer Genome WorkBench (http://cgwb.nci.nih.gov), an integrated bioinformatics platform able to perform high-throughput data analysis, construct mutation profiles, and permit evaluation of the effect of mutations on protein coding and 3-D structural changes. A novel algorithm, IndelDetector, has been developed to find insertion/deletion changes. It is integrated with SNPdetector, a software tool we developed previously for finding substitution variations, in an automated tumor mutation analysis pipeline. Somatic mutations, germline mutations and copy number changes in paired tumor-normal samples discovered by this pipeline are assembled into mutation profiles. These clinical mutation profiles are then integrated to the reference human genome in our enhanced version of UCSC genome browser. Using this approach we have analyzed three data sets. The first is resequencing of candidate genes in paired tumor-normal lung cancer samples. In this data set, a tumor sample can have a tumor-to-normal allele ratio as low as 20%. Clinical samples with two inactivated copies of tumor suppressor gene LKB1 (one inactivation caused by LOH and the other by somatic mutation) have been found. The second is a re-analysis of 156 genes resequenced by the SeattleSNPs. The results show that our method is able to find 90% of the 1,250 insertion/deletion polymorphisms manually identified by SeattleSNPs. In addition, 500 novel insertion/deletion polymorphisms have been discovered with a projected 90% validation rate based on manual data review. The third discovers germline mutations in familial ovarian cancer. Our experience demonstrates the feasibility of finding tumor suppressor genes that fit the double-hit model using an integrated analytical system. Access control of CGWB has been implemented and a user data submission portal is under development to facilitate large-scale data analysis centers as well as individual research laboratories.

Spinocerebellar ataxia type 8: bidirectional expression of CUG and CAG expansion transcripts and intranuclear polyglutamine inclusions. *T. Zu[1], M.L. Moseley[1], Y. Ikeda[1], W. Gao[2], A.K. Mosemiller[1], R. Daughters[1], G. Chen[2], M.R. Weatherspoon[1], H.B. Clark[3], T.J. Ebner[2], J.W. Day[1], L.P.W. Raumn[1]* 1) Inst. Hum. Genet; 2) Neuroscience; 3) Lab. Med. Pathology, Univ Minnesota, Minneapolis, MN.

We previously reported that a (CTG)n expansion causes spinocerebellar ataxia type 8 (SCA8), a slowly progressive ataxia characterized by reduced penetrance. The (CTG)n-expansion mutation is transcribed, alternatively spliced, and polyadenylated in the CTG orientation. Because initial sequence analysis revealed no likely ORFs spanning the repeat in either direction, and because of the known pathogenic properties of CUG expansion transcripts in myotonic dystrophy, we initially proposed that SCA8 is caused by an RNA gain-of-function mechanism. To elucidate the molecular events that cause SCA8, we developed a transgenic murine model in which the full length human SCA8 gene is expressed using its endogenous promoter. (CTG)116 expansion, but not (CTG)11 control lines, develop a progressive neurological phenotype and in vivo optical imaging studies show a loss of cerebellar cortical inhibition. Surprisingly, we found 1C2-intranuclear inclusions in Purkinje cells in SCA8 expansion mice and human SCA8 autopsy tissue result from translation of a nearly pure polyglutamine protein (ataxin 8) encoded on a previously unidentified anti-parallel transcript spanning the repeat in the CAG direction. Experiments are now being performed to define the sequences that control transcription and translation and to characterize the expression pattern of this newly identified gene (*ATXN8*). The neurological phenotype in SCA8 BAC-expansion but not BAC-control lines demonstrates the pathogenicity of the (CTGCAG)n expansion. Moreover, the expression of non-coding (CUG)n expansion transcripts (*ataxin 8 opposite strand*, *ATXN8OS*) and the discovery of intranuclear polyglutamine inclusions suggests SCA8 pathogenesis involves toxic gain-of-function mechanisms at both the protein and RNA levels.

Effect of *APOH* promoter SNPs on gene expression and plasma levels of APOH. *S. Suresh[1], R.L. Minster[1], F.Y. Demirci[1], M. Kenney[1], P. Shaw[2], A. Kao[2], C. Kammerer[1], F. Bontempo[3], S. Manzi[2], M.I. Kamboh[1]* 1) Dept. of Human Genetics; 2) Lupus Center of Excellence; 3) Dept. of Medicine, Univ. of Pittsburgh, Pittsburgh, PA.

APOH has been implicated in several physiologic pathways, including coagulation, production of antiphospholipid antibodies and lipid metabolism. Plasma APOH levels vary significantly among individuals, ranging from immunologically undetectable to as high as 35 mg/dL. Family and heritability data suggest that APOH plasma variation is under genetic control; however, the exact underlying molecular basis of this variation is largely unknown. Our initial studies demonstrated that the -32 promoter SNP, which is in LD with the codon 316 mutation, was significantly associated with changes in plasma APOH levels and in reporter (luciferase) gene expression. However, this did not preclude the possibility of other promoter variations regulating gene expression. The purpose of this study was to search for additional promoter functional variants that are associated with altered gene expression and plasma APOH levels. For this purpose, we have focused on a ~1.4 kb fragment in the immediate 5 flanking region of the APOH gene. Six promoter SNPs, including -32, were genotyped by Pyrosequencing in 238 Caucasian SLE women and 195 healthy Caucasian women and plasma APOH levels were determined by the modified capture-ELISA method. The functional effects of these promoter SNPs were analyzed using in vitro dual-luciferase reporter assays in COS-1 cells. The variant alleles of three SNPs at positions -1219, -643 and -32 showed lower luciferase gene expression (51%, 40% and 37%, respectively) as compared to the respective wild-type alleles. Due to LD between these 3 SNPs, only the -32 SNP showed significant association with plasma APOH levels in the ANOVA ($p<0.0001$). The variant alleles of the other two SNPs were in LD with the wild type allele of the -32 SNP that is associated with higher gene expression and thus nullifying the lowering effects of these two variants in ANOVA. The characterization of *APOH* promoter and its functional variants will help to further delineate the molecular basis of plasma APOH levels.

Prenatal diagnosis of inherited mental retardation: subtelomeric rearrangement mimicking an autosomal recessive condition. *A. Perez-Juana del Casal, M.A. Ramos-Arroyo, M. Artigas, S. Moreno, A. Alonso, A. Valiente* Dep. Genetics, Hospital Virgen del Camino, Pamplona, Navarra, Spain.

A 29 year-old epileptic woman was referred to the Genetics Clinic in her 8 week of gestation (twin pregnancy), because of her husbands family history of unexplained mental retardation. He (SG) had three out of his seven siblings, one man and two women, moderately mentally retarded. Their parents had normal intelligence and both of them came from the same small area in Navarra. Initially, the pedigree analysis suggested a recessive condition. We examined the oldest mentally retarded brother, no definite dysmorphy suggestive of any particular syndrome was observed. A routine karyotype and a Fragile-X study were normal. A DNA sample was checked for submicroscopic subtelomeric aberrations, using multiplex ligation dependent probe amplification MLPA (SALSA PO70 and PO36). This technique revealed a 20qtel duplication. Then, FISH analysis (ToTelVysion, Vysis) was carried out to confirm the rearrangement identified by MLPA, and an unbalanced subtelomeric translocation, between chromosomes 14 and 20 was identified: 46,XY,ish t(14;20)(qter+,qter+;qter-,qter+). It is remarkable that the recombinant chromosome 14 had not lost his qtel regions (confirmed by MLPA and FISH). This is probably due to the fact that this subtelomeric portion is highly repetitive and appears to be a hot spot for recombination.

Subsequently, karyotype and FISH analysis for subtelomeric regions of chromosomes 14 and 20 (ToTelVysion Vial 7 and 17), were performed on the healthy brother (SG) detecting a balanced translocation between both subtelomeric regions: 46,XY,ish t(14;20)(qter-,qter+;qter-,qter+). After all, an amniocentesis was performed. First, we analyzed the subtelomeric regions by FISH (ToTelVysion Vial 7 and 17) in uncultured amniocytes, showing a normal dosage of both subtelomeric regions (14qtel and 20qtel). Later, the study of cultured amniocytes, demonstrated a normal female karyotype, and the hybridization on the metaphase spread showed that the fetuses had not inherited the familial subtelomeric translocation: 46,XX,ish(14qtel,20ptel,20qtel)x2.

Detecting Copy Number Polymorphism in CGH Tiling Array Data from Multiple Individuals using Hidden Markov Models. *S. Zollner[1], N. Vinckenbosch[2], G. Abeçasis[1], H. Kaessmann[2]* 1) Dept Biostatistics, Univ Michigan, Ann Arbor, MI; 2) Cent Integrative Genomics, Lausanne, Switzerland.

Recent reports have revealed that considerable segmental copy number polymorphism (CNP) in the human genome contributes to genomic variation among individuals. CNPs range in length from a few to several hundred kb and often span genes. Thus they are functional candidates for common phenotypic traits and diseases. The distribution and allele frequencies of these polymorphisms is not yet well known, as large-scale CNP datasets are only beginning to emerge. Here, we used Comparative Genomic Hybridization (CGH) arrays together with a new statistical to model to examine the distribution of CNPs in a sample of 30 individuals. CGH arrays can detect copy number polymorphisms at high resolution. In this technology, the DNA sequence of a test subject is competitively hybridized to together with a reference sequence. Differences in hybridization intensity indicate a difference in copy number between the tested sequence and this reference. Analyzing CGH data is challenging, due to experimental noise and other confounding factors. In previous screens for CNPs, each hybridization experiment was analyzed separately. Since common CNPs are expected to occur in several individuals, jointly analyzing a population sample provides a more powerful method to detect CNPs from CGH data. Therefore, we have developed an approach based on a hidden Markov Model (HMM) where each hybridization experiment is treated as one realization of the same Markov process. The unobserved states represent the presence or absence of a CNP and the transition probabilities are dependent on its population frequency. We apply a variation of Baums algorithm to estimate these transition probabilities and the likelihood of each individual to carry a given CNP variant, combining information from all hybridization experiments. To assess the significance of a signal, we generate a null-distribution by permuting the hybridization intensities among loci. We present applications of our method to simulated data as well as to genomewide hybridization data generated for 30 humans using Nimblegen CGH arrays.

Comparison of a functional human gene network with a network reconstructed by applying genetical genomics.

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Gene networks could provide important clues to which genes may cause disease, by assuming that most of the causative genes in complex diseases are functionally closely related (Franke *et al*, AJHG 2006). To improve the reliability and extent of these networks, we used recent human genetical genomics data sources that combine genotypes and expression data from HapMap individuals. We first generated a network solely from these repositories and then compared it to our functional human gene network, available at www.genenetwork.nl. As expected, the majority of the most significant *trans* interactions had not been reported in the literature: Within this set of interactions, we saw an under-representation of interactions that were already described in KEGG, HPRD, BIND or Reactome. However, when we compared the identified significant genetical genomics interactions with the interactions predicted by our functional gene network, there was concordance. Additionally, the average distance between the significant interactions in our functional gene network was significantly shorter than expected, indicating that the majority of these inferred interactions represent indirect biological relationships that are yet unknown. Based on these observations, we propose a novel method that can help to reconstruct direct interactions, opening avenues for the generation of accurate regulatory gene networks that describe causal interactions. We show that these networks are helpful in identifying disease genes, allowing researchers to rely solely on microarray expression data. Such analyses result in sets of differentially expressed genes, for which the differences can be traced back to the causative genes, when using these regulatory gene networks.

Evidence for a susceptibility locus for mammographic density. *C.M. Vachon[1], V.S. Pankratz[1], J.M. Cunningham[1], E.C. Carlson[1], C.A. Hilker[1], A.H. Wang[1], R.L. Smalley[1], T.A. Sellers[2]* 1) Mayo Clinic, Rochester, MN; 2) H Lee Moffitt Cancer Center, Tampa, FL.

Introduction: Mammographic density, or the proportion of nonfatty tissue on the mammogram, is a strong risk factor for breast cancer and appears to be genetically influenced. We report the first genomewide linkage scan and follow-up fine mapping of mammographic density.

Methods: Families were selected from the Mayo Breast Cancer Family Study based on the availability of female relatives with mammograms. Percent density (PD) was estimated on both the Craniocaudal and Mediolateral oblique views using a thresholding technique. The mean PD from the two views served as the primary phenotype. The genomewide scan was based on 400 markers from the ABI Mapping Set, version 2.5, with an average spacing of 10 cM. Follow-up genotyping of 56 additional markers, with an average intermarker spacing of 1.7 cM in two regions implicated in the scan, was performed by deCODE Genetics. Multi-point IBDs were estimated using SIMWALK. Tests for linkage to PD were performed using EMVC, software for variance components linkage analyses. All analyses included covariates associated with PD in our sample.

Results: A total of 889 relatives (756 women, 133 men) from 89 families formed the sample for our linkage analyses. 658 (87%) women had available mammograms and estimates of PD. The maximum LOD score from the genomewide scan was 3.1, and a second highest peak reached a LOD of 2.5. The 1-LOD credible intervals for these regions identified by these peaks were 23.6 and 33 cM wide, respectively. Fine mapping resulted in stronger evidence for linkage in the first region (LOD=3.3) and a narrower 1-LOD interval (10.2 cM), but did not improve the strength of evidence for linkage in the second (LOD=2.3; 14.8 cM).

Conclusion: We provide evidence for a promising susceptibility locus for mammographic density. We are pursuing further studies in the region and hope to identify a gene accounting for our linkage signal while demonstrating an association with mammographic density.

24-Months on Treatment: Open-Label Phase I/II Long-Term Study of Enzyme Replacement Therapy (ERT) with Gene-Activated Human Glucocerebrosidase (GA-GCB) in Patients with Type 1 Gaucher Disease. *A. Zimran[1], G. Altarescu[1], C. Fratazzi[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 Gene-Activated human glucocerebrosidase (GA-GCB), a novel ERT for patients with type 1 Gaucher disease.

BACKGROUND: GA-GCB is produced in a continuous human cell line using proprietary gene-activation technology and has an identical amino acid sequence to the natural human enzyme.

METHODS: Ten of 11 patients who completed the Phase I/II study enrolled in the extension study. One patient discontinued treatment for reasons unrelated to GA-GCB. Patients continued to receive 60U/kg GA-GCB every other week for 12 months of treatment. At about Month 12, patients who qualified (based on Therapeutic Goals for ERT in type 1 Gaucher disease; Seminars of Hematology, 2004) began a step-wise dose reduction from 60U/kg to 45U/kg (for 13 weeks), then to 30U/kg.

RESULTS: GA-GCB was well tolerated at a 60U/kg dose every other week for approximately 12 months of treatment. Patients were dose reduced to 30 U/kg successfully and without incident. To date, no drug-related serious adverse events have been reported. One patient experienced an infusion-related adverse event (during the extension study) without interrupting treatment. Notably, no patient developed anti-GA-GCB antibodies by Month 18. Statistically significant mean increases from the core study baseline were observed for hemoglobin and platelet counts by Month 3 and continued through Month 18. Month 24 clinical activity, plasma chitotriosidase, and CCL18 data will be presented.

CONCLUSION: GA-GCB was well tolerated and demonstrated clinical activity in disease parameters in adult patients with type 1 Gaucher disease. These results suggest that GA-GCB holds promise as a new ERT and deserves further evaluation in clinical trials including in children.

Two ABCC6 gene polymorphisms are not associated with variation in plasma lipid levels in the Ludwigshafen Risk and Cardiovascular Health Study. *B. Struk[1], W. Renner[2], K. Lindpaintner[3], B.R. Winkelmann[4], B.O. Boehm[5], W. Maerz[2]* 1) Helios-Clinic, Charité and Max-Delbrueck-Centrum, Berlin, Germany; 2) Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University, Graz, Austria; 3) Hoffmann-La Roche Ltd, Roche Genetics, Pharmaceuticals Division, Basel, Switzerland; 4) Cooperation Unit Pharmacogenomics, Applied Genomics, University of Heidelberg, Heidelberg, Germany; 5) Division of Endocrinology and Diabetes, University of Ulm, Ulm, Germany;.

Mutations in the ATP dependent transporter protein ABCC6 cause Pseudoxanthoma elasticum (PXE), a mendelian disorder that affects the elastic tissue in skin, eye and the cardiovascular system and leads to premature cardiovascular disease. Other ABC transporters play a significant role in lipid transport. A recent study showed the association of the Q allele of the R1268Q polymorphism in ABCC6 with higher plasma HDL and lower plasma triglyceride levels in a small Canadian Inuit population. In contrast, HDL plasma levels showed a 25 % reduction in an ABCC6 knockout mouse model in comparison with the wild type. To further evaluate potential associations of ABCC6 single nucleotide polymorphisms (SNP) with plasma lipid levels in the general population, we genotyped two SNP (V614A, R1268Q) in a total of 3316 participants of the LURIC study, a prospective cohort study. There was no allelic association of V614A and R1268Q genotypes with total plasma cholesterol, plasma HDL, LDL, plasma triglycerides or plasma lipoprotein a (Lp a) levels within the study population. These results from the total study population did not differ in the sub group of study participants younger than 50 years (n=433) Our data do not demonstrate an allelic association of two common missense SNPs with variation in plasma lipid levels in a population of sufficient sample size. We conclude that common allelic variants of ABCC6, do not represent susceptibility markers for variation in plasma lipid levels.*R1268QV614A*, *R1268QV614AR1268Q*.

Mutations in the SPG8 gene cause hereditary spastic paraplegia. *P.N. Valdmanis[1], I.A. Meijer[1], A. Reynolds[2], A. Lei[1], P. MacLeod[3], D. Schlesinger[4], M. Zatz[4], P. Dion[1], P. Drapeau[2], G.A. Rouleau[1]* 1) Centre de recherche du CHUM, Hopital Notre-Dame, Montreal, Canada; 2) Center for Research in Neuroscience, Department of Neurology and Neurosurgery, McGill University, Montreal, Canada; 3) Children's & Women's Health Center, Vancouver, Canada; 4) Univ de Sao Paulo dept of Biology, Sao Paulo, Brazil.

Hereditary spastic paraplegia (HSP) is a debilitating upper motor neuron disorder which leads to a spastic gait phenotype. Thirteen genes and over thirty loci have been identified for this highly heterogeneous disorder. Three adult-onset dominant families with a pure HSP phenotype were used to map the *SPG8* locus to chromosome 8q23-24. We have identified three additional families by linkage analysis which map to this locus. Key recombinants in our families enable a candidate interval to be defined between markers D8S1832 and D8S1774, containing 10 known genes. These genes have been sequenced in their entirety, leading to the identification of two mutations in the *SPG8* gene. Both mutations occur in amino acids that are conserved across all species, and neither mutation is present in over 1000 control chromosomes. Three European families have a valine to phenylalanine missense mutation, and a Brazilian family has a leucine to phenylalanine change. Bioinformatic analysis and protein modeling of the expected *SPG8* protein product predict that these amino acids are adjacent in 3D space within an alpha-helix and that the mutations potentially disrupt this helix. Furthermore, studies in zebrafish indicate that knocking-down *SPG8* using morpholino oligonucleotides causes a severe morphological phenotype which can be rescued with a wildtype, but not mutant, human form of *SPG8*. Little has been described about the gene; however, cell transfection studies indicate a diffuse distribution of the *SPG8* protein in the cytoplasm, and its possible involvement in the cytoskeletal structure of the cell. *SPG8* is expressed ubiquitously, including in various regions of the brain. The elucidation of the nature of this gene and its potential interactions with other HSP genes will aid in the understanding of this neurodegenerative disease.

Estimation of X chromosome effects on human quantitative traits. *L. Pan, C. Ober, M. Abney* Department of Human Genetics, The University of Chicago, Chicago, IL.

Recently, we evaluated the sex specific genetic architecture for 17 quantitative traits in the Hutterites (Weiss et al., 2006 Nat Genet 38:218-222). Estimates of heritability revealed significant interactions with sex for five of the 17 traits examined. The sex specific genetic architecture of these traits could be due to autosomal genes that are differentially expressed in males and females, or to X-linked genes. In this study, we extended the variance component, maximum likelihood method to evaluate the relative contributions of sex specific effects on both the X chromosome and the autosomes, to overall estimates of heritability of 20 quantitative human phenotypes. Seven of the 20 phenotypes showed significant X-linked effects: systolic blood pressure (SBP), adult height, fasting insulin, triglycerides, lipoprotein (a) [Lp(a)], serotonin, and age at menarche. Three traits (SBP, adult height, and triglycerides) showed significant X-linked effects only in males. The proportion of the total heritability due to X-linked effects was 33% for SBP (broad heritability $H^2 = 0.48$), 10% for adult height ($H^2 = 0.90$), 47% for fasting insulin ($H^2 = 0.71$), 34% for triglycerides ($H^2 = 0.59$), 53% for Lp(a) ($H^2 = 0.88$), and 39% for serotonin ($H^2 = 1.00$). For the female specific trait age at menarche, the X chromosome accounted for 100% of the genetic effects ($H^2 = 0.46$). Our findings suggest that sex specific genetic effects may not only be common in human quantitative traits, but also that the X chromosome plays both a large role in these effects and has a variable influence between the sexes. This work was supported by NIH grants HD21244, HL56399, HL66533, HG02899 and DK55889.

Further delineation of the Williams syndrome genotype/phenotype correlation using a unique 3-generation familial deletion. *A.S. Parikh[1,3], E.S. Siwik[2], C.A. Curtis[1,3], L.J.B. Jeng[1,3], S.E. McCandless[1,2,3]* 1) Departments of Genetics; 2) Pediatrics; 3) and Center for Human Genetics, Case Western Reserve University, University Hospitals of Cleveland, Cleveland, OH.

Williams syndrome (WS, MIM 194050) is a microdeletion syndrome involving the cardiovascular, CNS, and other systems. The typical deletion at 7q11.23 is ~1.5Mb. Genotype/phenotype correlations have been suggested for deletion of specific genes. Isolated elastin (ELN) deletions produce autosomal dominant arteriopathy, while LIMK1 and WBSCR1 deletions may be associated with the characteristic personality and mental retardation in WS. The role of other gene deletions in the region is less clear. We identified an ELN deletion by FISH in a proband with apparent elastin arteriopathy, but no other findings of WS. We identified deletions in three additional individuals with learning difficulties and mild cardiovascular abnormalities in three generations of this family. The facial appearance of only one individual (grandmother) was suggestive of WS. Each individual had a physical examination, echocardiography, and neurocognitive testing when possible. FISH analysis with additional BACs allowed further characterization of the deletion to ~340Kb in the WS region. BAC RP11-52A3 is deleted and attenuated signals define proximal and distal breakpoints within RP11-731K22 and RP11-805G2, respectively. Deleted genes include ELN, LIMK1, and WBSCR1. Two genes recently suggested to play a role in the WS phenotype, CYLN2 and GTF2IRD1, are not deleted. Two genes, LAT2 and RFC2, are near the distal breakpoint defined by the BAC studies, and ongoing microsatellite analysis will further define the breakpoints and determine if these two genes are present. **Discussion:** Familial deletions of WS are rarely reported, and this unique 3-generation family with a variant of WS contributes valuable information about the role of several genes in the region to the phenotype. More importantly, the obvious intra-familial phenotypic variation associated with this deletion illuminates the need for caution when making genotype/phenotype correlations based on single or rare cases of chromosomal deletions and rearrangements.

Study of the Involvement of the Fanconi Anemia Proteins in ALT Telomere Maintenance. *H. Root*[1,2], *M.S. Meyn*[1,2] 1) Genetics & Genomic Biol, Hosp for Sick Children; 2) Mol & Med Genet, Univ of Toronto, Toronto, ON.

Fanconi anemia (FA) patients have short telomeres, suggesting that FA proteins may play a role in telomere maintenance. We have shown a role for FA proteins in the recombination-based Alternative Lengthening of Telomeres (ALT) pathways that function in ~10% of human tumors. We find that FANCA, G, D1, and D2 localize to telomeric foci in ALT, but not telomerase-positive fibroblasts. FANCD2 colocalizes with telomeric proteins within PML bodies in late S/G2 and co-IP experiments indicate ALT-specific in vivo interactions between FANCD2, BLM, and TRF2 in late S/G2. Some ALT telomeric foci contain H2AX, a marker of DNA damage, and FANCD2 is almost always present in these foci. This suggests that FANCD2 may aid in the response to dysfunctional telomeres.

We are now investigating the events required for FANCD2 localization to ALT telomeric foci. Following DNA damage and in S phase, FANCD2 forms nuclear foci, is monoubiquitinated and phosphorylated. Initial results suggest that FANCD2 localization to ALT telomeric foci is independent of the ATM kinase. The role of ATR in ALT-specific localization is currently under investigation. FANCD2 focus formation is thought to be dependent on a core complex of FA proteins. FANCA depletion disrupts the core complex and reduces both FANCD2 monoubiquitination and focus formation in ALT cells. Strikingly, FANCD2 foci remaining after FANCA depletion are 3 times more likely to be associated with telomeric foci than in controls, suggesting that FANCD2 monoubiquitination is not required for FANCD2 localization to ALT telomeric foci. Furthermore, siRNA depletion of FANCA does not affect the apoptosis, senescence or growth rate of ALT cells relative to controls. This may differ from FANCD2, which can be stably suppressed with shRNA in telomerase positive but not in ALT cells. Our data support a model in which FANCD2 plays multiple roles in ALT cells and can be recruited to sites of dysfunctional DNA in both a core complex dependent and independent manner. RNA depletion studies also indicate a critical role for FANCD2 in ALT telomere maintenance that is independent of the core complex.

Alcohol dehydrogenase genes, alcohol consumption and risk of orofacial clefts. *P.A. Romitti, K.L. Rose, L. Sun, K. Malville-Shipan, J.C. Murray* The University of Iowa, Iowa City, IA.

Studies of periconceptional alcohol exposure and orofacial clefts have been mixed perhaps due in part to unexplored genetic susceptibilities. Specifically, little attention has been given to the impact of variants in alcohol dehydrogenase (ADH) genes. Using Iowa data from a population-based case-control study, we examined the risk of clefting associated with potential ADH gene-alcohol interactions. Cases were deliveries diagnosed with a cleft lip palate (CL/P) or cleft palate only (CP) between 1997 and 2002 and controls were unaffected deliveries during the same time period. Case (n=156) and control (n=329) mothers provided reports of periconceptional alcohol use (one month prior through the first three months of pregnancy) by telephone interview, and birth parents and infants provided buccal samples by mail. Gene variants examined by Applied Biosystems TaqMan assays were those designed manually, ADH1B (rs1229984) and ADH1C (rs698), and those generated by the vendors design service, ADH4 (rs1800759, rs1800761, T-192A). Risk estimates for CL/P and CP associated with any maternal alcohol consumption, maternal genotype (1 or 2 copies of the allele of interest versus none) and the joint risk of each exposure were estimated using odds ratios (ORs) and 95% confidence intervals (CIs). For periconceptional alcohol use, ORs adjusted for infant sex and family history and maternal age, education, body mass index, smoking and folic acid use were 0.8 (CI=0.5,1.3) for CL/P and 1.4 (CI=0.7,2.5) for CP. For both phenotypes, ORs for maternal genotypes adjusted for infant sex and family history tended to differ by gene variant (ADH1BADH1CADH4), and a statistically significant increase in risk of CL/P was found for mothers who carried the C allele for ADH4 rs1800759 (OR=2,8; CI=1.2,6.7). The joint risk associated with each exposure was most elevated for mothers who drank and carried the C allele for ADH4 rs1800759 (OR=4.4; CI=1.1,17.8). Our findings suggest that risk of clefting associated with periconceptional alcohol exposure may be influenced by select ADH gene variants. We will attempt to replicate our analyses using data from a second Iowa case-control study.

***SUMO1* haploinsufficiency can cause cleft lip and palate.** *I. Saadi[1], F.S. Alkuraya[1], J.J. Lund[1], A. Turbe-Doan[1], C.C. Morton[2], R.L. Maas[1]* 1) Division of Genetics, Department of Medicine; 2) Departments of Obstetrics & Gynecology, Reproductive Biology, and Pathology, Brigham & Women's Hospital and Harvard Medical School, Boston, MA.

Human birth defects represent an example of a complex genetic disease, in which multiple genetic and environmental factors play a role. Cleft lip with or without cleft palate (CL/P) is among the most common human birth defects, with an incidence between 1/500 and 1/2000 births, depending on the population. Monogenic forms and variants in several genes have been identified that contribute to CL/P, but the full spectrum of such genes and whether and how they interact is unknown. As part of the Developmental Genome Anatomy Project (DGAP), we have studied *de novo* balanced translocation cases with CL/P to discover genes involved in CL/P that may be difficult to determine by other means. We ascertained a patient with isolated cleft lip and palate and a 46,XX,t(2;8)(q33.1;q24.3)dn that disrupts *SUMO1* in intron 2. To validate the role of *SUMO1* in clefting, we confirmed expression of *Sumo1* in the developing mouse lip and palate. We also established a hypomorphic *Sumo1* gene-trap allele, $Sumo1^{Gt}$, that produces an incompletely penetrant cleft palate phenotype. Furthermore, the *Sumo1* hypomorphic allele interacts genetically with a loss-of-function allele for *Eya1*, a gene involved in palatogenesis in mouse and human, such that $Sumo1^{Gt/+}/Eya1^{+/-}$ mice show a significant increase in the incidence of cleft palate (17%) compared to $Sumo1^{Gt/+}$ (5%) ($p<0.008$, Fisher exact test) or $Eya1^{+/-}$ (0%). We also demonstrate that Eya1 is sumoylated at multiple sites *in vivo*, and thus joins a growing list of proteins implicated in clefting that are sumoylated, including Msx1, Satb2, and Pax9. Sumoylation is a post-translational modification in which a SUMO peptide is transferred to a target protein, altering its transcriptional activity, nuclear localization or other functional properties. Our data suggest that through its potential impact on a number of proteins involved in palatogenesis, *SUMO1* haploinsufficiency may reduce the overall genetic threshold required to cause CL/P.

Mapping Genes Affecting Age-at-onset of Alzeihmer Disease in the Chromosome 8. *J. Vance, J. Deng, GM. Mayhew, J. Grimsley, X. Huo, MA. Pericak-Vance, YJ. Li* Prof, Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC.

Identification of genes that affect age-at-onset (AAO) of AD has profound impact in understanding the genetics of AD, and will potentially improve prevention and treatment in this aging-related neurodegenerative disorder. We previously conducted a whole genome screen for AAO of AD using 449 families and identified several linkage regions including chromosome 8q. The initial linkage region of chromosome 8q covers 46cM interval (between 119 to 165cM; LOD>1.0) with a peak multipoint LOD score of 2.09. To refine the interval, we genotyped the same four microsatellite markers under this linkage region on the additional new 158 families. We applied the variance component linkage analysis method implemented in SOLAR (Almasy and Blangero 1998) to screen the chromosome 8 using this expanded dataset (607 families). AAO was treated as a quantitative trait and sex was treated as a covariate. We obtained an updated two-point LOD score of 2.34 at the marker D8S1179. In addition, the new analysis narrowed the peak region to about 20cM (between 128 to149cM; LOD>1.0). These data strengthen the evidence for a potential gene(s) in this region contributing to the genetic etiology of AAO in AD. We then follow up the interval that covers one LOD score below the peak marker (1-LOD down region) using Illumina custom SNP arrays. A 1536 Illumina Goldengate SNP OPA will be used to fine map the ~24mb area between q23.3 to q24.23 in Chromosome 8 in these data. Identification of the chromosome 8 AD AAO gene will increase our understanding of susceptibility genes in AAO of AD.

Detection using QMPSF of partial deletions and duplication of the *NSD1* gene in Sotos syndrome. *P. Saugier-Veber[1], C. Coubes[2], M. Holder[3], V. Drouin-Garraud[1], J. Roume[4], D. Bonneau[5], S. Férenbach[1], T. Frebourg[1], L. Burglen[6]* 1) Department of Genetics, University Hospital, Rouen, France; 2) Department of Medical Genetics, University Hospital, Montpellier, France; 3) Department of Medical Genetics, University Hospital, Lille, France; 4) Department of Medical Genetics, Hospital of Poissy-Saint Germain en Laye, Poissy, France; 5) Department of Medical Genetics, University Hospital, Angers, France; 6) Department of Genetics, Trousseau Hospital, Paris, France.

Sotos syndrome is an overgrowth syndrome characterized by pre- and postnatal overgrowth with advanced bone age, macrocephaly, characteristic facial features and variable mental retardation. Large 5q35 microdeletions encompassing the *NSD1* gene detected by FISH, and intragenic *NSD1* mutations have been reported in 60-90 % Sotos patients and in some patients affected with Weaver syndrome. We and others recently reported partial deletions of the *NSD1* gene. We have now developed a QMPSF (Quantitative Multiplex PCR of Short Fluorescent Fragments) assay covering exons 2 to 23 of the *NSD1* gene and have integrated this QMPSF assay in the molecular diagnosis of Sotos syndrome. This QMPSF assay allowed us to identify 4 partial *NSD1* deletions removing exon 2, exons 6-7-8, exons 18-19 and exons 22-23 and the first partial duplication of *NSD1*, involving exon 4. All patients exhibited characteristic features of Sotos syndrome and cannot be distinguished from patients with point *NSD1* mutations. These results highlight the importance of systematic detection of such genomic rearrangements in the molecular diagnosis of Sotos syndrome by using molecular methods such as QMPSF since these *NSD1* genomic rearrangements cannot be detected by FISH. From our experience, we estimate that the partial deletions and duplications represent 5 % of the *NSD1* alterations whereas complete *NSD1* deletions represent 12.5 % of the *NSD1* alterations.

EMPIRICAL DETECTION OF NATURAL SELECTION AT TWO BRAIN-RELATED GENES (FOXP2 AND AHI1) BUT FAILURE TO CONFIRM EVIDENCE AT ASPM. *F. Yu[1, 2], R.S. Hill[2, 3, 4], A.A. Mignault[1], R.J. Ferland[3,4], C.A. Walsh[2,3,4], D. Reich[1,2]* 1) Dept Genetics, Harvard Med. School; 2) Broad Inst. of MIT & Harvard; 3) Div. of Neurogenetics & Howard Hughes Med. Inst., Beth Israel Deaconess; 4) Dept. of Neurology, Harvard Med. School.

A startling hypothesis is that genes cause neurodevelopmental defects have been subject to natural selection during human evolution. Dorus (2004) showed that this has been the case over the past tens of millions of years of primate history, and anecdotal studies have suggested that the selection may have continued into the last tens of thousands of years. The most striking evidence has been at FOXP2 (Enard 2002), ASPM (Mekel-Bobrov 2005), and Microcephalin (Evans 2005). However, the evidence is based on comparing the pattern of variation at the genes to computer simulations, which cannot capture all features of real data. To empirically assess whether the brain-related genes indeed stand out compared with random genomic regions, we studied two genes with previous evidence of selection, FOXP2 and ASPM, and two that cause neurodevelopment defects but with no previous evidence, AHI1 and GPR56. We resequenced 15-30 kb from each in 16 European Americans and 16 West Africans, and genotyped the SNPs in HapMap samples. This was identical as method used to study 5 ENCODE regions, providing a large empirical comparison data set. Both FOXP2 and the AHI1 stand out compared with the pattern of variation in European Americans in the ENCODE regions. The results at AHI1 constitute new evidence and are particularly striking, with the pattern more extreme than anything seen in comparable segments of the ENCODE regions by various tests(all $P<<0.006$). There is no evidence for selection ASPM, even though a recent study suggested positive selection within the last 6,000 years. Our analysis suggests it arose tens of thousands of years ago, and the gene does not stand out from empirical data by multiple tests. Thus, comparison to simulations can mislead inferences. To provide the most compelling arguments for selection, multiple lines of evidence should be brought to bear, and comparison to empirical data can be an important resource.

Examination of *LAMB1* and *EN2* as autism candidate genes. *D.A. Skaar[1], J.M. Jaworski[1], J.L. Benton[1], E.R. Martin[1], H.H. Wright[2], R.K. Abramson[2], M.L. Cuccaro[1], J.R. Gilbert[1], M.A. Pericak-Vance[1]* 1) Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) University of South Carolina School of Medicine, Columbia, SC.

Multiple linkage peaks for Autism have been identified on chromosome 7q, primarily from 7q21.3 to 7q31.3. Many candidate genes on 7q have been tested for autism association, mostly from this prime region of linkage peaks. *RELN*, *NRCAM*, *EN2*, and *WNT2* have all been examined based on their functions in neurological development, and brain and behavior phenotypes in mouse models of *RELN*, *EN2*, and *WNT2*, with significant association seen in all. Significant associations have also been seen for other candidates in the peak region, *UME2* and *LAMB1*. We have previously shown association in *RELN*, and have screened a 10Mb region of 7q containing several of the linkage peaks. This region is also centered around *RELN* and a chromosomal inversion seen in all autistic members of one family. This screen identified two significant SNPs near *LAMB1*, and stronger associations distal to *LAMB1*. These results, combined with studies showing significant PDT associations for the *LAMB1* SNPs LAMB1x30 (p= 0.0466, Bonora et al., 2005) and rs2249956 (p=0.01, Hutcheson et al., 2004), led us to replicate the SNPs from these studies, and add tagging SNPs for blocks of linked SNPs in *LAMB1*. *EN2* was also examined in our dataset, based on PDT associations previously reported (rs1861973 and rs1861973, p=0.0296 and 0.0121, Benayed et al., 2005) and the unusual location of *EN2*, 7q36.3, well outside the usual focal region defined by the linkage peaks.

In 363 Caucasian families (multiplex and singleton) ascertained by Duke and USC, PDT association was calculated for these SNPs, with no significance seen for any *LAMB1* or *EN2* SNPs. Subdivision based on family history does show significant associations for *LAMB1* SNPs rs11770141 and rs3735599 (p=0.0159 and 0.0094) in families with no autism history. Similar subdivision still showed no significance for any *EN2* marker. These results in the Duke dataset indicate *LAMB1* may be a susceptibility gene for sporadic cases of autism, but do not support *EN2* as a candidate gene.

A functional SNP in proteolipid protein 2 (PLP2) promoter increases ER stress induced apoptosis and confers increased risk of neonatal hypoxic-ischemic brain injury. *L. Zhang[1], T. Wang[1], D. Valle[1,2]* 1) Dept Human Genetics, IGM, JHMI; 2) Howard Hughes Med. Inst., Baltimore, MD.

We identified a SNP (-113C>A) in the promoter of proteolipid protein 2 (PLP2) gene that results in significant reduction (> 4 fold) of PLP2 mRNA and protein. PLP2-(-113C>A) is over-represented in males with X-linked mental retardation (frequency: control 1.56%, XLMR 5.86%, P<0.001). To delineate PLP2 function in CNS, we generated PLP2 deficient mice by gene targeting. We found PLP2-deficient MEFs have elevated levels of pro-caspase 12 and cleaved caspase 12, an ER stress specific caspase, as well as increased GRP78, an ER chaperone, suggesting increased basal level of ER stress. By immunofluroescence and electron microscopy, we showed PLP2 deficient MEFs have dilated ER lumen and inefficient protein trafficking from ER to Golgi as visualized by ts-VSVG-GFP, supporting the idea of increased basal ER stress. Furthermore, we found the increased ER stress is specifically associated with enhanced eIF2-alpha signaling, as evident by the increased eIF2-alpha phosphorylation and increased expression of downstream genes, such as ATF4 and CHOP. PLP2-deficient MEFs and cultured primary neurons show increased apoptosis following ER stress induction, but are indistinguishable from control cells in response to intrinsic and extrinsic apoptosis stimuli. In addition, PLP2-deficient neurons show increased sensitivity to pharmacologically induced hypoxia. Using a model of neonatal hypoxia-ischemia, we demonstrated that the hippocampal injury of PLP2 deficient neonatal mice is 10 fold more than the wild type littermates. Finally, we found that male human fibroblasts with PLP2-(-113C>A) also have increased susceptibility to cell death when exposed to ER stressors. In summary, our results suggest that the human PLP2 polymorphism is an important modifier of genetic or environmental stress of the ER and that individuals with PLP2-(-113C>A) have increased risk to neonatal brain injury related to genetic and/or environmental stress on the ER machinery. This mechanism may account for the association of the PLP2 promoter polymorphism and XLMR.

Multiple genes in the A processing pathway have susceptibility alleles that show significant, replicable association with LOAD. *S.G. Younkin[1], S.G. Younkin[1], M.M. Carrasquillo[1], T.A. Dincman[1], R.I. Budjak[1], S. Singhal[1], M. Allen[1], F. Zou[1], N. Graff-Radford[1], R.C. Petersen[2], N. Taner[1,2], L.H. Younkin[1]* 1) Mayo Clinic, Jacksonville, FL; 2) Mayo Clinic, Rochester, MN.

The mutations in APP, PS1,and PS2 that cause early onset familial Alzheimers Disease increase A42, which aggregates to form senile plaques in AD brain. ApoE, which is the only widely accepted gene with susceptibility alleles for late onset AD (LOAD), also fosters A aggregation. Our working hypothesis is that novel LOAD genes have been difficult to identify because most LOAD genes have multiple susceptibility alleles with effects smaller than the well known ApoE alleles. To develop a paradigm for identifying novel LOAD genes, we analyzed many good candidate genes in the A processing pathway in multiple, large case control series. Functional variants are most likely to be found in regions conserved between human and rodent genomes. For this reason, we genotyped all variants that we could find in conserved regions of the A processing genes. These putative functional variants were analyzed thoroughly by logistic regression at the single variant, haplotype, and multilocus genotype level. As the size and statistical power of the case control series available to us increased, many genes in the A processing pathway began to show significant, replicable association. A good example of this is the APP gene, which has a set of variants in the conserved regions of two adjacent haplotypes blocks that show highly significant association with AD. At this meeting, we describe significant association for additional A proceesing genes in the abstracts by Zou et al. (MME), Carrasquillo et al. (IDE, PLAU), and Taner et al (VR22, LRRTM3).

Expression analysis of the IRF6 gene in placental tissue. *F. Rahimov*[1], *J.C. Murray*[2] 1) Interdisciplinary Genetics PhD Program, University of Iowa, Iowa City, IA, 52242 USA; 2) Department of Pediatrics, University of Iowa, Iowa City, IA, 52242 USA.

Non-syndromic cleft lip with or without cleft palate (CL/P) is the most common craniofacial malformation of multifactorial etiology in humans. Mutations in the gene for interferon regulatory factor (*IRF6*) have been shown to cause the dominant Van der Woude syndrome (VWS), a common syndrome displaying CL/P as a feature of its phenotype. VWS is an excellent model for studying the non-syndromic forms of clefts. Strong association of *IRF6* variants with non-syndromic CL/P have shown in multiple studies, however, no coding mutations in *IRF6* have been detected in non-syndromic cases. Haploinsuffiency of *IRF6* is thought to underlie the pathogenesis of VWS. Since no structural mutations were detected in *IRF6* in non-syndromic CL/P cases we hypothesize that genetic variants in the regulatory regions of the IRF6 gene might affect its expression level and could predispose to non-syndromic CL/P.

To test our hypothesis, we performed quantitative gene expression analysis in placental tissue. *IRF6* expression in the placenta was confirmed by *in situ* hybridization. Placental tissues from 16 newborns without any orofacial defects were examined for expression of IRF6 mRNA by quantitative real-time RT-PCR analysis to determine the association between different genotypes and expression level of *IRF6*. Three single nucleotide polymorphisms (SNP) (rs1319435, rs2013162 and rs4844880) in and around *IRF6* were chosen based on previously reported results showing an association with non-syndromic CL/P in Danish, Iowan and Filipino populations and tested for their correlation with the expression level of the gene.

Our preliminary results showed no significant ($p<0.7$) association between various genotypes (haplotypes) in and around *IRF6* and its expression level in the placental tissue. Further studies on *IRF6* expression in additional placental samples and in the palatal tissues collected during surgery from patients with cleft lip and palate is underway.

Patterns of linkage disequilibrium reveal genotyping errors and copy number polymorphisms. *P. Scheet, M. Stephens* Dept Statistics, Univ Washington, Seattle, WA.

While average genotyping accuracy for large-scale studies of population genetic variation is now very accurate, a small number of individual SNPs may exhibit unusually high error rates, either due to pathological patterns in the intensities on which genotype calls are based, or because the SNP lies within a copy number polymorphism (CNP). Simple filters, particularly a test for Hardy-Weinberg equilibrium (HWE), are usually applied to attempt to identify any suspicious SNPs. Here we introduce a new filter based on identifying SNPs whose genotypes produce unusual patterns of linkage disequilibrium. By using a flexible model for variation in a sample of unphased multilocus genotypes, we scan the observed genotype data, evaluating the potential for a marker position to have at least one error by constructing a likelihood ratio (LR) statistic for each SNP in the sample. We also calculate the expected number of errors at each SNP among all sampled individuals. We test these criteria for error identification and quantification on two independent data sets. We applied the method to unfiltered data of unrelated CEU individuals from the HapMap Project, computing LRs at SNPs which showed a signature of genotype error and comparing these to the number of Mendelian incompatibilities (MI) obtained from trio information. Sites with multiple MIs show a different distribution for the criteria than do SNPs with 0 or 1 MI. Inspection of raw genotype data confirmed the presence of errors or a CNP at the 1 relevant SNP for which we had access to the intensities. Additionally, we scanned data from the early phase of an association study, consisting of 192 people typed at 105,000 SNPs genome-wide. We were able to confirm the presence of errors and CNPs at suspicious SNPs from the genotype intensities. Although these data are sparse compared with forthcoming studies, our method identified SNPs which would have been overlooked by deviations from HWE alone. For the more dense HapMap data, our method offers considerable improvement over deviations from HWE. These methods have been incorporated into fastPHASE and will be available in a future release of the software.

Multiplex, quantitative analysis of learning and memory related gene expression in rat hippocampus. *Y. Wu[1], L. Tong[2], H.C. Chi[1], J. Luo[1], C.W. Cotman[2], S.K. Boyer[1]* 1) Nucleic Acid Testing Business Center, Beckman Coulter, Inc., 4300 N Harbor Blvd, Fullerton, CA 92834; 2) 1226 Gillespie Neuroscience Facility, Institute for Brain Aging and Dementia, University of California, Irvine, CA 92697-4540.

Quantitative analysis of multiplex gene expression in a single reaction from a limited amount of total RNA is of great interest to research scientists. Currently available techniques either utilize arrays to detect the expression of a high number of genes per reaction at high cost and low throughput, or utilize real-time PCR† to detect the expression of a few genes at moderate throughput. However, researchers are more often interested in 20 or more genes in a given signal transduction or metabolic pathway. Here we present a highly multiplexed method that can quantitatively detect the expression of 34 genes related to learning and memory in a single reaction from as little as 25 ng of total RNA. This method is capable of producing over 7600 gene expression results in 24 hours. The genes for this study were selected based on the current evidence that their biological role is directly or indirectly related to learning and memory process. This multiplex method was used to test the effects of selective cytokines on neurotrophic factor or neurotransmitter-induced gene expression in hippocampal slide cultures. † The PCR process is covered by patents owned by Roche Molecular Systems, Inc. and F. Hoffman-LaRoche, Ltd.

Prioritizing disease genes through a protein map of yeast mitochondria. *F. Perocchi* gene expression, European Molecular Biology Laboratory, Heidelberg, heidelberg, Germany.

A challenge in systems biology is to enable a transition from the study of individual genes in a pathway to the understanding of entire sub-cellular processes as complex networks of interconnected components. Here we reconstruct the protein network of an organelle, the yeast mitochondrion, by integrated analysis of a comprehensive set of genome-scale data. We predict 346 proteins as novel candidates for mitochondrial localization and support our prediction experimentally. The functional network covers the known mitochondrial biology to greater extent than existing databases, and it brings together annotated and new modules into one framework. Based on the defined map, properties of the system were derived for the network connectivity, mutant phenotypes, regulation, evolution of yeast mitochondria and their conservation in humans. We propose human candidate genes for mitochondrial disorders through assessment of network properties of yeast orthologs to human disease genes.

Investigation of common disease-associated polymorphisms within Asian Indians. *T.J. Pemberton, N. Mehta, H. Allayee, P.I. Patel* Institute for Genetic Medicine, University of Southern California, Los Angeles, CA.

Asian Indians display a high prevalence of diseases linked to changes in diet and environment that have arisen as their lifestyle has become more westernized. In the case of coronary artery disease, Asian Indians exhibit unique characteristics that distinguish them from the other populations, suggesting that unique causative factors underlie this and possibly other related diseases. Recent STRUCTURE analysis using 1200 genome-wide polymorphisms in 432 individuals from 15 Asian Indian language groups has shown that: (i) Indians constitute a distinct cluster, and (ii) despite the geographic and linguistic diversity of the groups they exhibit a low level of genetic differentiation. We have now investigated the prevalence of disease-associated polymorphisms that have recently been reported to be risk factors for atherosclerosis, hypertension, diabetes, prostate cancer, Hirschsprung disease, and age-related macular degeneration within 576 individuals from the same Indian groups. All of these diseases have a high prevalence in Asian Indians. Analysis of the data revealed no allele frequency differences between the different language groups. We did however find allele frequency differences between this Asian Indian cohort and previously reported populations for certain polymorphisms. Most striking was the high abundance of the DG8S737 allele associated with an increased risk of prostate cancer (24.5%) but only a relatively moderate abundance of the disease-associated allele of the SNP, rs1447295 (12.9%). These two polymorphisms were found to be in high linkage disequilibrium (LD) within the other populations; however our data suggests that they are not in LD within the Asian Indian population. Further studies in Asian Indian disease cohorts will be needed to confirm an association between DG8S737 and the SNP rs1447295 with prostate cancer in Asian Indians.

TAPVR: A Cardiac Defect Associated with Alagille syndrome. *P.A. Sanchez-Lara[1,2], I.D. Krantz[1,2], N.B. Spinner[1,2]*
1) Division of Human Genetics and Molecular Biology, The Childrens Hospital of Philadelphia, Phila., PA; 2) The University of Pennsylvania School of Medicine, Phila., PA.

Alagille syndrome (AGS) is a genetically heterogeneous disorder caused by mutations in either Jagged1 or NOTCH2. Cardiovascular anomalies are present in almost all AGS patients (94%) with the vast majority (82%) having right sided lesions; branch pulmonary artery stenosis being the most common. Only one patient has been reported with both AGS and total anomalous pulmonary venous return (TAPVR). Here we present three additional cases in two unique families that give insight to genetic and environmental factors involved in TAPVR.

TAPVR is a rare congenital heart defect that occurs when all four pulmonary veins connect to the systemic venous circulation. Its frequency is 6.8 cases per 100,000 live births and accounts for 1.5% of children with congenital heart disease. TAPVR is usually an isolated anomaly but has been associated with cat-eye syndrome, Holt-Oram syndrome and asplenia syndrome. There is also evidence from the Baltimore-Washington infant study (BWIS) of a possible association between paternal lead exposure and TAPVR.

We reviewed records from our cohort of 245 AGS families with JAG1 mutations and found two with TAPVR. The first had paternal transmission of both a JAG1 mutation (c.693delAG) and a defect of the pulmonary venous system. The father had peripheral pulmonic stenosis, an atrial septal defect and partial anomalous pulmonary venous connection. The proband had infra-cardiac TAPVR requiring surgical repair. In the second family, identical AGS twins with a JAG1 splice site mutation (c.2916+1G>C) had discordant heart phenotypes. Twin A had a physiologic murmur with no significant gradient while Twin B had cor triatriatum and TAPVR.

The fact that identical twins were discordant with respect to their heart phenotype points to other environmental factors (e.g. placental insufficiency) which may also modify cardiac defects. Our findings suggest that Jagged1 plays a role in the development of TAPVR although a mutation alone is not sufficient.

Achievement of twin pregnancy with spermatozoa from a globozoospermic man after ICSI treatement. *A.B. Sallemi[1], Dh. Sallemi[3], A. Amouri[2], J. Cherif[1], T. Rebai[1], N.B. Abdelmoula[1]* 1) Laboratory of Histology, University of Medicine, Sfax, Tunisia; 2) Laboratory of Cytogenetics, Pasteur Institute, Tunis, Tunisia; 3) Private Sector.

Abnormal sperm morphology is associated with male infertility globozoospermia is an uncommon severe form of teratozoospermia characterized by round-headed sperm with an absence of acrosomes. Family cases of globozoopermia suggest that this pathology has genetic origins, but the mode of inheritance remains unknown. So far, no responsible genes have been identified. Pregnancies and live birth are rarely reported even with ICSI treatment. We report a successful achievement of a twin pregnancy in a Tunisian couple in which the male partner had globozoospermia and referred to us for cytogenetic evaluation. A 35 year old man and his 24 year old partner, a consanguineous couple sought assisted conception after 2 years of primary infertility. Semen sample revealed the following characteristics: volume 4 ml; sperm concentration 121.2x106/ml; 25 percent progressive motility at 30 min and 65 percent vitality. All the spermatozoa were round-headed. Hormonal profile was within normal range. The karyotypes of the two partners were normal. The couple proceeded, after a first unsuccessful cycle of ICSI, to a second cycle of ICSI in a Belgium centre. Of 12 oocytes retrieved, 11 were micro-injected and 6 fertilized normally. Four compacting embryos with eight or more cells were obtained. Two embryos were transferred 72 h after micro-injection and two others were cryopreserved for future use. A twin clinical pregnancy results and culminates in a spontaneous vaginal delivery of live twin, a female and a male. The infant's birth weight was respectively 2.3 and 2 kg. The twin show normal development after 3 months. Genetic counselling was very hard in this situation. Infact, if some genetic causes of male infertility have been identified and are clearly explained to patients, many others genetic facors of infertility remain largely unexplored.

Array-based comparative genomic hybridization (array CGH) for rapid prenatal diagnosis of cytogenetic abnormalities. *I. Van den Veyver[1,2], T. Sahoo[2], C. Shaw[2], S. Kang[2], D. Del Gaudio[2], S. Darilek[2], A. Patel[2], P. Ward[2], J. Li[2], C. Chinault[2], B. Roa[2], J. Lupski[2], A. Beaudet[2], S. Cheung[2], C. Eng[2]* 1) Dept Ob-Gyn; 2) Dept Mol Hum Genet, Baylor Col Medicine, Houston, TX.

We have shown in a prospective validation study that an array CGH test was highly accurate for rapid detection of chromosomal aneuploidies and deletions or duplications on fetal DNA samples in a clinical prenatal diagnostic setting. Here we present our updated "post-validation phase" experience with this test. All women underwent chorionic villus sampling (CVS) or amniocentesis for standard indications, received genetic counseling and provided informed consent. DNA was prepared directly or after whole genome amplification (WGA) from CVS and amniotic fluid (AF) and tested for maternal cell contamination (MCC). Back-up cell cultures were established and standard karyotypes performed for all samples. A blood sample from both parents was requested to determine the origin of copy number variants (CNV) detected in the fetus if needed. After initial validation on 98 samples, 45 additional clinical cases have been referred. Of these 45, there were 36 AF, 23 of which were analyzed after WGA only, 5 after WGA with confirmation on cell culture DNA. For 8 AF, only cultured-cell analysis was requested. There were 9 CVS, 3 were analyzed after WGA, 3 on direct DNA preparations and 3 on cell culture DNA. We detected one trisomy 21 and identified a marker to have originated from chromosome 12. There was 100% concordance with the karyotype. We also found one level II mosaic trisomy 11 that most likely arose during extended cell culture. There was no MCC. We detected 9 benign CNVs, inherited from a phenotypically normal parent (overall CNV rate was 19/143 or 13.2%). The combined data on all samples establishes a detection rate of 7/143 (4.9%) for cytogenetic abnormalities. In 90% the result was available before the karyotype. In conclusion, our data confirms that array CGH yields highly accurate results on CVS and AF in 2 weeks in most cases. Further large scale studies are needed to assess whether array CGH can replace karyotyping and FISH for rapid and expanded prenatal detection of chromosomal imbalances.

A unique case of Alveolar Rhabdomyosarcoma with two translocations:t(2;13) and t(1;13), and amplification of N-myc in a 5-year-old child. *R. Mosely[1], M. Liu[1], B. Myers[1], M.A. Thompson[2], T. McCurley[2], S. Shankar[3], V.G. Dev[1], A. Yenamandra[1]* 1) Genetics Associates Inc., Nashville, TN; 2) Dept of Pathology, Vanderbilt University School of Medicine, Nashville, TN; 3) Dept of Medicine,Vanderbilt University School of Medicine, Nashville, TN.

The most common type of rhabdomyosarcoma is alveolar rhabdomyosarcoma (ARMS), characterized by the specific translocation t(2;13)(q35;q14) or its rarer variant, t(1;13)(p36;q14), producing the fusion genes PAX3-FKHR and PAX7-FKHR, respectively. The N-myc gene is also reported to be amplified in a few cases of ARMS. We report a previously healthy, 5-year-old girl who presented with a four-week history of severe back pain and parasthesias in lower extremities. Radiographs of her spine showed a collapsed vertebral body. Further evaluation revealed a large paraspinal mass with intraspinal extension and spinal cord compression. She had widespread metastatic disease involving multiple bony sites and bone marrow. The differential diagnosis was sarcoma or neuroblastoma. The histopathological studies of the biopsy demonstrated that the tumor cells were positive for the muscle marker MyoD1, desmin, CD99, and NSE. The specific neuroendocrine markers chromogranin and synaptophysin were negative, as were antibodies to lymphoid markers and to keratin. Cytogenetic analysis of the patient revealed the translocations t(2;13) and t(1;13). FISH with FKHR breakapart DNA probe revealed that the 3 end of the FKHR sequences of one chromosome 13 had moved to 2q35 whereas the 3 end of the second chromosome 13 that had moved to 1p was deleted. N-myc gene was amplified 15-20 times in about 50% of the cells. PAX3-FKHR and PAX7-FKHR overexpression has been reported to result from two distinct genetic mechanisms. The PAX3-FKHR from altered transcriptional regulation and the PAX7-FKHR from fusion gene amplification. In our case PAX3-FKHR fusion gene seems to be intact while the PAX7-FKHR gene appears to be deleted at the 3end of FKHR along with the N-myc gene amplification. Further analysis of the regulation mechanisms will lead to the understanding of the critical level of gene product for the oncogenic effects of these fusions.

Genetic and cellular analysis of Meckel syndrome. *R. Punyashthiti[1], B. Consugar[1], C.J. Ward[1], V. Kubly[1], R. Bacallao[2], V.H. Gatton II[2], V.E. Torres[1], P.C. Harris[1]* 1) Nephrology, Mayo Clinic , Rochester, MN; 2) Indiana University School of Medicine, Indianapolis, IN.

Meckel Syndrome (MKS) is a lethal autosomal recessive disorder, involving bilateral cystic kidney, hepatic fibrosis, CNS malformations, and polydactyly. MKS is genetically heterogeneous with 2 loci identified. MKS1 has 18 exons with 70% of described MKS1 patients homozygous for a 29-bp deletion in intron 15, a founder mutation originating in the Finnish population. MKS3 has 28 exons and different mutations were found in five consanguineous families of south Asian origin. The MKS3 protein product, meckelin, is predicted to contain 7 transmembrane domains with a cysteine rich domain. Both gene products are widely expressed and may have some primary cilia involvement, similar to other cystic kidney disease-related proteins. To further analyze the mutations associated with MKS, DNA from a cohort of 8 families has been collected, 4 with at least 2 affected sibs. All cases have PKD, and encephalocele and/or polydactyly was noted in 6 families. Most cases were of northern European origin and identified in the US. Initial analysis by PCR revealed that 2 out of 6 families were heterozygous for the Finnish type MKS1 mutation. Total gene sequencing of the remainder of MKS1 and MKS3 is now underway to identify the importance of these two loci in this population. To study the location and the possible role of MKS proteins, we generated a full-length rat Mks3 construct with a V5 tag inserted after the signal peptide of the protein to facilitate detection. Western analysis revealed a protein size of ~110kDa, with the dimerized form of 220kDa. Deglycosylation reduced the protein size, consistent with the predicted five N-glycosylation sites. The construct was stably transfected into IMCD cells, a polarized mouse collecting duct cell line, to study the protein sub-cellular localization. Preliminary analysis demonstrated signals from the pericentriolar region in addition to plasma membrane, suggesting possible ciliary/ basal body involvement in the disease pathogenesis. Analysis of the endogenous protein in MDCK cells with a meckelin antibody is also consistent with a ciliary localization.

Pleiades Promoter Project: Genomics Resources Advancing Therapies for Brain Disorders. *E.M. Simpson[1,2,3], W.W. Wasserman[1,2], R.A. Holt[3,4], S.J. Jones[2,4], D. Goldowitz[5], S. Ward[6], S. Kingsley[7]* 1) Centre for Molecular Medicine and Therapeutics, Child & Family Research Institute; 2) Department of Medical Genetics, University of British Columbia (BC), Canada; 3) Department of Psychiatry, University of British Columbia, Vancouver, BC, Canada; 4) Canada's Michael Smith Genome Sciences Centre, BC Cancer Agency, Vancouver, BC; 5) University of Tennessee Research Center of Excellence in Genomics and Bioinformatics, Department of Anatomy and Neurobiology, University of Tennessee Health Sciences Centre, Tennessee, USA; 6) School of Journalism, University of British Columbia, Vancouver, BC, Canada; 7) International BioPharma Solutions Ltd., Vancouver, BC, Canada.

Brain disorders represent an enormous growing social and economic burden and a major unmet treatment need. However, gene therapy directly or via stem cells, holds great therapeutic promise. The Pleiades Promoter Project (www.cisreg/pleiades.org) aims to generate 160 fully characterized, human DNA MiniPromoters (less than 4 kb) to drive gene expression in defined brain regions of therapeutic interest for diseases such as Alzheimer, Parkinson, Huntington, Amyotrophic Lateral Sclerosis, Multiple Sclerosis, Spinocerebellar Ataxia, Depression, Autism, and Cancer. As part of the Mouse Atlas project (www.mouseatlas.org), seventeen brain regions of therapeutic interest have been isolated from mouse by laser capture microscopy and SAGE (Serial Analysis of Gene Expression) libraries have been prepared. We are presently using these data and existing genomic expression resources to identify genes with medium- to high-level, region-specific expression. For each gene, four MiniPromoter constructs will be generated by PCR (often stitching together non-contiguous DNA) and cloned into gene-targeting vectors to make 200 new stains of knock-in mice. Detailed histological analyses will be conducted to document the region and cell-type specificity of these MiniPromoters. Intermediate deliverables will include embryonic stem cells, promoter constructs, and promoter-design software. Thus, we will develop a richly characterized tool-set of resources for both basic and therapeutic research.

Oculo-Facio-Cardio-Dental (OFCD) syndrome: two female patients with novel mutations in the BCOR gene. *S. Whalen[1], S. Manouvrier[2], P. Bitoun[3], M-P. Cordier[4], I. Bailleul-Forestier[5], M. Goossens[1], A. Verloes[5], I. Giurgea[1]* 1) Dpt génétique and INSERM 654, Hôpital Henri Mondor, Creteil, France; 2) Service de génétique clinique, Centre Hospitalier Régional Universitaire de Lille, France; 3) Dpt génétique, Bondy, France; 4) Dpt génétique, Lyon, France; 5) Dpt génétique médicale, Hôpital Robert Debré, Paris, France.

OFCD syndrome is caracterised by congenital cataract, microphthalmia, dysmorphia, cardiac septal defects, dental and digital abnormalities. Dental abnormalities are oligodontia, radiculomegaly, delayed tooth eruption and dental fusion. OFCD syndrome results from mutations in the BCOR gene, located on Xp11.4, which is a key transcriptional regulator during early embryogenesis, particularly in the eye, skeleton and central nervous system. OFCD syndrome is an X-linked dominant condition with skewed X inactivation in female carriers and presumed male lethality. However, a missense mutation was described in males from a family with Lenz microphtalmia syndrome. To delineate further the clinical spectrum of OFCD syndrome, we studied two girls with OFCD syndrome and two boys with Lenz microphtalmia. Patient 1, a 12 years old girl, presented with bilateral congenital cataract, oligodontia, radiculomegaly, scoliosis, camptodactyly and syndactyly of the toes. She had no developmental delay, or cardiac malformation. Patient 2, a 14 years old girl, presented with congenital cataract, microphthalmia, glaucoma, dysmorphia, oligodontia, dental fusion and subnormal developmental delay. She had no heart defect. BCOR gene mutations were screened by direct sequencing, and intragenic deletions by QMF-PCR. Both female patients carried a deletion of a single nucleotide, creating a premature stop codon: c.863delC (p.Pro288ArgfsX90) for Patient 1 and c.570delC (p.Pro190ProfsX26) for Patient 2. No mutation or deletion in the BCOR gene were found for the male patients with Lenz microphtalmia. In conclusion, we report two novel frameshift mutations in the BCOR gene in two unrelated females presenting an OFCD syndrome, without heart defect. These results broaden the clinical spectrum of OFCD syndrome associated with BCOR gene, and suggest underdiagnosis of the disease.

The Effect of Genetic Essentialism and Exceptionalism on Law and Policy. *M.W. Sharp, T.A. Caulfield* Health Law Inst, Univ Alberta, Edmonton, AB, Canada.

Introduction: Genetic determinism and genetic essentialism are ideas that have been part of the latter half of the twentieth century. Some believe these ideas have shaped a reductionist approach to genetic research, including the human genome project - an ethos that has been criticized by many in the ELSI community. Genetic exceptionalism is associated with the idea that genetic material and information is different from other types of biological material or medical information and requires special governance for its acquisition, possession, and use. As a result of genetic exceptionalism, laws and policies around the world in the areas of reproductive technologies, informed consent, privacy, and discrimination, have been influenced in different ways and at varying levels. The purpose of our study is to examine this influence. Methods: An analysis of pertinent legislation, policy statements, and case law was performed to examine the extent to which essentialist and exceptionalist messages have been adopted. Background justifications for the incorporation of these messages into law and policy were also examined. Results: The field of genetics has been afforded special treatment in many laws and policies around the world. This has often been done in a proactive manner, to prevent future potential harms rather than to address specific, current, issues. Conclusions: Contemporary genetic theory has moved well beyond the one gene-one trait paradigm into the multifaceted network model of understanding gene expression and the complex role of environmental factors. Genetic essentialism and reductionism have been largely abandoned, but the idea of genetic exceptionalism is still prevalent and informing law and policy. These new laws, for the most part, remain untested in the courts and their impact is unclear. The courts could take into account the current understanding of genetics when applying these laws and can treat genetic material or information the same as other biological material or medical information. A more detailed analysis of specific laws and policy statements can lead to policy reform recommendations that would reflect the more complete, current, genetic theory.

45,X/46,XY Turners syndrome girl with complex congenital heart defect history. *A. Sahnoun[1], I. Trabelsi[2], A. Ahlem[3], M. Meddeb Cherif[4], S. Kammoun[2], T. Rebai[5], N.B. Abdelmoula[5]* 1) Urology department, Habib Bourguiba Hospital, Sfax, Tunisia; 2) Cardiology department, Hedi Chaker Hospital, Sfax, Tunisia; 3) Cytogenetic laboratory, Pasteur Institute, Tunis, Tunisia; 4) Genetic laboratory, Tunis, Tunisia; 5) Histology laboratory, University of Medicine, Sfax, Tunisia.

Congenital heart defects occur in approximately 30% of patients with Turner syndrome. Left-sided obstructive defects predominate, especially bicuspid aortic valve (BAV) and coarctation of the aorta (COA). Aortic root dilatation is uncommon, but potentially devasting if rupture occurs. It is usually associated with a risk factor such as BAV, COA or hypertension. Herein, we report the case of a 16-years-old girl for whom it was conducted a cytogenetic evaluation as part of our screening project of genetic abnormalities in congenital heart defects. She had a COA associated to a BAV not repaired since she develops an ascending aortic aneurysm and an acute aortic dissection evolution at the age of 5 years. She had repair of COA and aneuvrysm rupture in two-staged resection-interposition grafts at the ascending and isthmus levels. Immediate postoperative chest X-ray showed a dissected hematoma in the interposed isthmus graft but which is quiet until now. She remains also with an hypertension requiring beta-blocking drugs treatment. At genetic counselling, the girl has characteristic physical features of Turners syndrome with short stature, pubertal delay and dysmorphic stigmata especially very important neck webbing. Cytogenetic evaluation at lymphocytic level showed a mosaic formula 45,X[8]/46,XY[42]. FISH analysis is conducted to investigate Y chromosome structure and to estimate more precisely the mosaicism ratio. An adequate management based on international recommendations is also performed to increase this girls chances of the best quality of life. Due to a high prevalence of cardiovascular malformations in Turner syndrome, karyotype should be considered earlier in these cases.

Choosing the optimal platform and analysis for prostate cancer array CGH. *A. Pearlman[1,2], T. Anantharaman[2], B. Mishra[2], H. Ostrer[1]* 1) Dept Pediatrics, NYU Sch Medicine, New York, NY; 2) Courant Inst of Mathematical Sciences, NYU, New York, NY.

Prostate cancer cells incur somatic alterations that can be measured as chromosomal gains and loses with genomic DNA microarrays. These events may be associated with the initiation and progression of disease. Numerous array CGH platforms and analysis tools have been developed in recent years, presenting researchers with uncertainty in designing experiments. In deciding on an approach we tested Affymetrix 10K (affy), Nimblegen 347k tiling (ngen), and 19K BAC (bac) arrays with 3 sets of Gleason 7 tumors with matched normals. For analysis, we used segmentation algorithms vMAP (PNAS. 2004; 101:16292-7) and DNAcopy (Biostatistics. 2004; 5:557-72.). These tools performed comparably using default parameters on a simulated benchmark dataset (Bioinformatics. 2005; 21:3763-70). However, vMAP outperformed DNAcopy with lower signal to noise data when each corresponding algorithms threshold parameter was optimized. In this context, we improved the analysis by incorporating a function that automatically selects the threshold parameter used for the segmentation thus, minimizing the false discovery rate. Analysis of the three tumors assayed across three platforms revealed 33 segments (16 amplifications and 17 deletions). Forty percent of the events were concordant across all three array platforms whereas 61% percent were concordant with at least two platforms. Three, five and six platform unique segments were called with affy, ngen, and bac respectively. The 40% discordance is attributed to variable probe representation resulting in false negative and false positive calls. The overall performance of the arrays as gauged by signal to noise (ratio of segment mean to standard deviation) resulted in affy having the highest, bac second and ngen lowest. Also, affy provides genotype information that could be used for loss of heterozygosity and snp association analysis. In conclusion, arrays providing the greatest density and highest signal to noise will greatly influence the choice of platform while dynamic analysis tools are needed to adjust for sample and platform dependencies.

Detecting genomic regions subject to natural selection by examining deviations of genealogies from neutral models. *Y. Wang, B. Rannala* Genome Center and Section of Evolution and Ecology, University of California, Davis, CA.

There is much interest in detecting genomic regions that have been under natural selection because of the potentially functional importance of selected loci. Different types of natural selection have different signatures in DNA sequence and single nucleotide polymorphism (SNP) data, reducing or maintaining local genetic polymorphism in natural populations. For mutilocus data the effect of selection and recombination are combined. Given the observed pattern of genetic variation within a chromosome interval containing an allele that has been under recent selection, the underlying genealogy of the sample will deviate from that expected under neutral coalescence and recombination models. We developed a Bayesian framework to identify genomic regions that depart from neutral genealogical models by comparing the distances between the prior and the posterior probability distributions of the genealogy underlying a sample of chromosomas, and by examining variations of the estimated posterior distributions of the population size under neutrality among chromosomal intervals. The performance of the method is examined by simulation, and the method is applied to SNP data from the International HapMap project.

Gene expression profiles and pathway analysis of early mouse inner ear development. *S.A. Sajan[1], M. Warchol[2], M. Lovett[1]* 1) Depts of Genetics and; 2) Otolaryngology, Washington Univ School of Medicine.

To identify new inner ear genes and pathways, we have expression-profiled the early stages of embryonic mouse inner ear development at half-day intervals on Affymetrix MOE430Av2 chips. From E9 to E10 (early category) whole inner ears were profiled, and from E10.5 to E12 (middle category) the cochleae and vestibular organs were profiled separately. In the late category, from E12.5 to E15, the cochleae, utricles, and saccules were profiled individually. We first identified genes over-expressed by 1.5-fold or more in all possible unique and pair-wise combinations of these categories with an estimated false discovery rate of less than 0.5%. Some genes over-expressed in the early category included *Trp53*, *Slc2a3*, *Lin28*, and *Hoxb1*, out of a total of 1,312 genes. Estrogen receptor, PI3/AKT, and chemokine signaling pathways, among others, were significant in this category. We then analyzed the middle and the late categories through analysis of variance (ANOVA) tests to identify genes differentially-expressed based on tissue-type, developmental-stage, or both. In the middle category, genes such as *Rgs3*, *Kctd10* and *Nfkb2*, out of a total of 240 genes, were highly expressed in younger stages (E10.5 and E11) while some of the 326 genes high in older stages (E11.5 and E12) were *Six5*, *Nfib*, and *Lnx2*. Significant pathways included B-cell receptor and cardiac beta-adrenergic signaling in younger stages, and TGF-beta and apoptosis signaling in older stages. In the late category, the cochlea had high levels of *Clu*, *Irx5*, *Gata3*, and *Scara3*, out of a total of 90 genes; the utricle had *Pthlh*, *Timp3*, *Hes5*, and *Foxp1*, out of a total of 162 genes; the saccule had *Otoa*, *Foxd1*, *Ror2*, and *Sall3*, out of a total of 178 genes. A burst of expression for 212 genes at E14 relative to all other late stages was also observed. Such genes included *Fzd6*, *Pdgfc*, *Trim26*, and *3110056o03Rik*. We have validated expression patterns of numerous genes by RNA *in-situs*. Of the 5,136 genes differentially-expressed across all time-points, 11.2% currently have no known function, and 14.4% fall within uncloned human genomic deafness intervals, serving as candidates for deafness-causing genes.

Deletion of the entire FBN1 gene is a recurrent mutation in Marfan syndrome. *G. Pals, R. van Spaendonk, A. Nygren* Dept Clinical Genetics, VU Medical Ctr, Amsterdam, Netherlands.

Large deletions or duplications in the FBN1 gene are a frequent cause of Marfan syndrome. Using MLPA (multiplex ligation dependent probe amplification) tests for the FBN1 gene we detected a large deletion or duplication in 23 independent families (4.5% of mutations in FBN1 in a series including 445 point mutations). Most of these were unique deletions or duplications of one or more exons, but deletion of the entire gene was the most common mutation and was found in seven independent families. We have used DNA repeat markers and additional MLPA probes to determine a possible founder mutation and get information about the size of the deletion. The results show no common haplotype for patients that have a deletion of the entire FBN1 gene. The size of the deletion is variable between families and ranges from more than2 Mb 5 to more than 3 Mb 3 of the FBN1 gene. The gene itself covers approximately 240 kb of genomic DNA. The deletions encompass at least 44 other genes. Despite the large number of heterozygously deleted genes there are only a few phenotypical differences between families with different sizes of the deletion.

Mutations in *PLA2G6* cause infantile neuroaxonal dystrophy and neurodegeneration with brain iron. *S.K. Westaway[1], N.V. Morgan[2], A. Gregory[1], S. Sonek[1], J.E.V. Morton[2], J. Coryell[1], P. Gissen[2], H. Cangul[2], N. Canham[3], S. Pasha[2], N. Nardocci[4], D. Rodriguez[5], I. Desguerre[6], B. Wilmot[1], P. Kramer[1], J. Gitschier[7], S.J. Hayflick[1], E.R. Maher[2]*
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Neurodegenerative disorders with high brain iron include Parkinson and Alzheimer disease as well as the genetic neuroaxonal dystrophies. These include infantile neuroaxonal dystrophy (INAD), neurodegeneration with brain iron accumulation (NBIA) and Schindler disease, all of which show the distinctive pathologic feature of distended axons (spheroids) throughout the central nervous system. INAD is characterized by progressive motor and sensory impairment with spheroids found also in peripheral nerves, while NBIA is characterized by neurodevelopmental regression and dystonia with basal ganglia iron deposition. We performed genetic linkage studies in families with INAD and NBIA and mapped a locus for both to chromosome 22q. We found mutations in *PLA2G6*, encoding a calcium-independent phospholipase A2, in 38 families representing a broad clinical spectrum. We found 44 unique mutations throughout the gene, including missense, nonsense, splice and deletion mutations, most occurring at highly conserved positions. Our findings enable the molecular diagnosis of INAD and NBIA, obviating the need for a tissue biopsy. This phospholipase A2 is involved in membrane lipid remodeling, cellular signaling, and the mitochondrial apoptotic cascade. Our gene discovery links the pathogenesis of NBIA with INAD and ties these as well to pantothenate kinase-associated neurodegeneration, a related form of NBIA caused by defective mitochondrial coenzyme A biosynthesis. Moreover, this discovery implicates phospholipases in the pathogenesis of neurodegenerative disorders with brain iron dyshomeostasis and identifies a new target for neuroprotection in common diseases.

Second trimester prenatal triple marker screening for Down syndrome: the associations in the levels of serum markers in successive pregnancies. *A.M. Summers, T. Huang* Genetics Program, North York General Hosp, Toronto, ON, Canada.

Women who have been screen positive for Down syndrome by triple marker screening in the second trimester in one pregnancy tend to have a positive screening result again in their next pregnancy. This reflects the consistency between markers from one pregnancy to the next. Various adjustment methods have been proposed to eliminate recurrent false positives; however, the influence of the adjustments on detection rate (DR) has not been well evaluated due to insufficient data available from affected pregnancies. This study investigated the association between the levels of second trimester serum markers in first and subsequent pregnancies and assessed the impacts of including a previous screening result in the current risk estimation on the false positive rate (FPR) and DR of the current pregnancies. The study was based on 57,151 women who had triple marker screening in two or more singleton pregnancies in the Ontario Maternal Serum Screening Program between October 1993 and September 2000. Screening results were compared between different pregnancies in the same individual. Associations in the levels of serum markers were estimated using correlation analysis. A published method was used to adjust the current risk estimation for previous screening results and the effect of this adjustment was assessed by comparing DR and FPR before and after the adjustment. Women who screened positive in one pregnancy were twice as likely to be screen positive in subsequent pregnancies. Adjusting for previous screening results may reduce the risk of a recurrent false positive from 26.4% to 10.7% without significantly altering the DR (72.5% and 74% before and after the adjustment respectively). In conclusion, the risk estimation for the current pregnancy may be adjusted for the screening results from a previous pregnancy. This adjustment may significantly reduce recurrent FPR without compromising DR.

Genotype-phenotype correlation: a comparison between MPS II sibilings. *T.A. Vieira, I.V. Schawrtz, L.L.C. Pinto, M.V. Muñoz, A. Pires, R. Giugliani* Medical Genetics Service, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil.

PURPOSE: to compare clinical findings between MPS II affected brothers. METHODS: data was obtained from medical charts of four pairs of siblings attending at Medical Genetics Service-HCPA. RESULTS: Sibling pair A: mutation p.P231L, proband A1: 20 yrs and A2: 15 yrs, respectively presenting iduronate sulfatase activity (IDS) 2 nmoles/4h/m and undetectable, GAG urinary excretion: 1.56 and 4.1 fold normal upper limit(FNUL), FVC: 52.4% and 48.7% predicted normal, 6MWT: 540m and 480m. Cardiology findings: (A1) incomplete right bundle branch block, moderate aortic/mitral valve regurgitation and (A2) mild mitral/moderate aortic valve regurgitation. Both present normal intelligence. Sibling pair B: mutation A85T, proband B1: 18 yrs and B2: 14 yrs, respectively presenting IDS 6.2 and 2.4, GAG urinary excretion: 4.8 and 2.4 FNUL, FVC: 54.6% and 58.8% predicted normal, 6MWT: 427m and 404m. Cardiology findings: B1 incomplete right bundle branch block, mild aortic/mitral/tricuspid valve regurgitation and B2 mild mitral/aortic valve stenosis, left ventricle relaxing deficit and left atrium overload. Both siblings present normal intelligence. Sibling pair C: mutation c.708G-A, proband C1: 9 yrs and C2: 7 yrs, respectively presenting IDS 1.2 and 1.2, GAG urinary excretion: 4.4 and 2.8 FNUL, FVC: 62.7% and 46% predicted normal, 6MWT: 456m and 405m. Cardiology findings: C1 1st grade atrium-ventricle block, moderate aortic/ mild mitral valve regurgitation and C2 mild mitral/aortic valve regurgitation. IQ was 53 and 78. Sibling pair D: mutation unknown, proband D1: 14 yrs and D2: 10 yrs, respectively presenting IDS 10 and 5, GAG urinary excretion: 3.4 and 2.9 FNUL, FVC: 28.8% and 45% predicted normal, 6MWT: 412m and 475m. Cardiology findings: D1 mild aortic valve stenosis, mild mitral/aortic valve regurgitation and D2 moderate aotic and mild mitral valve regurgitation. IQ was 50 and 50. CONCLUSION: All pair of siblings (A-D) presented different phenotypes which can not be explained by genotype. Albeit, except for sibling pair C, such differences were not enough to alter neurological phenotype.

Skeletal Muscle Phenotype on Autopsy in Paternal Uniparental Isodisomy of Chromosome 14. *M.F. Wangler[1], E.M. McKay[2], M.B. Bhattacharjee[2], V.R. Sutton[3]* 1) Pediatrics, Baylor Col Medicine, Houston, TX; 2) Pathology, Baylor Col Medicine, Houston, TX; 3) Molecular and Human Genetics, Baylor Col Medicine, Houston TX.

Paternal uniparental disomy of chromosome 14 (upd(14)pat) produces a characteristic phenotype that includes axial skeletal anomalies, dysmorphic facial features, joint contractures and developmental delay. Although the condition is associated with a poor prognosis, no autopsies on children with this condition have been reported and no microscopic examination of skeletal muscle has been reported. We present a case of upd(14)pat in a Latin-american male who expired due to complications of sepsis and respiratory failure. At autopsy, a striking variability in muscle fiber size was noted on microscopic examination. Muscle phenotypes are present in the two animal models of upd(14)pat . In the ovine Callipyge model there is loin muscle hypertrophy due to a relative increase in number and size of fast-twitch glycolytic (type II) fibers and in the murine upd(12)pat, abnormally large fibers with centrally located nuclei are reported but there is no detailed histology published. This case suggests that in humans with upd(14)pat, as in the animal models, there is an imprinted region on chromosome 14 that is involved in skeletal muscle development during embryogenesis that results in abnormal fiber size and development. The hypotonia in upd(14)pat may be due, in part, to myopathy.

Data Quality Assessment of STR Fragment Analysis on the 3730xl DNA Analyzer. *M. Vogel, D.V. Walker, D.D. Einum, C.L. Mouritsen* Research & Development, Sorenson Genomics, Salt Lake City, UT.

Multiplex PCR analysis of short tandem repeats (STRs) for genetic discovery, clinical diagnosis and human identification applications is nearly ubiquitous in high throughput laboratories. The Applied Biosystems 3730xl DNA analyzer is a recently developed capillary electrophoresis platform designed to increase sensitivity, accuracy, precision and throughput compared its predecessor, the 3700. To optimize the use of the 3730xl for human identification applications, an internally validated multiplex PCR panel was utilized to examine multiple quality parameters of STR fragment detection. Direct sensitivity studies using a commercial sizing standard revealed that optimal fragment sizing quality occurs on the 3730xl with 40% less DNA than is required for optimal sizing on the 3700. Instrument accuracy was examined by processing samples with known genetic profiles on the 3730xl using a Y chromosome STR panel and high levels of concordance were obtained. Precision of the 3730xl was also assessed and reveals that the sizes of PCR fragments generated from the same templates processed on distinct instruments in independent runs deviate by an average of 0.07 base pairs, thus underscoring the high degree of data reproducibility exhibited by the 3730xl. Finally, the sample failure rate was 0.3% on the 3730xl and is 40-fold lower than that exhibited by the 3700. In summary, this study provides evidence that the 3730xl is a reliable detection platform for PCR fragment analysis in human identification applications. Together with the high throughput component (96 capillary capability), the significant data quality improvements of the 3730xl will position the instrument as a indispensable tool for the myriad disciplines that benefit from the power of genetic analysis.

Genome-wide mapping of susceptibility to epilepsy-related photosensitivity identifies a novel locus on chromosome 13q13. *D. Pinto[1], U. Tauer[2], S. Lorenz[3,4], H. Muhle[2], B.A. Neubauer[5], S. Waltz[6], K.P. Lenzen[3,4], G. Rudolf[7], G-J. de Haan[8], D. Lindhout[1], B.P. Koeleman[1], T. Sander[3,4], D.G. Kasteleijn-Nolst Trenité[1], U. Stephani[2]* 1) Complex Genetics Section, DBG -Dept. Medical Genetics, University Medical Center Utrecht, Utrecht, The Netherlands; 2) Clinic for Neuropediatrics, Kiel Univer sity, Germany; 3) Gene Mapping Center, Max -Delbrück-Center, Berlin, Germany; 4) Dept. Neurology, Charité University Medicine, Berlin, Germany; 5) Clinic for Neuropediatrics, University Giessen, Germany; 6) Childrens Hospital, Cologne, Germany; 7) Strasbourg University, Strasbourg, France; 8) Stichting Epilepsie Instellingen Nederland, Heemstede, The Netherlands.

Photosensitivity or photoparoxysmal response (PPR) is an abnormal cortical response to visual stimuli that can be evoked by standardized intermittent photic stimulation. It is a highly heritable EEG trait, which occurs frequently associated with IGEs. Given its strong association with IGEs, PPR is a potential endophenotype for IGEs. Recently two genome-wide linkage scans on PPR identified 4 susceptibility loci at 7q32, 16p13 and 6p21, 13q31, varying on the familial background of epilepsy syndromes. In this study, we searched for risk factors that are independent of the familial epilepsy background by pooling genotype data from all 75 multiplex PPR families and performing a combined nonparametric linkage (NPL) analysis. To account for genetic heterogeneity, family members were classified under a broad (all PPR types I-IV) and a narrow (only PPR types III-IV) trait definition. We found evidence for a novel, shared locus on 13q13.2 at D13S1493 under broad ($P_{NPL} = 3.64x10^{-4}$) and narrow ($P_{NPL} = 6.81x10^{-5}$) trait definitions, achieving empirical genome-wide significance for the narrow model ($P_{gw} = 0.0132$). Accentuated linkage under the narrow trait definition (generalized PPRs) suggests an increase in phenotypic homogeneity, underscoring the close genetic relationship between generalized PPRs (types III-IV) and IGEs. Our findings suggest that the locus at 13q13.2 represents a common genetic component for visually-induced and spontaneously occurring generalized spike-and-wave EEG discharges, underlying generalized PPR in IGE patients.

Genome-wide detection of human copy number variations using high density DNA oligonuceotide arrays. *M.H. Shapero[1], F. Shen[1], W. Chen[1], V. Truong[1], K.R. Fitch[1], D. Komura[2], S. Ishikawa[2], J. Zhang[1], G. Liu[1], H. Aburatani[2], K.W. Jones[1], J. Huang[1]* 1) Genotyping Research, Affymetrix, Inc. Santa Clara, CA; 2) Genome Science Division, Research Center for Advanced Science and Technology, University of Tokyo.

DNA sequence diversity within the human genome may be more greatly affected by copy number variations (CNVs) than single nucleotide polymorphisms (SNPs). Although there are increasingly clear examples of how CNVs can influence , for example, drug responses and disease susceptibility, a global biological understanding of the roles of CNVs is not yet currently available. We have previously reported a comprehensive view of CNVs among 270 HapMap samples using high density (500K) SNP genotyping arrays which revealed greater than 1,000 CNVs spanning a broad size range from less than 1kb to over 3Mb. In order to improve probe coverage for genome-wide CNV identification, especially in regions such as segmental duplications that are rich in CNVs, an array has been synthesized which uncouples copy number detection from SNP genotyping. A probe selection algorithm has been used to design 8 independent non-polymorphic probes for each *in silico* predicted Nsp I restriction fragment in the 200-1000bp size range. This Nsp I fragment array, in conjunction with PCR-based, complexity-reduced DNA target, provides high resolution, querying 1,330,354 restriction fragments, which translates to a 3.24 fold increase in marker density as compared to the 500K arrays. The median inter-marker distance is reduced from 2,709bp to 776bp and has allowed the continuing identification of CNVs in the human genome. QPCR has been used as an independent method to verify array-based CNV calls. Initial results suggest that non-SNP-based arrays can detect both simple and complex CNVs in the HapMap set. The increasing precision of CNV boundary delineation should allow a more complete analysis of their genomic organization.

***LRRK2* G2019S in Families with Parkinson's Disease Originating from Europe and the Middle East: Evidence for Two Distinct Founding Events Beginning Two Millennia Ago.** *C.P. Zabetian[1,2], C.M. Hutter[3], D. Yearout[1,2], A.N. Lopez[1,2], S.A. Factor[4], A. Griffith[5], B.C. Leis[5], T.D. Bird[1,2], J.G. Nutt[6], D.S. Higgins[4], J.W. Roberts[7], D.M. Kay[8], K.L. Edwards[3], A. Samii[2,9], H. Payami[8]* 1) GRECC, VA Puget Sound HCS, Seattle, WA; 2) Dept of Neurology, Univ of Washington, Seattle, WA; 3) Dept of Epidemiology, Univ of Washington, Seattle, WA; 4) Parkinsons Disease & Movement Disorder Clinic, Albany Medical Center, Albany, NY; 5) Booth Gardner Parkinsons Care Center, Evergreen Hospital, Kirkland, WA; 6) Dept of Neurology, Oregon Health & Science University, Portland, OR; 7) Virginia Mason Medical Center, Seattle, WA; 8) Genomics Institute, Wadsworth Center, NY State Dept of Health, Albany, NY; 9) PADRECC, VA Puget Sound HCS, Seattle, WA.

The Leucine-Rich Repeat Kinase 2 (*LRRK2*) G2019S mutation is the most common genetic determinant of Parkinson's disease (PD) identified to date. It accounts for approximately 1-7% of PD in patients of European origin, and has a remarkably high prevalence (20-40%) in Ashkenazi Jews and North African Arabs with PD. Previous studies concluded that families from these populations shared a common founder who lived in the 13th century. We sought to test this hypothesis by performing a haplotype analysis of 22 families (13 of European and nine of Jewish ancestry) with G2019S recruited from movement disorder clinics in North America. We genotyped 25 markers (12 SNPs, 13 microsatellites) that spanned 9 Mb across the *PARK8* region and used the program PHASE to reconstruct haplotypes. We discovered two distinct haplotypes in our sample. Haplotype 1 was present in 19 families of Jewish and European ancestry and was consistent with that reported in the literature. Haplotype 2 occurred in three European-American families and spanned a minimum distance of 6 Mb. Using a maximum likelihood method we estimated that the families with haplotype 1 shared a common ancestor approximately 2,000 years ago, while those with haplotype 2 shared one more recently. Our data suggest two separate founding events for G2019S in these populations, beginning at a time far earlier than the 13th century which coincides with the end of the Jewish diasporas.

Use of Array-CGH for Analysis of Patients with a Laboratory Referral Diagnosis of Autism Spectrum Disorder.
T. Sahoo, W. Wells, S.W. Cheung, A. Patel, A.L. Beaudet Dept Human & Molec Gen, Baylor Col Medicine, Houston, TX.

Children with autism and related diagnoses are often found to have obvious cytogenetic abnormalities such as sex chromosome aneuploidy or isodicentric (15)(q11.2). In addition, subtle cytogenetic abnormalities are present in a small percentage of cases including dup(15)(q11q13) and a variety of telomeric deletions, particularly 22q. Array comparative genomic hybridization (CGH) provides a new opportunity to diagnose these more subtle abnormalities with greater sensitivity, particularly for submicroscopic duplications such as 15q11q13 which would not be detected even by metaphase FISH. The Cytogenetic Laboratory at Baylor College of Medicine has offered array-CGH as a clinical service since Feb. 2004, with 4065 samples analyzed through May 2006. Of these, 254 samples were received with a sole diagnosis of autism and 44 with a complex diagnosis including autism or autistic features. This referral information was based solely on the information provided with submission of the sample. Normal array-CGH results were obtained for 254 of the 304 cases. There were 12 definite abnormalities including four cases with a previous abnormal kayotype [two 47,XXY, one dup(21)(q), one del(7)p14.1)], and all were confirmed by array-CGH. Of the 8 cases with previous normal karyotype, there were 3 males with duplication of the RETT gene at Xq28 (Mat pending), one del(10)(p12p14) (parents pending), one de novo dup(17)(p13.3), one de novo del(22)(q13.33), one dup15q11q13 of maternal origin, and one del(9)(q34.3). There were 38 autistic cases with copy number variation, most of which were benign familial variants, and some still awaiting parental studies. Other findings of interest in which autism was not mentioned in the referral included one case with previously identified marker chromosome that proved to be isodicentric 15q, two cases of dup(15)(q11q13) (parents pending), 18 cases with duplications and 12 cases with deletions of the breakpoint 1-breakpoint 2 interval of the PWS-AS critical region at 15q11.2, three del(22)(q13.33), and nine cases (5 female and 4 male) with gain of the STS region at Xp22.32; all of which were familial in cases where parents were analyzed.

The effect of heating rate on DNA duplex melting: implications for mutation scanning and real-time PCR. *O. ELENITOBA-JOHNSON[1], R. M. WATSON[2], C. T. WITTWER[1]* 1) PATHOLOGY, UNIVERSITY OF UTAH SCHOOL OF MEDICINE, SALT LAKE CITY, UT; 2) ROCHE MOLECULAR SYSTEMS INC ALAMEDA, CA.

High-resolution DNA melting analysis is a powerful tool for genotyping and mutation scanning. The effect of heating rate on melting curve results is unclear. Historically, high quality melting data was obtained using absorbance at rates of 0.1 - 1C/min. Today, it is more common to monitor melting with fluorescence in conjunction with real-time PCR at rates of 0.1 - 1.0 C/s. Prior work suggests that melting temperatures (Tms) of PCR products increase by up to 1-3C as the heating rate increases. A high-resolution melting instrument (HR-1, Idaho Technology) and a custom prototype fluorimeter were used to study the effect of heating rate on Tm and melting curve shape. Rates between 0.01 and 1.0C/s were examined using PCR products and synthetic oligo duplexes in the presence of the dsDNA dye, LCGreen 3 (Idaho Technology). The custom fluorimeter replaced the HR-1 optics with a custom bifurcated fiber optic cable (04-08719, RoMack), a wavelength variable monochromator (DeltaRam, PTI), an emission spectrograph (FCS 77443, Oriel) and a CCD camera (DV 420-OE, Andor). A J-type thermocouple (TT-J-40-50, Omega), was inserted into each 10 ul sample for temperature monitoring. Using both the HR-1 and custom fluorimeter, the Tms of homozygous PCR products varied less than 0.2C over the heating rate range of 0.01 - 1.0C/s. The melting curves showed changes in shape over this range with apparent broadening of the transition at higher rates, possibly due to internal sample temperature heterogeneity. Results with amplified heterozygotes depended on duplex size and melting rate. Heteroduplexes were best observed at faster rates, particularly with short amplicons. Melting rates between 0.01 and 1.0C/s do not affect the Tm of homoduplexes determined by fluorescent melting analysis and results from amplified heterozygotes suggest that melting analysis for mutation scanning is best performed at higher melting rates. Still, melting analysis during each cycle of real-time PCR should be possible given adequate instrument resolution and accurate temperature monitoring of the sample.

Discovery of novel recurrent genomic disorders from the duplication architecture of the human genome. *A.J. Sharp[1], S. Hansen[1], R.R. Selzer[2], Z. Cheng[1,7], R. Regan[3], J.A. Hurst[4], C.A. Fitzpatrick[5], T.A. Richmond[2], D. Pinkel[6], P.S. Eis[2], S. Schwartz[5], S.L. Knight[3], E.E. Eichler[1,7]* 1) Genome Sciences, Univ. of Washington, Seattle, WA; 2) NimbleGen Systems Inc, Madison, WI; 3) Wellcome Trust Centre for Human Genetics, Oxford, UK; 4) Dept of Clinical Genetics, Churchill Hospital, Oxford, UK; 5) Dept of Human Genetics, Univ. of Chicago, IL; 6) Comprehensive Cancer Center, UCSF; 7) Howard Hughes Medical Institute.

Genomic disorders are characterized by large, highly homologous flanking segmental duplications that predispose these regions to recurrent rearrangement. Based on the duplication architecture of the genome, we mapped 130 intervals that we hypothesized as candidate sites for novel genomic disorders. We tested 330 patients with mental retardation and congenital abnormalities using a custom BAC array targeted to these sites (Sharp 2005, Am J Hum Genet 77:78). We identified 19 pathogenic rearrangements that were never observed in 316 controls. These included six patients with an identical *de novo* microdeletion of 17q21.31, identifying this as a novel site of common recurrent rearrangement. Two of these were ascertained on the basis of a similar phenotype to the first four cases. We used high-density oligonucleotide arrays to refine the breakpoints of this microdeletion, identifying multiple copy number polymorphisms in this region and defining a 478kb critical region containing six genes that was deleted in all six cases, but invariant in controls. Breakpoints were mapped to flanking paired segmental duplications that likely mediate this rearrangement. We also characterized five other pathogenic rearrangements using oligo arrays. In four of these, the breakpoints also localized to large clusters of flanking segmental duplications, strongly suggesting that these are also sites of recurrent rearrangement. In common with the 17q21.31 deletion, we observed that in each case the breakpoint regions are also sites of copy number polymorphism in controls, suggesting that these may be inherently unstable genomic regions prone to chromosomal breakage. Our strategy based on genomic architecture represents a powerful approach for the identification of novel genomic disorders.

Newborn Screening Programs in Asia - Program Implementation and Strategic Policy Formulation. *C. Padilla*
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Newborn Screening is a well recognized public health program aimed at the early identification of infants who are affected by certain genetic/metabolic/infectious conditions. Early identification of these conditions is particularly crucial, as timely intervention can lead to a significant reduction of morbidity, mortality, and associated disabilities in affected infants. The establishment of newborn screening programs in developing countries poses major challenges as it competes with other health priorities like control of infectious diseases, immunization, malnutrition, etc. Despite this, it is imperative that developing countries recognize the importance of newborn screening as it has been proven through decades of experience, that it can save thousands of babies from mental retardation, death and other complications. Some of critical factors necessary for the success of a national newborn screening program are 1) inclusion of newborn screening among the government priorities; 2) funding of newborn screening fees; 3) general public acceptance; 4) cooperation of the health practitioners; 5) government participation in institutionalizing the newborn screening system. This paper will present 1) highlights of the newborn screening programs (statistics, disorders, problems, strategies etc) and 2) policies that supported the implementation of newborn screening in both developed and developing countries. Data from the following countries will be presented -Australia, Bangla Desh, China, Indonesia Japan, Korea, Malaysia, Mongolia, New Zealand, Philippines, Singapore, Thailand , Vietnam.

The Familial Mediterranean Fever (FMF) gene (MEFV) as a disease susceptibility gene in Inflammatory Bowel Disease (IBD). *A.C. Villani[1], M. Lemire[2], E. Louis[3], M. Silverberg[4], Y. Renaud[2], S. Brunet[2], C. Collette[1], G. Fortin[1], C. Libioulle[3], A. Bitton[1], D. Gaudet[5], A. Cohen[1], D. Langelier[6], J. Rioux[7], P. Rutgeerts[8], G. Wild[1], S. Vermeire[8], T.J. Hudson[2], D. Franchimont[1]* 1) McGill University Health Center, Canada; 2) McGill University & Genome Quebec Innovation Centre, Canada; 3) CHU of Liège, Belgium; 4) Mount Sinai Hospital IBD Center, Canada; 5) Centre Hospitalier de la Sagamie, Canada; 6) Centre Hospitalier de Sherbrooke, Canada; 7) University of Montreal, Canada; 8) KUL, Belgium.

FMF is a hereditary auto-inflammatory disease caused by mutations in the MEFV gene. Since epidemiological studies revealed a higher prevalence of IBD in FMF non Ashkenazi Jewish patients, we evaluated MEFV as a susceptibility gene for IBD. **Methods**: DNA samples were obtained from 5 centers and divided into 3 cohorts: Belgian (Liege 190 IBD trios;Leuven 435 IBD trios), Canadian (Quebec 125 IBD trios;Toronto 190 IBD trios), Edinburgh (521UC,300 controls). The haplotype structure and fine-mapping of MEFV was done by genotyping 65 SNPs over 96.5kb using SNPstream UHT, FP-TDI and sequencing technologies. Standard measures of LD were estimated using EM algorithm, and association testing was done using TRANSMIT and Chi-Square test. **Results**: Association analysis revealed that haplotype A,covering the MEFV promoter to intron 2 and its 5 flanking region,was positively overtransmitted in both the Belgian ($p<0.03$),Canadian ($p<0.018$),and combined UC cohorts ($p<0.0012$). Although present,Haplotype A was not found to be significant in the Edinburgh cohort. Haplotype B,also covering the same region, was found to be positively overtransmitted in the Belgian ($p<0.27$), Canadian ($p<0.0031$), and combined CD cohorts ($p<0.005$). All exons and promoter regions of genes encompassed by haplotypes A and B were sequenced and the 6 observed coding variants were genotyped in the 3 cohorts. No coding variants could explain the observed associated haplotypes A and B. **Conclusion**: MEFV appears to be a disease susceptibility gene for IBD. Interestingly, this study suggests that a gene associated with a rare inflammatory disorder could be implicated in the susceptibility of more common inflammatory diseases, such as IBD.

Identification of a variant RAR gene rearrangement in a patient with acute promyelocytic leukemia. *B.M. Shearer[1], J.G. Keefe[1], C. Rubin de Celis[2], A. Vendrell[2], R.F. McClure[1], E.C. Thorland[1], R.P. Ketterling[1]* 1) Dept. of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN; 2) Brackenridge Hospital, Austin, TX.

Acute promyelocytic leukemia (APL) comprises approximately 10% of all acute myelogenous leukemias (AML). The hallmark genetic aberration is a translocation between chromosomes 15 and 17 resulting in the gene fusion of PML (promyelocytic leukemia) at 15q22 and RAR (retinoic acid receptor alpha) at 17q21 in approximately 95% of cases. Infrequently, RAR gene fusion with variant gene partners has been observed in APL. Variant gene partners include PLZF at 11q23, NuMA at 11q13, NPM at 5q35, and Stat5b at 17q21.2. Treatment with all-trans retinoic acid (ATRA) induces a transient complete remission and when consolidated with conventional chemotherapy produces a cure in 70-90% of patients. Herein, we describe a 42 year old male presenting with AML whose bone marrow contained nearly 100% promyelocytes with secondary granules and Auer rods. Chromosome analysis revealed a 46,XY karyotype and both RT-PCR and D-FISH failed to demonstrate PML-RAR fusion. However, interphase FISH analysis demonstrated a small split of the RAR probe, which was found to co-localize on the abnormal chromosome 17 by metaphase FISH. Break-apart FISH for RAR revealed a deletion of the centromeric portion of the probe. In combination, these results suggest a cryptic rearrangement on chromosome 17q, which may represent an inversion/deletion mechanism resulting in aberrant expression of RAR with a new partner. A candidate gene of interest includes Stat5b for which a sole APL patient demonstrated Stat5B-RAR fusion due to an interstitial deletion of 17q. However, since subsequent ATRA treatment of our patient induced remission and the previously described Stat5b-RAR patient was refractory to ATRA, a novel gene may be the target of RAR upregulation in our patient. Additional studies to identify the partner gene have been initiated. Detailed genetic results for this patient, a review of the literature, and a discussion of the pathogenetic model for this variant RAR gene rearrangement will be presented.

Genomic convergence to prioritize candidate genes for primary open-angle glaucoma. *V. Raymond[1], N. Boivin[1], P. Belleau[1], E. Deilhes[1], A. Marquis[1], R. Arseneault[1], J. St-Amand[2], E. Calvo[2], M.A. Rodrigue[1], J.L. Anctil[3]* 1) Ocular Genetics & Genomics, CREMO, Laval University Hospital (CHUL) Res Ctr, Québec City, PQ, Canada; 2) Endocrinology & Oncology (CREMO), CHUL Res Ctr; 3) Ophthalmology dept, St-Sacrement Hosp, Québec City.

Eleven *GLC1* loci have been mapped for primary open-angle glaucoma (POAG), but only 3 genes have been characterized: *TIGR/myocilin*, *OPTN* and *WDR36*. Five French-Canadian families displayed haplotypes compatible with segregation of a disease gene at three *GLC1* loci. However, the relatively small sizes of these families hampered confinement of the loci. We exploited genomic convergence, a multistep approach that combines gene expression analysis with genetic linkage, to prioritize candidate genes for POAG within these loci. Serial analysis of gene expression (SAGE) and Affymetrix microarrays (HG U133 plus 2.0) were used to probe transcripts in the retina and ciliary body. Tissues were obtained from 1 asymptomatic 67 year old male, 1 asymptomatic 75 year old female and 1 POAG female (76 year old). 21,025 species of SAGE tags were detected in a total of 46,096 sequenced tags from the retina. Specific tags, detected at least 5 times, corresponded to 784 transcripts of which 9 mapped (7 genes) within 1 of the 8 *GLC1* loci encoding a POAG gene not yet characterized. In our microarrays experiments, 684 and 421 transcripts were differentially expressed, respectively, in the retina and ciliary body of our patient as compared to controls. The transcripts that mapped to 1 of the 8 *GLC1* loci, for which a POAG gene remains to be identified, corresponded to 30 genes. Five of these genes localized within *GLC1B*, 3 within *GLC1C* and 2 within *GLC1D*. None of the 7 genes identified by SAGE corresponded to the 30 characterized by microarrays. The most interesting genes were *REEP1* (*GLC1B*), *TFDP2*, *ZBTB38* (*GLC1C*) and *ENY2* (*GLC1D*). In conclusion, our transcriptomic analyses of the retina and ciliary body revealed 37 genes that mapped within *GLC1* loci encoding unknown glaucoma genes. Twelve of these genes mapped at *GLC1B, 1C or 1D* and therefore represent primary candidate genes to be investigated in our families.

Noonan's syndrome children with valvular pulmonary stenosis and left hypertrophic cardiomyopathy: clinical assessment and cytogenetic analysis. *I. Trabelsi[1], S. Kammoun[1], M. Meddeb[2], T. Rebai[3], N.B. Abdelmoula[3]* 1) Cardiology department, Hedi Chaker Hospital, Sfax, Tunisia; 2) Genetics Laboratory, Tunis, Tunisia; 3) Histology Laboratory, University of Medicine, Sfax, Tunisia.

Noonan's syndrome is an autosomal dominant dysmorphic syndrome in characterized by craniofacial anomalies with hypertelorism, a downward eyeslant and webbing of the neck; as well as skeletal deformities such as short stature and other organ anomalies, mainly cardiac valve disease. Diagnosis of Noonan's syndrome still remains on its clinical features but mutations in PTPN11 gene which maps to 12q24.1 have been reported in approximately 40 percent of patients. As part of our screening project of genetic abnormalities in congenital heart defects, a prospective study about valvular pulmonary stenosis is conducted. We report the first part of this study in which we describe clinical features of nine children (four males and five females) in whom Noonan's syndrome is suspected. In three of these nine children, mother or father, seem to be affected with cardiac murmura and, or facial dysmorphy. Two of them are a twin. All children have a normal karyotype. All have valvular pulmonary stenosis associated with left hypertrophic cardiomyopathy.

The human single-strand DNA binding protein RPA rapidly associates with DNA breaks *in vivo*. *I. Pasic*[1, 2], *M.S. Meyn*[1, 2] 1) Genetics & Genomic Biology, Hosp for Sick Children; 2) Molecular & Medical Genetics, Univ of Toronto, Toronto, ON.

RPA is a single-strand DNA-binding protein involved in DNA metabolism. In response to DNA double-strand breaks (DSBs), its RPA2 subunit is phosphorylated by the damage response kinase ATM. While phosphorylation alters RPA's interactions with replication and repair factors, it is unclear how it affects interactions between RPA and DSBs. We therefore studied the effect of ATM deficiency on recruitment of RPA2 to photo-induced DSBs in human fibroblasts.

Endogenous RPA2 is detectable at DSBs by 30s and remains localized to DSBs 150s post-irradiation. RPA2 phosphorylated at T21 (T21~P) is also present at DSBs 30s post-irradiation, but by 90s is diffusely distributed throughout the nuclei of irradiated cells. This suggests that unphosphorylated RPA2 rapidly associates with DSBs, where it is then phosphorylated, thereby promoting RPA dissociation from DSBs. In support of this notion, the association of a T21DS33D phosphomimetic GFP-RPA2 mutant with induced DSBs is impaired and a T21DS23DS33D phosphomimetic GFP-RPA2 mutant associates with DSBs very poorly.

To further examine the negative role for phosphorylation in control of RPA's association with DSBs, we studied the association of RPA2 with DSBs in ATM-deficient cells. While RPA2 is seen at DSBs in ATM-deficient cells 30s post-irradiation, RPA2-T21~P is not detectable, suggesting DSB-induced phosphorylation of RPA2 at T21 is ATM-dependent, but T21 phosphorylation is unnecessary for RPA2s association with DSBs. Interestingly, in the absence of ATM, GFP-RPA2 associates with DSBs more slowly than in wild type cells. This may reflect a defect in an early processing step needed for RPA2 to associate with DSBs rather than lack of RPA2 phosphorylation. Such a step may involve the MRN complex, which promotes nucleolytic processing of DSBs. In support for this idea, we find that the association of GFP-RPA2 to DSBs in NBS1-deficient cells is severely impaired. Our results suggest that the association of RPA with DSBs is rapid and MRN-dependent; enhanced by ATM-dependent activation of MRN; and decreased by phosphorylation of RPA2, which likely occurs at DSB sites.

Bruck Syndrome Seen in Consecutive Siblings. *A..M. Zoumberakis[1], D.L. Broome[1], R.S. Lachman[2], R.W. Hassan[1]* 1) Dept Genetics, Kaiser Permanente, Downey, CA; 2) Cedars-Sinai Medical Center Los Angeles, CA.

Genetic counseling was done for a family who had two consecutive infants with multiple anomalies. The first child was born at 36 weeks by cesarean section for breech presentation with oligohydramnios. At birth, multiple anomalies were seen including contractures of hands and shoulders, microcephaly, hip dislocation, atrial and ventricular septal defects, small pulmonary artery and facial anomalies. The baby died 2 hours post delivery. Autopsy report revealed lung hypoplasia, with abnormal rotation of the forearms and hands, cauliflower appearing ears and lower limb deformities as well as the above findings. Chromosome studies were normal. The mother presented to us for genetic counseling during her second pregnancy. A targeted ultrasound and fetal echocardiogram were performed and reported as normal. The baby was born at 34 weeks by repeat cesarean section for breech presentation with intrauterine growth retardation and oligohydramnios. The baby was noted to have similar anomalies as in the previous infant. Additional findings included multiple bone fractures, 11 ribs and joint ankylosis. The baby died at 2 months of age due to respiratory distress. X-ray films were sent to the International Skeletal Dysplasia Registry through Cedars-Sinai Medical Center. Findings included very severe generalized osteoporosis, very thin long bones, thin ribs and dentinogenesis imperfecta. The diagnosis was consistent with Bruck syndrome. Bruck syndrome is a rare, autosomal recessive syndrome that consists of a combination of arthrogryposis multiplex congenita and osteogenesis imperfecta (OI). It is a progressive disease that leads to severe limb deformities, multiple fractures and short stature. Therefore, it is imperative to obtain a radiological assessment to rule out a possible underlying diagnosis of OI when arthrogryposis is suspected.

Gene Expression Differences between Patients and Controls in Type 1 Diabetes weakly correlates with Gene Divergence Rate. *A. Solidar[1], K. Kover[2], S. Svojanovsky[3], W.V. Moore[2], G.J. Wyckoff[1]* 1) Div Molec Biol & Biochemistry, Univ Missouri, Kansas City, Kansas City, MO; 2) Childrens Mercy Hospital & Clinics Kansas City, MO; 3) 2University of Kansas Medical Center Kansas City, KS.

Type 1 Diabetes is a multigenic disorder with a high prevalence, and identifying and characterizing genes and their products associated with Type 1 diabetes is a major goal of the Kansas City Diabetes Consortium. We are also interested in understand the large scale trends surrounding genes involved with this disease. Using microarray technology we have generated a gene expression profile of patients with new onset Type 1 diabetes. Peripheral blood was collected from 4 females and 4 males (7 -14 yrs old) upon diagnosis of Type 1 diabetes. Our analysis shows that of the 22,277 genes probed 6341 genes were upregulated and 5558 were down regulated in males with Type 1 diabetes compare to control males while 5415 genes were upregulated and 6740 were down regulated in females with Type 1 diabetes compared to female controls. The intersection showed 2627 genes were upregulated and 2921 were down regulated; upregulated genes include interferon induced genes, immunoglobulin light chain , MHC Class II DRB3, integrin beta 3 while down regulated genes include cell division related genes, homeobox protein NKX3, cathepsin W, and granzyme B. Several significantly differentially regulated genes show signs of positive selection. We analyzed the divergence of these proteins, looking at evolutionary rate measures (Ka/Ks) between humans and rodents to determine if differences in expression were correlated with differences in evolutionary rate. We found a small but significant ($r^2=0.04$, p-value < 0.05) correlation between expression difference and Ka/Ks, with genes having a higher expression difference more likely to have a high Ka/Ks between species. Recent studies suggested that evolutionary rate correlates with disease involvement (Kondrashov; Smith and Eyre-Walker). We suggest that the evolutionary plasiticity of a gene, measured in terms of scaled protein change rate, may correlate with disease involvement, and may have utility in picking candidates for further study in multigenic diseases.

Brain Imaging As A Strategy for Genome Wide Scan Data Reduction for Neuropsychiatric Illness. *S. G. Potkin[1], F. Macciardi[2], L. Friedman[1], J. Turner[1], J.H. Fallon[3], W. Bunney[1], D. Keator[1], D. Goldstein[4], N. Schork[5]* 1) Psychiatry and Human Behavior, UCI, Irvine, CA; 2) Dip. di Scienze e Tecnologie Biomediche, Università degli Studi di Milano, Milan, Italy; 3) Anatomy and Neurobiology, UCI, Irvine, CA; 4) Center for Population Genomics and Pharmacogenetics, Duke University, Durham, NC; 5) Psychiatry, UCSD, San Diego, CA.

Genetic association studies look for genetic contributions related to diagnostic status. These are inevitably small effects for complex disorders and require unrealistic sample sizes. The integration of intermediate phenotypes in imaging genetics holds promise for finding more specific and larger genotypic effects. This integrative approach incorporates 500+K SNPs and 20+K imaging voxels but creates additional statistical concerns. We utilize an ordered analysis incorporating candidate genes and genomewide strategies, a novel extension of a method for identifying significant results, and the use of a number of replication/validation strategies. Initially, imaging phenotypes are defined from the fMRI activation patterns that distinguish schizophrenic from healthy controls. The effects of genotype and diagnosis on the imaging phenotype is then analyzed. This analysis is not an association of genotype with diagnosis, but a combined general linear model (GLM) analysis of the effects of genotype, diagnosis, and their interaction, on the quantitative trait of the imaging phenotype. Initial attention in the ordered analysis is focused on a priori selected candidate genes, and followed with a genome-wide analysis, with appropriate significance adjustments for each level of analysis. Replication of results in new samples is essential. This approach combines the advantages of both the genome-wide scans and the candidate gene approach and addresses the concern of false positives in combining imaging and genome-wide scan approaches. Given the known importance of genetics in brain function, and the role of neuroimaging in revealing brain dysfunction, the integration of genetics with brain imaging can enhance our fundamental understanding of disease.

The Founder Contribution to the Old Order Amish of Lancaster County. *T.I. Pollin[1], R. Agarwala[2], A.A. Schäffer[2], A.R. Shuldiner[1,3], B.D. Mitchell[1], J.R. O'Connell[1]* 1) University of Maryland, Baltimore, MD; 2) NCBI, NIH, DHHS, Bethesda, MD; 3) Baltimore VA Medical Center, Baltimore, MD.

We previously confirmed the accuracy and completeness of the genealogical records of the Old Order Amish (OOA) of Lancaster County by analyzing the transmission of Y chromosome short tandem repeat genotypes through male lineages. We now provide a comprehensive analysis of the founder structure and the founders' genetic contribution to the 3,615 individuals (approximately 10% of the Lancaster OOA) who are currently enrolled in our ongoing studies of diabetes, osteoporosis, cardiovascular disease and other conditions at the University of Maryland. Using the Anabaptist Genealogy Database, we constructed a 14 generation pedigree containing 7,499 individuals that connected our 3,615 study subjects through all common paths to their common ancestors. All of these individuals were descended from a total of 275 founders (106 men and 169 women). We measured the average expected genetic contribution of each of the 275 founders to each of the 3,615 descendants and found that 76 founders (31 men and 45 women) accounted for over 95% of the average founder contribution. Each of these 76 founders was a direct ascendant of between 78 and 3598 study participants, with the average expected contribution ranging from 0.2% to 7.8%. All 76 were born in 1867 or earlier, and 69 were born prior to 1800. Only 75 founders were estimated to be born in 1800 or later, and these accounted for only 5% of the founder contribution; the 20 founders born in 1900 or later accounted for 0.5% of the founder contribution and were generally not active members of the Amish Church. The number of female founders (169 total, 45 of the top 76) may be an overestimate due to the difficulty of tracking relationships between women with different married surnames. Further genealogical studies and mitochondrial studies, currently in progress, may reduce the estimate of the number of female founders. These data quantify the extent to which the OOA are a closed founder population ideal for elucidating the role of genetic variation in complex diseases.

CHARACTERIZATION OF AORTIC DISEASE CAUSED BY MYH11 MUTATIONS. *H. Pannu[1], V. Tran-Fadulu[1], S. Scherer[2], R. Gibbs[2], D. Divecha[1], C. Papke[1], Y. Liu[1], S. Duraisamy[1], D. Guo[1], A. Estrera[1], H. Safi[1], A.J. Marian[2], M. Buja[1], D.M. Milewicz[1]* 1) The Univ of Texas Medical Sch, Houston, TX; 2) Baylor College of Medicine, Houston, TX.

Mutations in the smooth muscle myosin heavy chain gene, *MYH11*, have been reported in two families with thoracic aortic aneurysms/dissections (TAAD) and patent ductus arteriosus (PDA). To characterize *MYH11* mutations in familial TAAD, *MYH11* was sequenced in 96 unrelated TAAD probands, including 3 families with TAAD and PDA. Missense mutations in the ATPase head region and the C-terminal coiled-coil domain of MYH11 were identified in 2 families with TAAD/PDA (R712Q, L1264P); no disease causing variants were identified in the other 94 families. Aortic aneurysms in these families involved the ascending aorta distal to the sinuses of Valsalva, unlike aneurysms due to *FBN1*, *TGFBR1*, and *TGFBR2* mutations. Pathologic examination of an aneurysm due to a *MYH11* mutation (no dissection) showed typical loss of elastic fibers and increased proteoglycans. Atypical for TAAs was the observation that smooth muscle cells (SMCs) in the media were in disarray, with cells oriented at oblique angles to each other, similar to cardiac pathology observed for hypertrophic cardiomyopathy (HCM) due to cardiac myosin heavy chain mutations (*MYH7*). Unlike HCM, the SMCs in the mutant aorta were not hypertrophied as assessed by merosin staining. Another atypical finding was that aneurysm was highly vascularized with the vasa vasorum increased in the adventitia and penetrating into the media. Inflammatory CD68+ and CD3+ cells were observed in the initima, adventitia, and periadventitial media. Cytokine/chemokine analysis of aneurysm tissue by organ culture using the Luminex cytokine 25-Plex kit revealed a 5-8 fold increase in macrophage inflammatory proteins (MIP and), confirming inflammation. Increased staining of nuclear phospho-Smad in aneurysm versus control aortas indicated the TGF- pathway was activated. These findings suggest that *MYH11* mutations alter similar molecular pathways as *MYH7* mutations in cardiac tissue in HCM, including disarray of SMCs, increased angiogenesis, inflammation, and TGF- activation.

Haplotype variation and linkage disequilibrium within 36 genomic regions in two Indian language groups. *P.I. Patel[1], T.J. Pemberton[1], J.D. Wall[2], J.K. Pritchard[3], N.A. Rosenberg[4]* 1) Institute for Genetic Medicine and; 2) Department of Biological Sciences, University of Southern California, Los Angeles, CA; 3) Department of Human Genetics, University of Chicago, Chicago, IL; 4) Department of Human Genetics, University of Michigan, Ann Arbor, MI.

We have recently studied variation at 1200 polymorphic loci (729 microsatellite and 471 indel polymorphisms) in 432 individuals from 15 language groups across India. STRUCTURE analysis of these data revealed (i) Indian populations constitute a distinct cluster when compared to 52 other worldwide populations and (ii) despite the vast geographical region surveyed, there was very little genetic differentiation among the Indian language groups. A detailed analysis of haplotype structure and linkage disequilibrium within Indian populations would be useful in guiding the design of disease association studies. Together with similar information from other worldwide populations, such an analysis would also be valuable in the detection of natural selection as well as in inference of the history of migrations to and from India. Towards this goal, we have obtained genotypes at 3024 SNPs in 30 unrelated individuals who were part of the initial genome-wide survey - 15 whose grandparents were Bengali (from W. Bengal) and 15 Tamilians (from Tamil Nadu). The SNPs are spaced across 36 genomic regions, including 32 autosomal regions and 4 regions on the non-pseudoautosomal X chromosome, covering a total of ~12 megabases. To facilitate inferences about both fine-scale and long-range LD, each genomic region contains a central high-density core of 60 SNPs at 1.5 kb average spacing, as well as additional SNPs at lower density outside the core region, extending 120 kb in each direction at 10kb spacing. Hence each region spans a total of ~330 kb. To maximize the degree to which the regions were representative of the human genome, they have been chosen across the range of local gene densities and meiotic recombination rates. Analysis of these data is in progress and will include a comparison to genotypes of the same SNP set in 927 unrelated individuals from 52 populations in the HGDP-CEPH Human Genome Diversity Cell Line Panel.

microRNA Variants in patients with neuropsychiatric disorders. *J. Yan[1], J. Feng[1], K. Noltner[1], C. Katz[1], R. Schroer[2], C. Skinner[2], C.E. Schwartz[2], S.S. Sommer[1]* 1) Dept Molecular Genetics, City of Hope, Duarte, CA; 2) J.C. Self Research Institute, The Greenwood Genetic Center, SC.

MicroRNAs (miRNAs) are small (~18-24 nt) noncoding RNAs that are cleaved from larger (~80 nt) precursors. They negatively regulate gene expression at the post-transcriptional level and play important regulatory roles in animals and plants by targeting mRNAs for cleavage or translational repression. Alterations in microRNAs or target sites for miRNAs may be a significant but unrecognized source of human genetic disease, including psychiatric disorders. To explore the possibility that structural variants in the microRNA genes could predispose to neuropsychiatric disorders, 18 microRNA genes on the X chromosome were scanned in patients with these disorders. We used DOVAM-S (Detection of Virtually All Mutations-SSCP) to scan the precursor genomic region of the 18 microRNA genes on the X chromosome in 216 unrelated patients (96 with schizophrenia; 72 with mental retardation and 48 with autism). Two promising variants and nine other variants were identified. The first promising variant was identified in a patient with schizophrenia. It is a single base substitution at mature miRNA Let-7f-2. This site is highly conserved through C. elegans. This variant is not present in 6,700 controls and 616 patient samples (330 schizophrenia; 149 psychosis; 41 schizoaffective; 96 autism). Another promising variant is a deletion of microRNA hsa-mir-384 in a male patient with mental retardation. While more work is necessary to prove causation, the data herein are consistent with a role for microRNA mutations in the etiology of neuropsychiatric disorders.

Single Gene Transcriptome Analysis of Combinatorially Spliced Calcium Channels. *X. Zhong[1], M.C. Emerick[1], D.A. Hanck[2], W.S. Agnew[1]* 1) Department of Physiology, Johns Hopkins University School of Medicine, Baltimore, MD; 2) University of Chicago School of Medicine,Chicago,IL.

Alternative RNA splicing may greatly diversify the proteome. For a growing number of genes unexpectedly large numbers of variable sites permit combinatorial expression of large arrays of alternative products - mini-transcriptomes, or single-gene transcriptomes (SGTs) -- with mechanistically different proteins expressed in different cells. Single-gene transcriptome analysis, which includes cloning of hundreds or thousands of full-length cDNA products, reveals the inventory of physiological forms, variations in molecular function, and patterns of developmental expression. The human pacemaker calcium channel genes CACNA1G and CACNA1H can potentially generate thousands of alternative transcripts encoding core subunits of multimeric channel complexes. Full-length cloning reveals the expression of scores of functional variants with biophysical differences sufficient to markedly alter neuronal firing. Analyzed at the protein level, some domains alter channel biophysics in modular fashion, while others interact in cooperative ensembles. Combinatorial splicing must thus be regulated in concert and patterns of splicing must be gene specific. Each full-length cDNA constitutes a diary of concerted splicing decisions made in expressing cells. Surveys of full-length cDNA libraries reveal changes in variable domain linkages invisible to microarray tiling methods that identify individual variable sites. Multivariate analyses reveal highly concerted splicing patterns: for CACNA1G, each of 30 human brain reading frames are strongly developmentally regulated. We present a paradigm for the integrated regulation of splicing at multiple sites. Surveys of exonic splicing enhancer and silencer sites (ESEs, ESSs) reveal hundreds of candidate regulatory motifs near both constitutive and alternatively spliced junctions. Mutations of CACNA1H linked to human Idiopathic Generalized Epilepsies include SNPs that alter protein coding and channel gating, and others that alter ESE and ESS patterns, suggesting the relevance of the splicing regulatory paradigm in genetic disease analysis.

Genetic study of a large Chinese motor neuron diseases (MND) family. *Y.Q. Song[1, 2], G.C.Y. Fong[3], W.L. Ho[3], H.H. Kwok[3], C.Y. Chu[3], Y. Li[1], P. Sham[2, 4], S.L. Ho[3]* 1) Dept Biochemistry; 2) Genome Research Centrre; 3) Dept Medicine; 4) Department of Psychiatry, Univ Hong Kong, Hong Kong, China.

We have identified a large multigenerational Chinese family in Hong Kong with motor neuron diseases (MND). So far, we have documented over 130 members and collected 70 DNA in this four-generation family. Most interestingly, a major branches of this family displayed typical ALS, with onset in the 30s to 40s of mixed upper and lower motor neuron degeneration, bulbar dysfunction and rapid progression to respiratory failure. However, a part of this family has been revealed that the hereditary motor sensory neuropathy II (HSMN II, also known as Charcot-Marie-Tooth disease II- CMT2) phenotype. We have screened all five exons of the SOD1 gene for mutations using both single-strand conformational polymorphism (SSCP) and direct nucleotide sequencing methods. A mutation of T/C transition, at I149T in exon 5 was identified and this mutation is thought to affect the formation of dimers in SOD1. This SOD1 mutation was not detected in all affects of the CMT2 branch. We also sequenced for mutations in a known causative gene (mitofusin) to determine whether mutations in this gene were responsible for this neuropathic phenotype (CMT2). No mutations were detected in the CMT2 branch. At the same time, we have done a genome wide screen using the Illumina 5,900 SNP panel. Two suggestive results were detected in the genome-wide survey. One was on the chromosome 15 at 49 cM (NPL score of 1.46), while the other was on chromosome 10 at 165 cM (NPL score of 1.41). We are collecting additional family members for future linkage studies to clarify these results.

Elevated rates of sister chromatid exchange at chromosome ends. *K. Rudd, C. Friedman, E. Linardopoulou, B. Trask* Fred Hutchinson Cancer Res Ctr, Seattle, WA.

Subtelomeres are the transition zones between chromosome-specific sequences and the arrays of telomere repeats at the end of chromosomes. The frequency of inter-chromosomal exchanges at subtelomeres combined with enrichment of markers of double-strand breaks at chromosome ends led us to postulate that subtelomeres might be subject to more double-strand breaks than elsewhere in the genome. Using chromosome orientation fluorescence in situ hybridization (CO-FISH), Cornforth and Eberle (2001) observed an unusually high frequency of sister chromatid exchange (SCE) as compared to harlequin chromosome studies, which they attributed to the terminal ~10 Mb of chromosomes. We designed a series of CO-FISH experiments to measure the amount of SCE in subtelomeres and telomeres of human chromosomes. In normal lymphoblastoid cells, we find 16% of all SCEs occurred within the most distal 100 kb of the chromosome. Analyzing over 1000 chromosomes per experiment, we found that 0.6% of chromosomes have an SCE in the telomere and 1.3% of chromosomes have an SCE in the terminal 100 kb region (subtelomere + telomere). Only 1.6% of chromosomes have an SCE in the distal 10 Mb of the chromosome; thus, the majority of SCE occurs in the subtelomeric and telomeric regions. When the rate of SCE is calculated per basepair for each interval, we find a very sharp reduction in SCE rate with increasing distance from the telomere. The telomere, distal 100 kb (subtelomere + telomere), and distal 10 Mb have rates of ~4000 x 10-9, ~400 x 10-9 and ~4 x 10-9 SCE/bp, respectively. Thus, the terminal 10 kb of a chromosome incurs over 1000 times more SCEs than an equivalently sized interval in the body of the chromosome. We also find evidence of SCE "clustering"; there are more double SCEs per chromosome than expected if SCEs are independent events. The enrichment of SCEs in the subtelomeres and telomeres suggests that either chromosome ends are subject to more double-strand breaks or that they are more likely to be repaired by SCE. These data, coupled with our experiments on subtelomeric inter-chromosomal sequence transfers, suggest that subtelomeres are hotbeds of exchange and DNA repair.

Succinate dehydrogenase deficiency associated with chronic neutropenia. *F. Scaglia[1], A.M. Adesina[2], J.V. Hunter[3], L-J.C. Wong[1]* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Dept Pathology, Baylor Col Medicine, Houston, TX; 3) Dept Radiology, Baylor Col Medicine, Houston, TX.

Neutropenia has been previously reported in mitochondrial cytopathies, namely in Barth syndrome (MIM # 302060) and Pearson marrow pancreas syndrome (MIM # 557000). However, it has not been reported in association with succinate dehydrogenase deficiency. We report a 2-year-old Native-American child born to consanguineous parents. The proband presented at 2 months of age with hepatomegaly and cholestasis that eventually subsided. Urine organic acid analysis revealed large amounts of 3-methylglutaconic and smaller amounts of 3-methylglutaric and glutaric acids. Acylcarnitine profile exhibited increases of hexanoylcarnitine and decenoylcarnitine. Global developmental delay, hypotonia and persistent neutropenia were noted at 8 months of age. Bone marrow aspiration and biopsy were done and revealed predominance of early myeloid precursors in bone marrow with significant maturational delay of myeloid series. Episodic treatments with filgrastrim reversed episodes of severe neutropenia. Brain MRI revealed moderate cerebral volume loss, abnormal signal in the globus pallidi and ventrolateral thalami, cerebellar volume loss, and a region of restricted diffusion in the right posterior temporal and occipital lobes. No mutations were found in the coding regions of *G4.5* ruling out Barth syndrome. Mitochondrial DNA mutation analysis in blood did not reveal common point mutations, duplications or deletions ruling out Pearson syndrome. On muscle biopsy, there was increased deposition of glycogen but no mitochondrial abnormalities on electron microscopy. Mitochondrial enzyme assays exhibited significant reproducible deficiency of complex II. The presence of persistent hematological abnormalities in this subject with complex II deficiency demonstrates that neutropenia can be observed in other mitochondrial cytopathies besides Barth syndrome and Pearson syndrome expanding the clinical spectrum of mitochondrial cytopathies. Sequencing of genes encoding subunits of succinate dehydrogenase is ongoing to ascertain the molecular basis of this condition.

X-linked heterotaxy: a disease of the cilia? *S. Ware, S. Wang* Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Abnormal function or assembly of cilia is associated with congenital anomalies including hydrocephalus, neural tube defects, polydactyly, and situs abnormalities. In addition, recent data suggest a requirement for normal cilia function in convergent extension movements during gastrulation and neurulation. Previously we demonstrated that mice with loss of function of Zic3, the gene mutated in X-linked heterotaxy (MIM 306955), have gastrulation defects and situs anomalies consistent with heterotaxy. We further demonstrate that morpholino mediated knock down of Zic3 expression in Xenopus embryos results in convergent extension defects, suggesting ciliary dysfunction. Zic3 is a zinc finger transcription factor member of the Gli superfamily that mediates hedgehog signaling. The data demonstrate that Zic3 and Gli3 synergize to activate transcription in vitro and co-localize in vivo. In addition to its role as a transcription factor, we show that Zic3 can shuttle from the nucleus to the cytoplasm via canonical signaling domains, and localizes to cilia in the node of the early embryo. Further immunohistochemical analyses of node cilia indicate that Gli3 is also present, suggesting that Zic-Gli interactions within cilia of the node may be important for sonic hedgehog signal transduction. Scanning electron microscopy and immunohistochemistry of node cilia in Zic3 null mouse embryos indicate that absence of Zic3 does not disrupt cilia formation, but rather results in abnormal node morphology in approximately 60 percent of null embryos. Node cells are smaller than those of wild type mice and arranged in irregular foci accompanied by disordered peripheral cells. These results suggest that Zic3 contributes to situs determination through regulation of node formation and structure, and indicate a novel role for Zic3 and Gli3 in cilia of developing embryos.

Quantitative in vivo mouse model for evaluating translational bypass therapy: geneticin multi-day response. *C. Yang[1], J. Feng[1], W. Song[1], J. Wang[1], B. Tsai[1], Y. Zhang[1], K.A. Hill[1], K.A. High[2,3], S.S. Sommer[1]* 1) Beckman Res Inst, Molec Gen, City of Hope, Duarte, CA; 2) Department of Pediatrics, university of pennsylvania School of Medicine and Division of Hematology, the children's Hospital of Philadelphia, philadelphia, PA, USA; 3) Howard Hughes Medical Institute, philadelphia,PA,USA.

Aminoglycosides have been reported to suppress nonsense mutations (translational bypass therapy, TBT). Since about 15% of inherited severe genetic disease is due to nonsense mutations and, since there are 20,000 genes in the human genome, the potential of TBT is immense. However, initial results demonstrate the need for more potent or efficacious drugs. In addition, nonsense mediated decay and sequence context effects may be problematic. No quantitative in vivo system is available. We described such a system for quantitating TBT in the presence of and absence of nonsense mediated decay. We demonstrate that geneticin is much more efficacious in vivo than gentamicin. After two doses of geneticin, residual factor IX antigen (two hours half-life) can be detected after three weeks. The mechanism of this dramatic response is unclear. These data demonstrate the utility of the mouse system for evaluating nonsense suppressors in vivo. Geneticin may have utility in the treatment of hemophilia B in developing countries where factor IX replacement is unavailable. Furthermore, geneticin or metabolites should be evaluated as a general treatment of severe genetic disease due to nonsense mutations.

Human somatic microindels. *W.A. Scaringe[1], K. Li[1,3], D. Gu[1], L. Chen[1,3], K.D. Gonzalez[1], K.A. Hill[1,2], S.S. Sommer[1]*
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Little is known about microindels. We present the first analysis of somatic microindels in an endogenous gene in human cancer. P53 somatic microindels were identified in the literature and analyzed in the context of other p53 mutations. 65 of 66 somatic microindels were unique, although an additional 3 pairs showed the same deletion with a similar insertion. Gender and tissue effects were seen ($p = 0.009$ and 0.04, respectively). Analysis of the microindels suggests size distributions and sequence contexts different from pure microinsertions and pure microdeletions. Microindel sequence contexts suggest heterogeneous mechanisms including insertions of nearby, but not directly adjacent, sequence in either the sense or antisense direction. The insertions are sometimes imperfect repeats, indicating an error-prone process. Despite the ascertainment of these p53 indels in cancers, analysis of endogenous microindels in the Human Germline Mutation Database and available somatic microindels in the HPRT gene show general similarities with p53 somatic microindels in cancer. We conclude that microindels are distinct from pure microinsertions and pure microdeletions and occur by heterogeneous mechanisms.

Polymorphisms in the Vitamin D Receptor (VDR) that influence levels of intact parathyroid hormone have pleiotropic effects on bone mineral density and calcification of the coronary arteries and aorta. *E. Rampersaud*[1], *D. McBride*[1], *E. A. Streeten*[1], *A. R. Shuldiner*[1, 2], *B. D. Mitchell*[1] 1) EDN, University of Maryland, Baltimore, MD; 2) GRECC, VAMC, Baltimore MD.

Vitamin D metabolism is critical to bone health, and arguably to cardiovascular health. To assess the impact of sequence variation within the vitamin D receptor (VDR) gene on these outcomes, we genotyped 9 SNPs in VDR and evaluated their associations with bone mineral density (BMD), intact parathyroid hormone (iPTH), and aortic/coronary artery calcification in 940 subjects from large Amish families. BMD was measured by dual energy x-ray absorptiometry (DXA), and calcification by electron beam computed tomography. Linkage disquilibrium (LD) among these SNPs was modest, with pairwise r2 ranging from 0.2 to 0.7. SNP association analyses were performed using a variance components framework implemented in the SOLAR program, which accounts for the relatedness of family members. After adjusting for age, gender and body mass index, two intronic SNPs included in our analyses were significantly associated with iPTH. The homozygous rare TT genotype for rs1540339 was significantly associated with increased levels of iPTH ($p<0.001$) and with decreased aortic artery calcification ($p=0.025$). In contrast, the homozygous rare TT genotype for BSM1 was significantly associated with decreased levels of iPTH ($p=0.025$) and decreased BMD at the ultradistal radius (RUS) ($p=0.021$). In sex-stratified analyses, the FOKI nonsynonymous coding polymorphism was significantly associated with increased coronary artery calcification ($p=0.008$) and increased aortic artery calcification ($p=0.025$) in women only. BSM1 ($p=0.033$) and rs1540339 ($p=0.001$) remained significant for iPTH in the subset of women, but not in men. Our analyses suggest that the VDR BSM1 polymorphism is jointly associated with decreases in both iPTH and RUS, lending support to a pleiotropic effect of vitamin D metabolism. Additionally, rs1540339, which is not in LD with BSM1, is jointly associated with increased levels of iPTH and RUS, and with decreased aortic artery calcification. These findings appear to be particularly pronounced among females in our study.

hnRNP E1 transcriptional and translational regulation profiles. *N. Zhong[1,2], L.R. Huo[1], J.H. Zou[1], M. Yan[1], D. Wu[1], W. Ju[2]* 1) Peking University Center of Medical Genetics, Beijing, China; 2) New York State Institute for Basic Research in Developmental Disabilities.

The hnRNP E1 [heterogeneous ribonucleoprotein E1, also referred to as poly(rC)-binding protein1 (PCBP1) or aCPs] contains three KH domains and is a member of the hnRNP family. The family members are involved in several RNA-related biological processes, such as transcription, pre-mRNA processing, mature mRNA transport to the cytoplasm, and translation. The hnRNP E1 also has been described as regulating initiation of RNA replication and translation. Recent studies have shown that hnRNP E1 may increase the activity of the internal ribosome entry segment (IRES) of Bag-1 mRNA suggesting that hnRNP E1 is essential for IRES function. It was also shown that hnRNP E1, along with hnRNP D and K, have a remarkable binding specificity for the cystosine-block [(CCCTAA)n] repeated motif, which may be involved in telomere functions during cell replication. Furthermore, hnRNP E1 could play a critical role in mRNA processing and transport through interaction with nuclear actin in the hnRNP complex. Our recent study showed that hnRNP E1 is one of the progerin-interactive partner proteins. To study the transcriptional and translational regulation of hnRNP E1, we have investigated the RNAs and proteins profiles at a condition either the endogenous hnRNP E1 has been knocked down with lentiviral vector mediated siRNA or an exogenous hnRNP E1 was overexpressed. RNAs or proteins were extracted from the conditioned cells that had been sorted and cultured. The RNAs were analyzed by hybridization with a microarray chip and the proteins were fragmented with PF2D. As anticipated, differential expression profiles of both RNAs and proteins were obtained in hnRNP E1 knocked down and overexpression cells. These differentially expressed RNA or protein profiles suggested they have been up- or down-regulated by hnRNP E1.

LMNA mutations associated with familial and sporadic cases of dilated cardiomyopathy in Koreans. *K. Song[1], M.-P. Dube[2], J. Lim[1], I. Hwang[3], I. Lee[3], J.-J. Kim[4]* 1) Dept Biochem & Molec Biol, Univ Ulsan Col Medicine, Seoul, Korea; 2) Institut de Cardiologie de Montréal, Université de Montréal, Quebec, Canada; 3) Dept Pathology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea,; 4) Dept Internal Medicine, Asan Medical Center, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea.

Dilated cardiomyopathy (DCM) is a disorder characterized by cardiac dilation and systolic dysfunction. So far sixteen genes have been shown to cause autosomal dominant familial dilated cardiomyopathy (FDC). We identified a large Korean family from the Cheju island showing a clear Mendelian inheritance of FDC. A genomewide linkage scan at 9 cM marker density identified a peak multipoint LOD score of 2.82 at D1S195. Haplotyping of the region with 15 additional markers defined a candidate interval that included LMNA, a known candidate gene encoding the lamin A/C. Sequencing of the LMNA exons revealed one missense mutation at C568T (Arg190Trp) in the -helical rod domain of the LMNA gene cosegregating with FDC with conduction-system disease. We found the same mutation in patients of a second Korean family with FDC without conduction-system disease. We then screened 14 sporadic DCM cases showing similar pathology to the familial cases. One had a previously described (Glu161Lys) mutation and two had novel (Glu53Val and Glu186Lys) mutations. Our results suggest that variable genotypes of laminopathy are implicated in not only familial but also considerable proportion of sporadic DCM.

Novel mutations in medium-chain acyl-CoA dehydrogenase gene. *B.Z. Yang, J.H. Ding, L. Sweetman, C.R. Roe* Inst Metabolic Disease, BRI, Baylor Univ Medical Ctr, Dallas, TX.

Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency is the most common inherited defect of fatty acid oxidation, characterized by episodes of illness in early childhood. The disorder may present after fasting with symptoms resembling Reye Syndrome, coma, hypoglycemia, hyperammonemia, fatty liver, and sudden death. The reported cases show significant phenotypic heterogeneity of MCAD deficiency, even with the same genotype. In this report, two patients with MCAD deficiency have recently been detected by newborn screening . Acylcarnitine levels in dried blood spots were measured by tandem mass spectrometry (MS/MS), which showed highly elevated octanoyl carnitine and decenoyl carnitine, indicating MCAD deficiency. To investigate the molecular aspect, all 12 exons and their flanking intronic sequences were amplified from probands DNA. The PCR products were purified and sequenced. Sequence analysis revealed that both patients are compound heterozygous with a novel mutation 1011_1012ins 13-bp and 385 T>G respectively. These novel mutations were verified by a PCR/restriction test, but were not detected in the normal control subjects. In our group, eighteen unrelated babies with MCAD deficiency were detected by newborn screening and confirmed by mutation analysis. The common mutation 985A>G presented in 69% of all defective alleles. In addition, the patients follow-up had been discussed.

Mutation spectrum in non-ketotic hyperglycinemia. *J. Van Hove[1, 2], G. Scharer[1], E. Spector[1], V. Mahieu[2], E. Schollen[2], G. Matthijs[2], C. Freehauf[1], C. Wilson[3]* 1) University of Colorado at Denver Health Sciences Center, Denver, CO; 2) Catholic University Leuven, Leuven, Belgium; 3) Starship Children's Hospital, Auckland, New Zealand.

Patients with non-ketotic hyperglycinemia (NKH) have mental retardation and seizures. NKH is a disorder of glycine catabolism, characterized by deficient glycine cleavage enzyme activity. This system is composed of 4 peptides, the P, T, H and L proteins. Here we describe the mutational spectrum of patients with proven NKH. Methods: We characterized mutations in 58 patients with proven NKH by direct sequencing of all exons and intron-exon borders of the AMT, GLDC, and GCSH genes. Parental studies identified the phase. To identify deletions in GLDC, we analyzed a set of 6 highly polymorphic markers in the core family, and we used a new quantitative real-time PCR-based method. Results: Mutations were found in the AMT gene in 14 patients (24%), and in the GLDC gene in 40 patients (69%). In 4 patients (5%), most with proven deficient enzyme activity, no mutations were found in AMT, GLDC and GCSH. In AMT, 93% of the alleles were identified. In this gene, 68% of the alleles were missense mutations, with the common R320H accounting for 21% of the alleles. A founder mutation I106T in the Dutch population was associated with a mild phenotype. In GLDC, 95% of the alleles were identified. There were 43 alleles with missense mutations, 9 alleles with nonsense mutations, 11 alleles with splice-site mutations, and 2 small deletions. There was proof of a large deletion in 9 alleles varying in length and position, 6 of them including the 5' site of the gene. Recurring mutations were R515S, A389V, R424X, IVS19-1AG. A common mutation was present in the New Zealand patients. Patients with mutations with known residual activity had milder phenotype. This testing allows confirmation of diagnosis and has been successfully used in prenatal diagnosis. It further aids in the identification of patients affected with a milder disease.

Genetic characterization of the SCN5A gene in Korean patients with Brugada syndrome. *E.J. Seo[1, 2], J.O. Lee[2], K.J. Kim[2], G.B. Nam[3], Y.H. Kim[3], K.H. Han[3], I.S. Park[2,4]* 1) Dept. of Laboratory Medicine, Univ. of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea; 2) Genome Research Center for Birth defects and Genetic disorders, Asan Medical Center, Seoul, Korea; 3) Dept. of Internal Medicine, Univ. of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea; 4) Dept. of Pediatrics, Univ. of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea.

Mutations in the cardiac sodium channel gene SCN5A cause various arrhythmia syndromes such as Brugada syndrome and long QT syndrome type 3. In addition, common polymorphisms of the SCN5A gene have been known to alter electrophysiology and S1103Y of them was associated with increased arrhythmia clinically. Therefore, we investigated whether genetic variations of the SCN5A gene implicate in risk of Brugada syndrome. Mutational analysis was performed in the SCN5A gene in sporadic 24 patients with Brugada syndrome by PCR based sequencing method. Nine common polymorphisms (R34C, R481W, S524Y, G552R, H558R, P1090L, S1103Y, R1193Q, V1951L) were genotyped in those patients and 100 healthy controls. We identified only one mutation among 24 patients (4.2%). The mutation was a novel heterozygous missense, c.874G>A:G292S, which was not found in 100 controls. Although H558R, P1090L and R1193Q are frequent in Asians, any common polymorphisms except H558R were not found in Korean population. The allele frequency of H558R was 8.3% in patients and 8% in controls, respectively, which was in Hardy-Weinberg equilibrium and not different between two groups. Our findings suggest that the SCN5A gene might not be the major cause of Brugada syndrome in Korean patients and common SCN5A polymorphisms also rare in Korean population.

Measured genotype analysis of neuropeptide Y levels in early-onset coronary artery disease. *S. Shah[1,2], C. Newgard[3], L. Wang[2], M. Muehlbauer[3], E. Dowdy[1], S. Nelson[2], C. Haynes[2], J. Rombaut[2], G. Ginsburg[5], P. Goldschmidt-Clermont[4], J. Vance[2], W. Kraus[1], S. Gregory[2], E. Hauser[2]* 1) Dept Medicine, Duke Univ Med Ctr, Durham, NC; 2) Center for Human Genetics, Duke University Medical Center, Durham NC; 3) Sarah W. Stedman Nutrition & Metabolism Center, Duke University School of Medicine, Durham NC; 4) Miller School of Medicine, University of Miami, Miami FL; 5) Institute for Genome Sciences and Health Policy, Duke University, Durham NC.

Introduction. We have previously reported linkage and association for NPY gene variants with early-onset CAD in two independent datasets, with stronger association seen in very early-onset CAD. We hypothesized that NPY and NPY receptor gene variant alleles and genotypes are associated with quantitative differences in NPY levels.

Methods. We identified 74 cases with very early-onset CAD (age-of-onset <38) and 74 sex- and race-matched controls with no CAD (age>60), from subjects undergoing cardiac catheterization. NPY and NPY receptor single-nucleotide polymorphisms (SNPs) were genotyped using Taqman. NPY levels were measured from fasting frozen whole blood collected at time of catheterization. Samples were assayed for NPY using a commercial RIA kit. Counts were measured with a Wallac Wizard 1470 automatic gamma counter. NPY concentration was determined as the average of two replicates, calculated relative to calibrated standards. Measured genotype analysis with ANOVA was used to assess significance in differences in NPY levels, by multiple NPY and NPY receptor SNPs.

Results. NPY SNPs previously shown to be associated with early-onset CAD were not associated with NPY levels. A rare NPY SNP showed a trend for association (p=0.06). However, two NPY1R SNPs were associated with differential NPY levels (rs1574637, p=0.02 and rs4518200, p=0.05).

Conclusions. NPY receptor, but not NPY, gene variants are weakly associated with quantitative differences in NPY levels. Further studies of the NPY pathway in early-onset CAD are ongoing.

Association study of conserved variants in MME with late-onset Alzheimers Disease (LOAD). *F. Zou[1], M. Raam[1], L. Ma[1], J. Lok[1], S.G. Younkin[1], M.M. Carrasquillo[1], L.H. Younkin[1], N. Graff-Radford[1], R.C. Petersen[2], S.G. Younkin[1]*
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MME, which encodes the A degrading enzyme neprilysin, is an excellent functional candidate gene for late onset Alzheimers Disease (LOAD). To identify MME variants likely to be functional, we screened publicly available databases for variants in conserved regions (>70% human vs. mouse) and identified 22 SNPs with minor allele frequencies >1%. While using DHPLC to investigate a known variant, we discovered an additional novel SNP in exon 1 that converts methionine to valine. Using logistic regression, 6 SNPs have so far been tested for association with LOAD in a combined set of three case/control series with a total of 1524 AD patients and 2125 controls between the diagnosis ages of 60 and 85. When the 6 single variants were tested in an allelic dosage model, three had p values 0.028, 0.098, and 0.002 in the combined series, and all three were found in one haplotype block. The most significant (p=0.002) was the previously unreported coding SNP (met/val) in exon 1. These three SNPs formed 4 haplotypes. In the combined series, the four haplotypes showed significant association (global p=0.007). This association was primarily due to the haplotype marked by the novel coding SNP, which showed replicable association in each of the three case/control series with ORs of 1.8 -2.4 and p values of 0.068, 0.028, and 0.016. In the combined series, the OR for this haplotype was 1.8 (1.3-2.5) with a p value of 0.001. Of the three remaining haplotypes, two showed no evidence for association and the third showed suggestive association in the combined series with an OR of 0.89 (0.77-1.03) and a p value of 0.116. In the combined series, the multilocus genotypes formed by the three MME SNPs also showed significant association (global p = 0.024). PLAU and IDE, two other genes that encode proteins involved in A degradation, also have susceptibility alleles for LOAD (see Carrasquillo et al., these meetings).

Genome-wide Scan of Factors for the Metabolic Syndrome in Non-diabetic Hong Kong Chinese. *C.H.T. Tam, M.C.Y. Ng, V.K.L. Lam, J.C.N. Chan* Department of Medicine & Therapeutics, The Chinese University of Hong Kong, Shatin, Hong Kong.

Recent studies suggest shared genetic factors contribute to different components of metabolic syndrome (MES). The aim of this study was to identify loci contributing to the factors of MES derived from principal component factor analysis.

Factor analysis on 15 MES-related traits yielded 6 factors accounting for 77% of the total variance of the original variables. These included adiposity factor (body mass index, waist circumference and body fat percentage), glucose factor (glucose at OGTT for 0, 30 and 120 min, insulin at 120 min), insulin factor (insulin at OGTT for 0, 30 and 120 min), blood pressure factor (systolic and diastolic blood pressure), TC+LDL factor and TG+HDL factor. Variance component-based linkage analyses on these factors were conducted with 355 microsatellite markers on 797 non-diabetic subjects ascertained through 179 families participating in the Hong Kong Family Diabetes Study.

Significant evidence for linkage of the adiposity factor was found on chromosome (chr) 1 at 189cM (LOD = 4.30). In addition, suggestive evidence for linkage (LOD = 2.51-3.12) were observed for adiposity factor (chr 11 at 33 cM), glucose factor (chr 7 at 155 cM), insulin factor (chr 5 at 21 cM, chr 11 at 142 cM) and TG+HDL factor (chr 7 at 155 cM). In summary, our findings suggest the presence of susceptibility loci that influence either single (chrs 1, 5 and 11) or multiple factors (chr 7) for metabolic syndrome in non-diabetic Hong Kong Chinese.

A Structurally Abnormal X Chromosome with a Neocentromere in a Girl with Mosaic Turner Syndrome. *B.T. Wang[1], P.E. Warburton[2], F. Cheung[2], X.J. Yang[1], M. Ayad[1], F.Z. Boyar[1], M. El Naggar[1], C. Zapata[1], J. Neidich[1], B. White[1], A. Anguiano[1]* 1) Cytogenetics Dept, Quest Diagnostics,Nichols Inst, SanJuanCapistrano, CA; 2) Department of Human Genetics Mount Sinai School of Medicine, NY.

Neocentromere is a rare human chromosomal aberration where a new centromere has formed in a previously non-centromeric location. We report the finding of a structurally abnormal X chromosome with a neocentromere in a 15 year-old girl with clinical features suggestive of Turner syndrome including short stature and primary amenorrhea. G-banded chromosome analysis revealed a mosaic female karyotype involving two abnormal cell lines. One cell line (84% of analyzed metaphases) had a structurally abnormal X chromosome with duplication of the long arm and deletion of its short arm, and a normal X chromosome. The other cell line (16% of cells) exhibited monosomy X. C-banding studies were negative for the abnormal X. FISH analysis revealed lack of hybridization of the abnormal X chromosome with both the X centromere-specific probe and the all human centromeres probe, a pattern consistent with lack of the X chromosome endogenous centromere. FISH study using XIST gene probe for detection of the XIST gene on the abnormal X chromosome and R-banding for evaluation of X-inactivation status are underway. An assay for centromeric protein C (CENP-C) was positive on both the normal and the abnormal X chromosomes. The position of CENP-C in the abnormal X chromosome defined a neocentromere, which explains its mitotic stability. The karyotype is thus designated as 46,X,neo(X)(qterq12::q12q21.2neoq21.2qter)[42]/45,X[8], which is consistent with stigmata of Turner syndrome. The mother of this patient has a normal karyotype; however, the father was not available for study. Over 75 examples of human neocentromeres have been described. To our knowledge this is the second example of an analphoid X-derived chromosome with a proven neocentromere.

Improved Detection of Genomic Disorders: Will Denser Arrays Identify New Syndromes? *T.H. Shaikh[1], L. Medne[1], D. McDonald-McGinn[1], S. Saitta[1], C. Bonneman[2], E.H. Zackai[1]* 1) Div. of Human Genet; 2) Div. of Neurology, Children's Hosp Philadelphia, Phila., PA.

Microdeletions and microduplications are a leading cause of genomic disorders. Many of these rearrangements are submicroscopic and not detectable by standard clinical tests like karyotype analysis and subtelomeric FISH. Microarray-based analysis of copy number alterations has recently gained widespread usage for the detection of genomic rearrangements. We have successfully used high-density, oligonucleotide arrays for the detection of copy number aberrations. In our study we analyzed 62 patients with multiple congenital anomalies and normal karyotypes and subtelomeric FISH results. We detected de novo copy number alterations in 16 (26%) cases. These include microdeletions and microduplications in chromosomes 1p, 1q, 2p, 3p, 6q, 7q, 10q, 15q, 16q, 17p and 22q, ranging in size from 600 Kb to 10 Mb. Most of these rearrangements are novel and do not overlap with previously characterized genomic disorders or syndromes. It is possible that some of these novel rearrangements detected in our study represent a new genomic disorder or syndrome in which a rearrangement can be associated with a specific spectrum of phenotypic features. This is exemplified by one of our cases, CH-217, a twelve year old boy with a history of developmental delay, short stature, hypotonia and obesity. He had a normal karyotype and although his phenotype resembled Prader-Willi syndrome, no aberrations were detected in 15q by FISH and DNA methylation testing. We detected a 10 Mb deletion in 6q16-q21 using our approach. This is the fifth reported case of a Prader Willi-like phenotype associated with aberrations in 6q. Thus, it is likely that these cases represent a new 6q16-q21 deletion syndrome. We expect that new genomic disorders will be identified in the future as a result of improvements in detection techniques, in the form of higher density arrays and analysis tools, combined with the clinical characterization of associated phenotypic features. The creation and maintenance of a comprehensive, public database of rearrangement-based disorders should further improve our ability to identify new syndromes.

Multiplex Genotyping by Small Amplicon Melting and Unlabeled Probes: Application to HFE Genotyping. *R.J. Pryor, C.T. Wittwer* Pathology, University of Utah, Salt Lake City, UT.

Simple methods of genotyping by melting analysis without labeled probes have recently appeared. Small amplicon genotyping requires only two PCR primers and a generic DNA dye. Unlabeled probe genotyping incorporates an additional 3-blocked unlabeled probe. Small amplicon and unlabeled probe genotypes can be extracted from the same melting curve after multiplex PCR. This combined method was applied to genotyping all common alleles of the HFE gene (HLA-H). Mutations in the HFE gene are responsible for hereditary hemochromatosis, an autosomal recessive disorder of iron metabolism.

PCR was performed on a LightCycler (Roche) and an HR-1 (Idaho Tech.) was used for high resolution melting analysis. The dsDNA fluorescence dye LCGreen Plus (Idaho Tech.) was used to monitor PCR amplification and subsequent melting analysis. The PCR reaction used two primer sets. One amplicon incorporates H63D and S65C and the other C282Y. The unlabeled probe covers the H63D mutation and T189C polymorphism but does not span the S65C site. An unlabeled probe is needed to genotype the H63D site because a C is exchanged for a G which, due to nearest neighbor parameters of that particular sequence, makes the Tm of the homozygous mutant the same as wild type.

There are three main mutations associated with hemochromatosis: C282Y (G845A), H63D (C187G) and S65C (A193T). There is also a polymorphism, T189C which is a wobble at the third base of a histidine codon. The nine most common genotypes of HFE were all differentiated, including wild type, homozygotes for C282Y and H63D, heterozygotes for C282Y, H63D, S65C and T189C and the compound heterozygotes C282Y/H63D and H63D/S65C. Multiplex genotyping by melting of small amplicons and unlabeled probes is simple, inexpensive, and rapid. Regions of considerable sequence variation can be genotyped in a single color, closed-tube system by rapid PCR followed by high-resolution melting analysis.

High prevalence of IVS14+1G>A mutation in the *AAAS* (Allgrove Syndrome) gene among Puerto Rican newborns. *M.C. Schneider[1], P.J. Santiago Borrero[2], A. Morales Reyes[2], S. Ireland[1], A. Shouse[1], R.G. Leon[1]* 1) SIU Dept Ped, Div Gen/Metabol, SIU Sch Medicine, Springfield, IL; 2) Department of Pediatrics, University of Puerto Rico School of Medicine, San Juan, Puerto Rico.

Allgrove syndrome (MIM 231550) is an autosomal recessive entity initially described with the cardinal signs of achalasia, alacrima, and ACTH-resistant adrenocortical deficiency (hence also called Triple-A syndrome). The phenotype is broader; patients often show mild growth failure and slowly progressive neurologic and autonomic dysfunction, including mental retardation, hypotonic hyperreflexic spasticity, optic nerve atrophy, and autonomic dysfunction. It had been noted that 6/8 cases identified in the United States occurred in individuals with Puerto Rican ancestry, and a single IVS14+1G>A mutation in the AAAS gene accounted for over 80%; of the Allgrove alleles in this population. Using site-specific PCR followed by restriction digest, the presence of this mutation was assayed among 972 independent island-wide blood spot samples, and seventeen heterozygote carriers were found. Unlike other recessive conditions such as Hermansky-Pudlak, which are restricted to mainly to certain towns, carriers for Allgrove were distributed throughout the island and the major metropolitan areas. Heterozygote frequency is estimated to be 1 in 57 in Puerto Rico, which predicts that 1 in 13,000 newborns are affected. In our experience, the condition is underdiagnosed, and can lead to early demise. Since the prevalence of affected exceeds that of other entities screened among newborns, and treatment of the adrenal failure can prevent death, we recommend either universal newborn or carrier screening for this entity should be assessed among individuals of Puerto Rican descent.

Genetic services in satellite clinics: A model of delivery in three different communities. *N. Stewart[1], B. Crawford[1], R. Lee[1], J. Mak[1], P. Conrad[1], M. Beattie[1], J. Luce[3], J. McLennan[1], J. Ziegler[1], V. Caggiano[2]* 1) Cancer Risk Program, University of California San Francisco Comprehensive Cancer Center, San Francisco, CA; 2) Cancer Risk Program, Sutter Cancer Center, Sacramento, CA, United States, 95816; 3) Division of Hematology-Oncology, San Francisco General Hospital, San Francisco, CA, 94110.

Genetic counseling services are routinely offered in Comprehensive Cancer Centers, but are less available in community cancer centers and county hospitals. Barriers include lack of insurance coverage, funding, trained counselors, and language and cultural differences. The Cancer Risk Program (CRP) at the University of California San Francisco (UCSF), began offering genetic counseling, testing and 20 year follow up in 1996 through a clinic based research protocol, serving primarily insured families of high socioeconomic status. To reach underserved populations, the UCSF CRP expanded services by partnering with Sutter Cancer Center (SCC), a community cancer center 90 miles away, and with San Francisco General Hospital (SFGH), a county hospital serving a low income multi-ethnic population. Financial barriers for low income families were removed though scholarship funds at UCSF and SCC and Avon Foundation grant support at SFGH. A total of 1451 patients enrolled in the protocol and tested for BRCA1/2 with mutation detection rates of 20-26% at all three clinics. Patients and their families were provided with current, evidence-based recommendations for surveillance and protocols for prophylactic surgeries in mutation carriers. Patients identified at SCC and SFGH were more likely to choose both mastectomy and oophorectomy, whereas carriers at UCSF were evenly split between oophorectomy alone and oophorectomy and mastectomy. Prophylactic surgery has been shown to reduce the incidence of breast and ovarian cancer in these high risk populations, therefore there is a greater need to identify high risk families of all ethnicities, education levels, and geography. This outreach model of satellite clinics provides the benefits of a clinical research program with multidisciplinary long-term follow up care to underserved and/or distant populations.

Genetic analysis of large four generation families with idiopathic adolescent scoliosis. *K. Ward, M.C. Meade, L.M. Nelson, V. Argyle, T. Berry, S. Murphy, J. Braun, J.W. Ogilvie* Axial Biotech, Inc, Salt Lake City, UT.

Our aim was to study the genetics of adolescent idiopathic scoliosis (AIS) in large Utah families. Four-generation pedigrees were obtained from 85 probands. 83/85 probands were surgically treated. In 69 of the 85 families, probands reported a positive family history of AIS within the 4 generations. AIS was confirmed in these 69 probands and in 174 affected relatives either by x-ray(n=107) or medical records(n=136). An additional 53 female and 18 male relatives were reported to have scoliosis, but the diagnosis was unconfirmed for these patients. 21 family members had other spinal deformities (12 females, 9 males). Females tended to have more severe curves;female:male ratio for the confirmed AIS cases is 3.8:1.

	Cobb Angle	Total Aff	Female	Male	%ParentAff	Mat Trans	Pat Trans
Severe	>40	91	83(91%)	8(9%)	22(24%)	56(62%)	27(30%)
Moderate	25-39	36	30(83%)	6(17%)	9(25%)	19(53%)	13(36%)
Mild	10-24	116	79(68%)	37(32%)	24(21%)	64(55%)	36(31%)
Unknown	Various	71	53(75%)	18(25%)	9(13%)	25(35%)	14(20%)
Total		314	245(78%)	69(22%)	64(20%)	164(52%)	90(29%)

Excluding probands; assuming AD inheritance, penetrance in females was 55%. Penetrance in males is 21%. There were 18 reported instances of male: male transmission (confirmed diagnoses in both males for only 2 of these). Four probands reported scoliosis in both parents families. Conclusions: This study confirms the familial nature of adolescent idiopathic scoliosis and expands our understanding of the genetic parameters.

Abnormalities in endochondral bone development in the Apert syndrome mouse model. *Y. Wang, M. Sun, F. Yang, S.A. Shulby, J. Elisseeff, D.L. Huso, E.W. Jabs* Inst Genetic Medicine, Johns Hopkins Univ, Baltimore, MD.

Fibroblast growth factor receptor 2 (FGFR2) plays an important role in skeletogenesis. FGFR2 S252W and P253R mutations are associated with Apert syndrome, an autosomal dominant disorder characterized by bone malformations of the skull and limbs. We created $Fgfr2^{+/S252W}$ and $Fgfr2^{+/P253R}$ heterozygous mice with craniofacial and skeletal defects that mirror the human Apert syndrome to study bone development in this condition. Previously, we reported the effect of these mutations on membranous bone. However, the effect of these mutations on endochondral bone formation is not well understood. Therefore, we studied the developing limbs of our mutant mice and littermates from E15.5 to P0. Histological analysis of mutant long bones revealed abnormal endochondral bone formation with disorganization of the growth plate, subtle irregularity of the hypertrophic zone, and more prominent cartilage mineralization of the ossification zone. TRAP staining showed a reduction in osteoclast number at the chondro-osseous junction of the growth plate, indicating a defect in recruitment of osteoclasts to the hypertrophic region. In situ hybridization of Col X revealed an abnormality in hypertrophic chondrocytes in the mutant growth plate. Alteration of expression of Mmp13, osteopontin and osteocalcin suggests abnormal cartilage matrix remodeling and osteoblast differentiation in the mutants. In vitro 3D hydrogel culture of osteoblasts isolated from the long bones of newborn mutant mice showed increased expression of osteoblastic markers collagen type I, bone sialoprotein and osteocalcin. Apert cells also demonstrated higher expression of Col X as compared to the controls. These in vitro results are consistent with our in vivo findings. The $Fgfr2^{+/P253R}$ heterozygous mice showed similar or less severe abnormalities and gene expression patterns of osteoblastic markers compared to those of $Fgfr2^{+/S252W}$mice, suggesting no clear genotype-phenotype correlation. Our data reveal that these two Fgfr2 gain of function mutations result in dysregulation of chondrocyte differentiation, cartilage extracellular matrix remodeling, and osteoblast differentiation during endochondral ossification.

Detection of mosaicism in pooled human genomic DNA by bi-directional pyrophosphorolysis activated polymerization allele-specific amplification. *J. Shi, Q. Liu, S. Sommer* Department of Molecular Genetics and Molecular Diagnosis, City of Hope National Medical Center, Duarte, CA.

The incidence of mosaicism is not well characterized. The frequency of mosaic mutations may be underestimated, because they are often missed during routine mutation analysis. Bi-PAP-A (Bi-directional Pyrophosphorolysis Activated Polymerization Allele-specific Amplification) is a derivative of PAP that uses two opposing pyrophosphorolysis activable oligonucleotides (Sleeping Beauties) with one nucleotide overlap at their 3 termini. Bi-PAP-A may amplify one copy of the mosaic mutation in the presence of 10^7~10^9 copies of wild type genome without any false positives. For large scale screening, six Bi-PAP-A assays with sensitivities of one molecular and selectivities equal to or greater than one part in 10^8 were utilized to detect somatic mosaicism in the *TP53* gene. Eight pooled DNA samples were initially screened, each containing 4 x 10^5 genome copies (1.3 g of genomic DNA). Each pooled sample contained human blood genomic DNA from 400 individuals. Positive samples were characterized further to identify the positive individual(s). Putative somatic mosaicism was detected. Bi-PAP-A can be utilized to estimate the frequency of somatic mosaicism for cancer predisposing somatic mutations in the *TP53* gene.

Rapid Analysis and Visualization of Genomewide Association Data with GWAVA. *J.E. Wigginton, G.R. Abecasis*
Center for Statistical Genetics, University of Michigan, Ann Arbor, MI.

The advent of the genomewide association (GWA) scan is clearly a watershed event for the field of complex disease mapping. While the opportunities presented by these data sets are considerable, sifting through and analyzing the enormous volume of data generated in a typical GWA scan will be a significant challenge and a barrier to success for many groups. Although a few existing software tools will be capable of handling GWA, most were not specifically designed for this purpose and will not be very efficient.

GWAVA (Genome-Wide Association Visual Analyzer) is an extensible software platform tailored to the unique computational and data storage requirements of GWA data sets. In addition to basic quantitative and qualitative tests of association, GWAVA implements a highly efficient EM algorithm for haplotype frequency estimation in SNP data with or without missing genotypes, and can use the resulting estimated haplotypes to test for association between genetic markers and one or more phenotypes using a linear model (for quantitative traits) or a logistic regression model (for discrete traits). GWAVA can execute quality control checks and incorporates a very rapid exact test of Hardy-Weinberg equilibrium. GWAVA can be run either as a command-line tool in batch mode or via a modern graphical user interface. The latter allows the user to interactively search, sort and filter data and analysis results, presenting raw data in table format and/or a variety of useful graphical summaries. Further information for individual tests or markers of interest may also be displayed with a simple mouse click, which will facilitate interpretation of results without presenting large volumes of extraneous information. GWAVA is specifically designed for rapid data input and output, is capable of handling large data sets with hundreds of thousands of markers on thousands of individuals, and balances computational efficiency with memory usage for graphics and analysis results. Source code, a tutorial and executables for Windows, Mac, UNIX, and Linux platforms are freely available at http://www.sph.umich.edu/csg/abecasis/gwava.

Genome-wide linkage scan for primary open angle glaucoma (POAG) loci in a Caucasian population. *A. Woodroffe[1], C.M. Krafchak[2], M. Soehnlen[1], N. Fuse[2], P.R. Lichter[2], S.E. Moroi[2], R. Schertzer[2], C.A. Downs[2], M. Boehnke[3], J.E. Richards[1,2]* 1) Dept Epidemiology, University of Michigan, Ann Arbor, MI; 2) Dept Ophthalmology, University of Michigan, Ann Arbor, MI; 3) Dept Biostatistics, University of Michigan, Ann Arbor, MI.

Glaucoma is characterized by neurodegeneration of the optic nerve, resulting in visual field loss and, if untreated, can lead to blindness. Over 60 million people world-wide are affected by glaucoma. Glaucoma has a strong genetic component. In addition to increased age, increased intraocular pressure (IOP), thin central cornea, and race, family history is one of the strongest risk factors for developing glaucoma. Several genes predisposing to glaucoma susceptibility have been identified, however these genes account for only a small proportion of glaucoma cases. Therefore, it is likely that more glaucoma genes will be mapped. To identify novel glaucoma loci, we conducted a genome-wide linkage scan on 25 multiplex, Caucasian families with primary open angle glaucoma. We included 167 individuals; 108 are affected. We tested 378 microsatellite markers across the genome, resulting in an average coverage of 7 cM. Preliminary results suggest a maximum multipoint non-parametric LOD (NPL) score of 2.0 at 176.2 cM on chromosome 4q and a maximum singlepoint NPL score of 2.6 at the same location. There was also modest evidence for linkage on 5p and 7p. We are following-up our results using microsatellites to fine-map the regions of interest.

Genome-wide search for linkage interactions: application to schizophrenia. *J. Shi[1], F. Amin[2], D.W. Black[3], N.G. Buccola[4], W.F. Byerley[5], C.R. Cloninger[6], R. Freedman[7], B.J. Mowry[8], J.M. Silverman[9], P.V. Gejman[10], D.F. Levinson[1]* 1) Stanford University, Stanford, CA; 2) Emory University, Atlanta, GA; 3) University of Iowa College of Medicine, Iowa City, IW; 4) School of Nursing, LSUHSC, New Orleans, LA; 5) UCSF, San Francisco, CA; 6) Washington University, St. Louis, MO; 7) University of Colorado School of Medicine, Denver, CO; 8) Queensland Center for Mental Health Research, Brisbane, Australia; 9) Mt. Sinai School of Medicine, New York, NY; 10) ENH/Northwestern University, Evanston, IL.

In common complex disorders, epistatic interactions among susceptibility genes are likely, but each locus could have an effect too small to be detected by linkage analysis. Therefore, it may be useful to search for interactions among loci. Here we do this with a score statistic, using genome scan data for 280 affected sibling pairs (ASPs) from 263 European-ancestry families (Molecular Genetics of Schizophrenia study, Suarez et al., AJHG 2006;78:315-33). For each ASP, IBD was computed (GENEHUNTER 2.1, multipoint analysis) at each autosomal marker location (N=385). We tested each pair of marker positions in the genome except pairs on the same chromosome. Z was computed for each marker pair, by taking the sum (across all ASPs) of [IBD(marker1)-1][IBD(marker2)-1], divided by the S.D. of the numerator. Interactions were considered distinct if they were separated by at least 36 cM (4 times the average inter-marker distance) on at least one chromosome. The four largest Z-scores (with marker locations in deCODE cM, and the SZ meta-analysis bin from Lewis et al., AJHG 2003;73:34-48, if it was significant) were: 4.13 (D2S405/44cM, D10S2325/29cM in bin 10.1); 3.97 (D5S1457/59cM, D19S245/49cM); 3.94 (D4S1647/92cM, D11S912/122cM in bin 11.5); and 3.84 (D10S2327/97cM, D12S1294/72cM). None of these interactions achieves genome-wide significance based on preliminary simulation studies. Computation of exact empirical significance levels is in progress. These results are exploratory, but they demonstrate the feasibility of this approach, which could prove useful for larger scans or for meta-analyses of multiple scans.

Identification of mouse miRNAs expressed exclusively or preferentially in retina. *P. Witmer[1], S. Xu[2], J. Mendell[1], D. Valle[1,3]* 1) Institute of Genetic Medicine, Johns Hopkins School of Medicine, Baltimore, MD; 2) Dept Ophthalmology/Neurological Sciences, Rush Univ Medical Ctr, Chicago, IL; 3) Howard Hughes Medical Institute.

MicroRNAs (miRNAs) are small non-coding RNAs about 22 nucleotides in length found in all metazoans. Since their discovery in 1993, at least 100 different miRNA genes have been recognized in the Drosophila and C. elegans genomes and about 300 in vertebrate genomes. miRNAs have been shown to provide a newly-recognized level of regulation of gene expression potentially important in a wide variety of biological processes. In spite of this progress, little is known about the miRNAs and their roles in the development and function of vertebrate retina and in retinal diseases. To investigate miRNA expression in retina, we purified miRNA-enriched total retinal RNA from adult mice and performed miRNA profiling using Combimatrix miRNA microarray of our design that includes 323 miRNAs and their precursors. To discover the miRNAs expressed exclusively or preferentially in retina, we used adult mouse brain and heart miRNA-enriched total RNA for comparison. Our result showed at least 75 miRNAs are expressed in mouse adult retina at various levels. Among these 23 were expressed preferentially or exclusively in retina including a cluster of 3 miRNA genes in a 4.5 kb intergenic region on mouse chromosome 6q3.3 (corresponding to human chromosome 7q32.2). These are among the highest in terms of expression level and appear to be specific for retina. We confirmed the tissue specificity of the major retina-specific miRNAs with quantitative RT-PCR. To determine if miRNAs are involved in the circadian cycles characteristic of retinal gene expression, we performed miRNA profiling with retinal RNA harvested at noon and midnight and identified a subgroup of miRNAs with diurnal variation in expression pattern. These miRNAs are candidates for involvement in the regulation of the circadian cycle and rhythmic metabolism of retina. Currently we are examining the predicted targets of the retina-specific miRNAs and performing functional studies to determine their role in normal development of retina and circadian cycle regulation.

Mitochondrial DNA polymorphisms in the Amerindian: Tarahumara, Huichol, and Purépecha, and in one Mestizo Mexican population. *L.. SANDOVAL-RAMÍREZ[1,2], M.T. MAGAÑA-TORRES[1,2], M. CASAS-CASTAÑEDA[2], J.M. OLIVA-ORTIZ[1,2], G. VACA[1,2], F. RIVAS[1,2], J.M. CANTÚ[2]* 1) División de Genética, CIBO, IMSS, Guadalajara, MÉXICO; 2) Doctorado en Genética Humana, CUCS Universidad de Guadalajara, Guadalajara, MÉXICO.

OBJETIVE: To describe and compare mtDNA haplotypes among Mexican populations: Mestizo of the West, Tarahumara, Huichol and Purépecha. METHODS: mtDNA of 200 unrelated individuals was analyzed; 50 Mestizos from Western Mexico and 150 native of the three American ethnic groups: 50 Southeastern of Chihuahua Tarahumaras, 50 North of Jalisco and East of Nayarit Huicholes and 50 lake region of Michoacan state Purépechas. Twenty-one polymorphic sites were analyzed using the PCR-RFLP technique. First, the haplogroups A, B, C, D and M were identified. The samples that the origin of their mitocondrial haplotype could not be identified, 12 additional polymorphic sites and the sequenciation of the HVI region were analyzed with the purpose to assign them to some of the following haplogroups: X (European-American); H, I, J, K and U (European), L (African) and F (Asian). RESULTS: In 200 samples, 15 different haplotypes were identified by the analysis of nine ethnic-specific polymorphic sites. This allowed the assignation of the haplogroups A, B, C and D in 188 samples. The distribution in the mestizo population was: A(34%),B(24%),C(10%),D(10%)and A/B (4%),in Tarahumara: A(12%),B(30%),C(54%) and D(2%),in Huichol: A(46%),B(26%),C(26%)and D(0%)and in Purépecha: A(44%),B(24%),C(18%),D(0%),A/D(6%)and C/D(6%). Twelve samples did not pertained to the haplogroups A,B,C and D(nine Mestizos, one Tarahumara, one Huichol and one Purépecha). Eight Mestizos were assigned to European haplogroups H, K and U. Haplogroup X was not observed among these four populations, and a high number of compound haplotypes (12%) was present in the Purépecha population. CONCLUSIONS: mtDNA was of Amerindian origin in its majority (82% Mestizos and 98% Tarahumaras, Huicholes and Purépechas). The genes flow of European origin that conform the cluster of Mexican pool genes came through males. There was no evidence of maternal African component in this population sample.

Loss of Y chromosome in patients with hematological disorders. *E. Shin[1], L. Zhang[3], Z. Yu[2], H. Tian[2], Y. Fei[2], R. Aldrich[2], J.J. Mulvihill[2], S. Li[2]* 1) Department of Pediatrics, OUHSC, Oklahoma City, OK; 2) Department of Pathology, OUHSC, Oklahoma City, OK; 3) Department of Hematology, the First Teaching Hospital of China Medical University, Shenyang, P.R. China.

To investigate whether a loss of Y chromosome is an age related phenomenon or a cytogenetic marker indicating a malignant change, we have studied twenty-four patients with a median age of 73 years-old (range 41-87) from a cohort of 592 consecutive male patients referred to us for chromosome evaluation because of hematological disorders. Two patients had acute myeloid leukemia (AML), 2 patients had chronic myeloid leukemia (CML), 2 patients had chronic myeloproliferative disorder (MPD), 3 patients had multiple myeloma, 5 patients had lymphoma, and 10 patients had myelodysplastic syndrome (MDS). Conventional cytogenetics studies were carried out according to our standard laboratory protocols. Fluorescence in situ hybridization (FISH) analysis was also performed utilizing DNA probes specific for the centromeres of chromosomes X and Y. The probes were purchased from a commercial source (Vysis, Downers Grove, Illinois, USA) and the tests were performed according to the manufacturer's protocols with minor changes. Twenty-one out of 24 patients had a loss of Y chromosome as sole anomaly and the remaining three had a loss of Y chromosome accompanied with other structural changes by conventional cytogenetic analysis. FISH analysis confirmed the routine cytogenetic results. All 24 patients had a loss of Y chromosome with a range of 17.5-98.5% of cells. Two of the patients one with AML and another with CML, had karyotype and FISH testing done both at the initial diagnosis and in the remission, and showed a loss of Y chromosome at initial diagnosis and had normal 46,XY karyotype during remission. In this study, it is demonstrated that the loss of Y chromosome is associated with a neoplastic change.

EGFR somatic mutations in lung cancer: of microindels, smoking and drug response. *S.S. Sommer[1,2], W.A. Scaringe[1,2], K. Li[1], J. Saldivar[1], K.A. Hill[1], Z. Chen[1], K.D. Gonzalez[1,2], D. Gu[1,2]* 1) Dept Molecular Genetics, City of Hope National Medical Center, Becker Research Inst, Duarte, CA; 2) Clinical Molecular DIagnostic Laboratory (CMDL), Department of Molecular Diagnosis, City of Hope National Medical Center, Duarte, CA.

We created an EGFR Molecular Epidemiology Database website that curates somatic EGFR mutations in non-small cell lung cancer (NSCLC) and associated epidemiological and methodological data including response to the tyrosine kinase inhibitors Gefitinib and Erlotinib. Herein, we analyze 809 mutations collected from 26 publications. The EGFR mutations are consistent with strong biological selection for gain of function. Four super hotspots account for 70% of reported mutations while two-thirds of 132 unique mutations have occurred only once, yet these single reports account for only 11% of reported mutations. The mutations are missense or in-frame microdeletions or microinsertions, consistent with gain of function mutations. Microdeletions and microindels are common in a small region of exon 19. Microdeletions are predominantly in-frame and delete 15 or 18 bp. Microindels, which account for 8% of mutations, have smaller inserted sequences (95% are 1 to 5 bp) and are elevated 16-fold relative to mouse somatic mutations and to human germline mutations in the Human Gene Mutation Database (HGMD). The 1-2 microindel commonly observed in mouse somatic mutations, human p53 somatic mutations and human germline mutations (HGMD) was NOT observed in EGFR. EGFR mutations in smokers DO NOT carry signatures of mutagens in smoke. Microdeletions/microindels are more frequent in responders to Gefitinib or Erlotinib (p=0.007). The pattern of mutation counts does not differ significantly with respect to gender, age, or tumor histology. The EGFR Mutation Database is designed as a central resource of EGFR mutation epidemiology data for clinicians, geneticists and other researchers. These data and data from animal studies suggest that the EGFR mutations found in elderly patient with lung cancer generally derive many years earlier from mutational events during lung development i.e. before direct exposure of the lung to cigarette smoke.

Enzyme replacement therapy on hypophosphatasia by using C-terminus-anchorless tissue-nonspecific alkaline phosphatase. *S. Tomatsu, H. Oikawa, T. Nishioka, M. Gutierrez* Dept Pediatrics, Ped Res Inst, St Louis Univ, St Louis, MO.

Hypophosphatasia is caused by deficiency of activity of the tissue-nonspecific alkaline phosphatase (TNSALP), resulting in a defect of bone mineralization. Plasma infusions were attempted but little clinical improvement was achieved. There is no definitive treatment available. Enzyme replacement therapy for hypophosphatasia was thought to be quite difficult since the TNSALP enzyme is membrane-bound and functions physiollogically when the enzyme is present at the cell membrane. The tissue TNSALP knock-out mouse is a model of infantile hypophosphatasia displaying impaired bone mineralization, epileptic seizures, apnoea, abnormal apoptosis in the thymus, abnormal lumbar nerve roots, and postnatal death before the weaning. To investigate clinical effectiveness with ERT for hypophosphatasia, we deleted the c-terminus of TNSALP cDNA and transfected into Chinese hamster ovary (CHO) cell line. The resultant secreted form of rhTNSALP secreted by CHO cells was purified and characterized in vitro. The weekly infusion of enzyme replacement therapy onto TNSALP knockout mice was administered. In vitro mineralization assays with the rhTNSALP in the presence of high concentrations of pyrophosphate provided evidence of bone mineralization with the bone marrow from a hypophosphatasia patient. Weekly intravenous administration of the purified rhTNSALP enzyme with 5 mg/kg dose into TNSALP knockout mice increased dramatically the life span with the increased body weight, showing that the treated mice lived at least 6 times as long compared to the untreated mice. Treated mice had no epileptic seizures at least till 3 months old. These results show the C-terminus-anchorless TNSALP functions bioactively in vivo and that is a potential therapeutic agent of ERT for hypophosphatasia.

Delineation of chromosome 9p rearrangements: A model for diverse chromosomal mechanisms of formation. *S. Schwartz[1], C. Crowe[2], M. Graf[3], H. Mashek[1], S. Lillis[4]* 1) University of Chicago, Chicago, IL; 2) MetroHealth Med Center, Cleveland, OH; 3) TGEN, Pheonix, AZ; 4) Children's Mercy Hospital, Kansas City, MO.

Over the past decade our knowledge of the phenotypic abnormalities associated with chromosomal abnormalities and the mechanisms responsible for the formation of these abnormalities has increased. While considerable work has been undertaken regarding many deletions, much less has been done with duplications. We have had the opportunity to study 24 chromosomal abnormalities involving the duplication of a portion of chromosome 9 of which 87.5% of the abnormalities were de novo and 12.5% of these were familial. Of the 24 abnormalities, seven different mechanisms have been identified as responsible for the abnormalities. This includes the formation of acentric, dicentric, segmental duplicated and duplicated/deleted chromosomes. A large proportion of these abnormalities are found to be more complex than expected from standard G-banding. Analysis of these cases along with other reported cases implicate an approximate 4 Mb region as a critical region for the characteristic phenotype in this syndrome. Results from these studies are interesting and reveal important information including: (1) Duplications of 9p are formed by a variety of mechanisms, probably due to both the lack of genes in 9p as well as the nature of DNA in 9p; (2) All of the accessory chromosome involving the entire short arm of 9p were dicentric containing all of 9p and two copies of the centromeric and pericentromeric region; (3) There are more complex abnormalities than expected. These complex rearrangements include acentric, dicentric and duplicated/deleted chromosomes. (4) The 4 Mb region identified as the critical region overlaps the critical region identified for the 9p deletion syndrome; (5) As the distal region of the chromosome arm is gene poor, much of the phenotype seems similar regardless of the amount of material duplicated, providing that the critical region is included. (6) One of the familial cases had a very mild phenotypes, in both the parent and proband, and did not involve a duplication of the critical region, but rather involved duplication of material distal to the critical region.

***PRODH* sequence variants in 22q11 deletion syndrome (22q11DS).** *A.S. Willis[1,2], J.A.S. Vorstman[3,4,5], M. De Sain-van der Velden[6], B. Dorland[6], B.S. Emanuel[3,7], D. Valle[1,2]* 1) Inst Genetic Medicine, Johns Hopkins Sch Med, Baltimore, MD; 2) Howard Hughes Medical Inst; 3) Human Genetics, Childrens's Hospital of Philadelphia, PA; 4) Child & Adolescent Psych, Univ Med Ctr, Utrecht, Netherlands; 5) Rudolf Magnus Inst of Neurosciences, Utrecht, Netherlands; 6) Metabolic Diseases, Univ Med Ctr, Utrecht, Netherlands; 7) Pediatrics, University of Pennsylvania Sch Med.

PRODH is located in the 22q11DS critical region and encodes proline oxidase (POX), which the first enzyme in the proline (Pro) degradation pathway. Several *PRODH* variants are known, and some have been suggested to contribute to the 25-fold increased risk 22q11DS patients have for schizophrenia (Sz). Previous studies have shown that about half of 22q11DS patients have hyperprolinemia. We are testing the hypothesis that sequence variants in the remaining *PRODH* allele account for the hyperprolinemia and relate to the risk for Sz and other neurological abnormalities in these patients. We sequenced the remaining *PRODH* allele in 53 22q11DS patients who have also undergone neuropsychiatric and psychophysiologic testing. We identified 11 missense and 9 synonymous variants. Two missense variants, Q19P and R185W, result from known SNPs and occur in 41 and 32 of the 53 *PRODH* genes, respectively, with 30 alleles having both. We have not considered these common variants in our initial analysis. We detected the remaining 9 missense variants in 1-6 individuals, each. Previously, we tested 5 of these for effect on enzyme activity (Bender et al, AJHG 76:409, 2005) and are in the process of testing the remaining 4. Pending full functional data, we divided the patient sample into thirds based on plasma Pro levels. We find that 8 of 17 in the high Pro group had missense variants as compared to 3 in the middle and 4 in the low Pro group, including 2 variants we previously showed to encode POX with increased activity. These results support our hypothesis that genotype at the remaining *PRODH* allele correlates with the plasma Pro levels and suggests that this variable should be considered in analyzing the phenotypic spectrum in 22q11DS.

A role for the developmental gene NOTCH2 in hepatoblastoma. *R.A. Schultz[1], G.E. Tomlinson[2,3,8], D. Rakheja[1], T. Chen[3], N.R. Schneider[1], K. Wilson[1], C. Echebiri[3], B.A. Hirschman[3], A.R. Brothman[4], P. Eis[5], T.A. Richmond[5], R.R. Selzer[5], J.H. Feusner[6], M. Malogolowkin[7], R.L. Stallings[9], Children's Oncology Group* 1) Dept Pathology, UT Southwestern, Dallas, TX; 2) Dept Pediatrics, UT Southwestern, Dallas, TX; 3) Hamon Center for Therapeutic Oncology Research, UT Southwestern, Dallas, TX; 4) University of Utah, Salt Lake City; 5) NimbleGen Systems, Inc. Madison, WI; 6) Childrens Hospital of Oakland, Oakland California; 7) Childrens Hospital of Los Angeles, Los Angeles, CA; 8) Children's Medical Center of Dallas, Dallas, TX; 9) Childrens Cancer Research Institute, UT Health Science Center at San Antonio, TX.

Hepatoblastoma, the most common malignant liver tumor in children, typically occurs between birth and five years of age, after which tumor development becomes uncommon. Histologically these tumors resemble fetal and embryonal liver elements in a disorganized growth pattern. We have determined that cytogenetically hepatoblastoma is characterized by numerical increases in chromosomes 2, 8 and/or 20 and by unbalanced translocations involving a recurrent breakpoint at 1q12. Using FISH, BAC-CGH arrays with 1 megabase resolution, and fine-tiling oligonucleotide array CGH with 60-80 base pair spacing, we have characterized tumors bearing chromosome 1q12 translocations. The results demonstrate that NOTCH2, a gene involved in liver development and differentiation, is disrupted in each of these tumors. Disruption appears to occur through a mechanism involving pericentric inversion prior to translocation. Immunohistochemical studies on hepatoblastoma tumor tissues and adjacent liver revealed strong staining for NOTCH2 in hepatoblastoma tumor cells and not in adjacent liver cells. Aberrant expression of NOTCH2 could contribute to tumor formation by expanding or maintaining that population of immature cells in the liver that are the substrate for hepatoblastoma. The results provide another strong link between alterations in normal development and the pathogenesis of embryonal tumors in children. The power of fine-tiling oaCGH in the characterization of translocation breakpoints in cancer is again demonstrated.

Structural and DNA binding differences between VWS and PPS mutations in IRF6. *N.K. Rorick[1,3], L. Gakhar[2], T.R. Waldshmidt[3], D. Schipper[3], J.C. Murray[3], B.C. Schutte[3]* 1) Genetics PhD Program, Univ Iowa, Iowa City, IA; 2) Biochemistry Dept, Univ Iowa, Iowa City, IA; 3) Pediatrics Dept, Univ Iowa, Iowa City, IA.

IRF6 is a member of the IRF family of transcription factors that contain a penta-tryptophan DNA binding domain (DBD) and a protein-binding domain. Mutations in Interferon Regulatory Factor 6 (IRF6) cause two dominantly inherited forms of cleft lip and palate, Van der Woude (VWS) and popliteal pterygium syndromes (PPS). Missense mutations in the DBD are observed in cases of both VWS and PPS; however, the phenotype of PPS is more severe, with skin and limb abnormalities along with cleft lip and palate. The goal of this study is to delineate potential differences in structural and functional effects of VWS- and PPS-associated missense mutations in the DBD. The affect of missense mutations on protein secondary structure was predicted with NNpredict and ProteinPredict . We observed that VWS mutations were more likely to perturb secondary structure than PPS mutations ($p = 0.037$). To test the predicted structural effects biochemically, circular dichroism spectrophotometry was performed on R84G (VWS-causing), R84C (PPS-associated), and wild-type DBDs. We observed a loss of secondary structure in the R84G, but no effect with the R84C mutation. These results support the structural effects predicted computationally. To examine the DNA binding function of the mutants, gel shift assays were performed on 5 VWS and 8 PPS mutants. Nearly all mutations abrogated DNA binding activity. Exceptions were normal binding with one VWS mutant, G70R, and very weak binding by two PPS mutants, L22P and Q82K. The general loss of DNA binding by VWS missense mutations is consistent with the predicted changes in secondary structure. The G70R exception suggests an alternative effect on IRF6 function. One possibility is an alteration in tertiary structure. Our data suggests that most PPS mutations retain their structure, but are unable to bind to DNA. The structural and functional data coupled with the phenotypic data are consistent with the hypothesis that PPS mutations have a dominant negative effect on IRF6 function.

Euchromatic duplication 9q21-9q22. A benign variant? *D.M. Stenberg, E.N. McDonald, G.S. Sekhon* Cytogenetics Lab., Genzyme Genetics, Santa Fe, NM.87505.

We identified 10 families (7 prenatal and 3 postnatal) with duplication of band 9q21-9q22. Four of the amniotic fluid samples were studied due to advanced maternal age, two due to nuchal thickening, and one for positive serum screen. The indication for all of the postnatal blood was phenotypic abnormalities. Chromosome analysis follow-up of the prenatal families showed that the duplication was of paternal origin in 4, maternal origin in 2 and de novo in 1. The parental follow-up studies of postnatal cases were performed in 2 of the families and in one of the family parents were not available. One was paternal and 1 was maternal. Using different banding techniques and fluorescence in situ hybridization with specific DNA probes, and C-banding, the structural rearrangements involved were considered. We suggest that possibly duplication of 9q21-9q22 can be regarded as euchromatic variant without any phenotypic effects as in the case of chromosome 9 variant with an extra G-dark band within the heterochromatic region. The strategy at present has been to offer parental chromosome study. If inherited from a phenotypically normal parent, it is most likely a euchromatic variant. In future studies, array comparative genomic hybridization (CGH) analysis will be a useful tool to more completely characterize the rearrangement.

Potential pharmacological chaperones for late-onset GM2 gangliosidosis. *M. Tropak[1], G.H.B. Maegawa[1,2], F. Kok[3], J.T.R. Clarke[1,2], D.J. Mahuran[1]* 1) Metabolism - Research Institute, Hospital for Sick Children; 2) Div of Clinical/Metabolic Genetics, Hospital for Sick Children, Toronto, ON, Canada; 3) Div of Neuropediatrics, Sao Paulo University, Brazil.

GM2 gangliosidosis (GM2) is an inherited neurodegenerative disorder caused by lysosomal -hexosaminidase A (Hex A) deficiency. Objective: test in fibroblast cell lines from patients diagnosed with late-onset GM2, both Tay-Sachs (TSV) and Sandhoff variants (SV), potential chemical compounds identified by high throughout screening (HTS). Results: Patients fibroblasts with diverse types of mutations were cultured in media with two compounds, N-acetylglucosamine thiazoline (NGT) and one of the hits from the HTS (H1). Following 5 days of treatment, Hex A activities were determined using MUGS. TSV cell lines with -G269S/null allele showed over 3-fold increases in Hex A activity with H1, and over 6-fold increase with NGT. Among the SV cell lines, one patient cell line homozygous for C137Y mutation showed close to 6-fold increase of Hex A activity in media with both H1 and NGT. The -R505E/IVS11+5G>A cell line showed 4 to 5-fold increased Hex A activity under H1 and NGT, respectively. The -G353R/IVS12-27G>A showed approximately 3-fold increase of Hex A under H1, and 2-fold increase under NGT. TSV patient cell lines with other juvenile mutations, such as R178H, R499C and R499H show mild Hex A activity to either H1 or NGT. Other lysosomal enzyme activities such as -galactosidase and -glucosidase remained unchanged. Western blots correlated with the observed Hex A activity increases revealed in cells exposed to the two studied compounds. Conclusion: The NGT and H1 can function as pharmacological chaperones for specific *HEXA* and *HEXB* mutations in late-onset GM2. We hypothize that these chemical compounds are able to stabilize the native-like conformation of the enzyme and, consequently, increasing the amount of Hex A exiting the endoplasmatic reticulum and reaching lysosomes. The identified compounds constitute a potential alternative as specific therapeutic approach for the subacute and late-onset forms of GM2. Key words: GM2 gangliosidosis; hexosaminidase; pharmacological chaperone.

Population pharmacokinetic modeling using diplotype configuration. *A. Saito[1], K. Tanikawa[2], M. Takeuchi[3]* 1) Central Research Laboratory, Hitachi, Ltd., Tokyo, Japan; 2) Life Science Group, Hitachi, Ltd., Saitama, Japan; 3) Division of Biostatistics, Kitasato University Graduate School, Tokyo, Japan.

For optimizing drug regimens in individual patients, it is important to predict individual pharmacokinetic behavior with high accuracy. The differences in individual pharmacokinetics are attributed to genetic information, although the effects of other factors, such as age, gender, and disease, are not negligible. To estimate the influences of genetic variation on pharmacokinetic differences, we present a new pharmacokinetic analysis method which combines population pharmacokinetic modeling with population genetic approach. The presented method is based on non-linear mixed effect modeling which is the most used method for population pharmacokinetic analysis. We include information of haplotype or diplotype configuration into non-linear mixed effect model as a covariate on fixed (population-typical) or random (population-variability) effect parameters. We have validated our method with the datasets from extensive simulation studies, comparing the estimated results to those obtained by the conventional ethnicity-based modeling. The results showed that our method could make more accurate prediction of individual pharmacokinetic behavior than the conventional methods.

A loss-of-function mutation in the *FTSJ1* gene causes non-syndromic mental retardation in a Japanese family. *K. Takano[1], E. Nakagawa[1], K. Inoue[1], F. Kamada[2], S. Kure[2], Y. Goto[1]* 1) Department of Mental Retardation and Birth Defect Research, National Institute of Neurology NCNP, Tokyo, Japan; 2) Department of Medical Genetics, Tohoku University School of Medicine, Sendai, Japan.

A prevalence of mental retardation (MR) is thought to be 0.6-1.3 % in Japanese children from three to twelve years old. Although the etiology of MR remains largely unknown, genetic abnormalities appears to be a major factor. Recent large-scale linkage studies mainly performed in European and North American countries, have successfully identified many X-linked mental retardation (XLMR) genes including *FTSJ1*. Here we identified a family with a mutation in *FTSJ1* through a screening of genetic abnormalities in XLMR genes using a cohort of 41 Japanese families. *FTSJ1* is a human homolog of the *Escherichia coli 2-O*-rRNA methyltransferase FtsJ/RrmJ gene, but its function in the central nervous system is unknown. The family has five affected males with non-syndromic mental retardation consisting with X-linked recessive inheritance. Sequence analysis of the proband and his mother revealed a GA substitution at the donor consensus splice site in intron 8 (IVS8+GA) in the *FTSJ1* gene. This mutation resulted in an abnormal transcript longer than the wild type fragment due to the involvement of the entire intron 8 into the transcripts. By conceptual translation, incorporation of the intronic sequence leads to a frameshift in the *FTSJ1* mRNA resulting in a premature termination codon in exon 9. Quantitative RT-PCR showed significant reduction of mutant *FTSJ1* transcript in patients lymphoblast cells, which was upregulated by suppression of nonsense-mediated mRNA decay (NMD) using cycloheximide. Therefore, this mutation resulted in a lack of *FTSJ1* transcript and the loss-of-function is likely responsible for the phenotype of this patient. The frequency of *FTSJ1* mutation in one out of 41 families in Japanese families appears to be compatible with the frequency described in a European study wherein four families have been found out of 249 non-syndromic XLMR families. Supported by the Research Grant (15B-4, 18A-5) for Nervous and Mental Disorders from the Ministry of Health, Labour and Welfare, Japan.

High resolution CGH of breast cancer. *D.N. Roberts[1], C.A. Carmack[1], A. De Witte[1], S. Milligan[1], E. Lin[1], J. Gao[1], S. Giles[1], S. Shchegrova[1], E. LeProust[1], P. Webb[1], D. Amorese[1], J. Gregg[2]* 1) Integrated Biology Solutions, Agilent Technologies, Santa Clara, CA; 2) UC Davis Medical Center, Sacramento, CA.

Array based Comparative Genomic Hybridization (CGH) provides a means for the determination of DNA copy number aberrations. We have designed a CGH array consisting of ~244,000 in situ synthesized 60-mer oligonucleotides spanning the entire human genome, resulting in an average genomic distance between probes of ~12 Kbp. Using these arrays, we performed an analysis of copy number changes in breast carcinomas and in breast carcinoma cell lines. In several cases displaying amplifications around ERBB2, we utilized a second, high definition CGH array with >20,000 probes dedicated to chromosome 17, with particular emphasis on the region encompassing ERBB2 (17q21.2-q21.3). Using these data in combination, we define multiple common aberrations in our sample set. Further, we analyze patient chromosomal/gene copy number with respect to the levels of ERBB2 protein, clinical classification, and outcome.

Whole Genome Association Studies in Admixed Populations. *N. Risch[1], H. Tang[2]* 1) Univ California, San Francisco, San Francisco, CA; 2) Fred Hutchinson Cancer Research Center, Seattle, WA.

Admixture mapping (AM) is a method that exploits ancestral allele frequency differences to map disease susceptibility genes in recently admixed populations such as African Americans and Latinos. With the advent of high density genotyping platforms with as many as 500K SNPs or more for whole genome association (WGA) studies, a question arises as to the relative power of direct association versus admixture mapping in such populations. Previously we have shown that even with lower density platforms (100K), it is possible to reconstruct the ancestry block structure of an admixed individual, making AM essentially completely informative. Here we show that in a direct association analysis comparing allele frequencies between cases and controls in an admixed population, the chi-square statistic can be partitioned into two nearly additive components, one chi-square (one df) corresponding to the local ancestry difference between cases and controls, and the other a Mantel-Haenszel (MH) chi-square (one df) stratified on the local ancestry of the chromosomes. We evaluate the relative magnitude of these two components across a range of values of disease allele frequency, admixing proportions, genotype relative risk, and the ancestral allele frequency difference (delta). We show that the MH component generally dominates the overall chi-square for delta values up to .6 (accounting for up to 65 percent); for delta values of .4 it accounts for 85 percent or more; for delta values greater than .7 (which are rare), the ancestry component begins to dominate. Hence, it is likely that in the large majority of cases, direct association will be more powerful than admixture mapping. However, this conclusion depends on the assumption that the causal SNP or one in very strong LD is included in the high density platform.

We also note an additional advantage of high density SNP analysis in admixed populations. The MH analysis stratified on local ancestry provides an internal cross-ethnicity replication study since the allelic odds ratios can be studied within each ancestral group of chromosomes (between cases and controls) and compared.

Genetic differences across Finland using genomewide SNP data. *C.J. Willer[1], L.J. Scott[1], H.M. Stringham[1], T.T. Valle[2], N.A. Rosenberg[3], R.N. Bergman[4], K.L. Mohlke[5], J. Tuomilehto[2,6], F.S. Collins[7], M. Boehnke[1]* 1) Dept of Biostatistics, University of Michigan, Ann Arbor, MI; 2) Dept of Epidemiology and Health Promotion, National Public Health Institute, Helsinki, Finland; 3) Dept of Human Genetics & Life Science Institute, University of Michigan, Ann Arbor, MI; 4) Dept of Physiology and Biophysics, Keck School of Medicine, University of Southern California, Los Angeles, CA; 5) Dept of Genetics, University of North Carolina, Chapel Hill, NC; 6) Dept of Public Health, University of Helsinki, Helsinki, Finland and South Ostrobothnia Central Hospital, Seinäjoki, Finland; 7) Genome Technology Branch, National Human Genome Research Institute, Bethesda, MD.

We are carrying out a genomewide association scan of 300,000 SNPs on 2363 Finnish individuals using the HumanHap300 BeadChip from Illumina. Here we evaluate possible geographic stratification of SNP allele frequencies across 12 historical provinces of Finland. Our sample consists of type 2 diabetic individuals and non-diabetic controls, matched for province of birth. Each province is represented by 78 to 300 genotyped individuals. In our initial analysis of autosomal SNPs genotyped for 1743 individuals, we find an excess of significant allele frequency differences among the 12 provinces and between all pairs of provinces. Even the most similar provinces show a 9-fold excess of significant allele frequency differences at p .0001. The pairs of provinces showing the strongest genetic differences are geographically distant (for example, Vaasa on the west coast and North Karelia in the east) and our results are in agreement with reported historical migration patterns and regional subisolates. These results suggest that careful case-control matching may be required to avoid false-positive results in genomewide association scans, even in populations often described as homogeneous.

Variance Component Linkage Analysis Allowing for Heterogeneity in Effect Sizes due to Measured Covariates.
J. Zheng, W.M. Chen, G.R. Abecasis Department of Biostatistics, University of Michigan, Ann Arbor, MI.

A common approach for quantitative trait linkage analysis in human pedigrees involves the use of variance component models. In a conventional variance components model, the genetic effect and environmental effect are modeled as random effects and the fixed effect due to covariates are assumed to be independent of random effects. In some situations, measured covariates may actually modify the size of genetic effects rather than directly affecting the trait mean. In these cases, especially when the interaction between the covariate effect and genetic effect are strong, failure to model the interaction may result in a much reduced power to detect linkage. In two recent papers, Pilia et al. (2006) found that among 98 cardiovascular and personality traits, about half showed heterogeneity in variance components by age, by sex or both, and Weiss et al (2006) also found substantial evidence for heterogeneity by sex in several human QTLs. Here, we extend the variance-component method to model genetic and random environmental variance components for each individual as linear functions of measured covariates. This model can conveniently incorporate the effects of binary or continuous covariates, or even multiple covariates in one analysis. Through simulations, we show that in the presence of interactions between measured covariate and genetic effects, our extended variance component model maintains correct type I error rates and demonstrates improved power to detect linkage. In contrast, the conventional variance component model only has very limited power to detect linkage when there is substantial heterogeneity in QTL effect sizes. For example, when we simulated traits whose heritability varied with age by a factor of 2 to 3 fold, we found several situations where the standard variance component model had <20% power but our model provided >80% power. We believe that our approach will be helpful in the mapping of the many human quantitative trait loci whose effects vary according to age, sex or another covariate.

Genetic dissection of male infertility-related molecular pathways by transcriptional profiling of testicular biopsies from patients with non-obstructive azoospermia. *A. Tajima[1], Y. Sakamoto[1], H. Okada[2], A. Tanaka[3], K. Shichiri[4], K. Tanaka[2], I. Inoue[1]* 1) Div Genetic Diagnosis, IMS, Univ Tokyo, Tokyo, Japan; 2) Dep Obstet & Gynecol, Niigata Univ School of Medicine, Niigata, Japan; 3) Saint Mother Obstet & Gynecol Clinic, Fukuoka, Japan; 4) Tachikawa Hospital, Niigata, Japan.

Male-factor infertility accounts for about half the cases in which assisted reproductive techniques are recommended. Many factors such as spermatogenic failure could cause male infertility, however, the etiologies and pathogenesis of this disease remain poorly understood. To identify new genes and/or pathways underlying male infertility with spermatogenic dysfunction, we performed a microarray-based gene-expression profiling in infertile testes. Infertile testicular biopsies were obtained under informed consent from non-obstructive azoospermia (NOA; n=47) and obstructive azoospermia (OA; n=11) patients. We found 2,611 transcripts as differentially expressed between NOA and OA testes, using Agilent Human 1A(v2) Oligo microarrays. Gene ontology-based profiling of the 2,611 transcripts revealed a significant association with biological processes involved in male gamete generation. To find novel NOA subclasses, the 2,611 transcripts were further examined with non-negative matrix factorization (NMF) method, a recently introduced clustering approach in class discovery. The NMF analysis provided three robust NOA subclasses, among which there were statistically significant differences in NOA-related clinical characteristics such as testicular pathological score. Subsequent statistical analysis showed that 149 transcripts (P<0.05) were differentially expressed among three NOA subclasses. The among-subclass differences in testicular expression for 53 transcripts with highly statistical significance (P<0.01) were confirmed by quantitative real-time RT-PCR method. These findings indicate that our strategy is successful in disclosing a group of transcripts related to spermatogenic defects and NOA classification. The 149 transcripts would therefore be diagnostic markers for NOA phenotypes, as well as potential candidates for susceptibility to NOA.

How mutations at the 3' end region of exons cause aberrant splicings? - *In silico* and *in vitro* analyses to answer the question -. *K. Sahashi[1,2], J. Shinmi[1], G. Sobue[2], K. Ohno[1]* 1) Division of Neurogenetics and Bioinformatics, Center for Neurological Diseases and Cancer, Nagoya University Graduate School of Medicine; 2) Department of Neurology, Nagoya University Graduate School of Medicine.

In the spliceosomal formation, U1 snRNA recognizes the 5 splice site and initiates pre-mRNA splicing. We constructed *SNCA*, *PARK2*, *PINK1*, and *PARK7* minigenes harboring exonic mutations causing Parkinson's disease, and tested if any of these mutations cause aberrant splicings. We found that an A-to-G substitution at position -2 of *PINK1* exon 6 predicting E417G and a G-to-C substitution at position -1 of *PARK7* exon 2 predicting E64D cause aberrant skipping of the mutation-harboring exon. We comprehensively analyzed nucleotide frequencies at positions -3 to +6 of the whole human genome comprised of 179,917 5' splice sites. The analysis revealed that non-consensus nucleotides at positions -3, -2, and -1 are complemented by consensus nucleotides at positions +4, +5, and +6. Next, we constructed minigenes harboring five exonic mutations of five different genes at position -2, which are known to cause aberrant splicings. We also constructed minigenes carrying four exonic mutations of four different genes at positions -3, -2, and -1, and found that these mutations also affect pre-mRNA splicings. Using the eleven minigenes above, we introduced a consensus nucleotide one by one, and found that a consensus nucleotide at position +4, +5, or +6 ameliorates aberrant splicings, whereas a consensus nucleotide at position -3 or -2 has no effect. We also confirmed that introduction of a single complementary nucleotide into U1 snRNA corresponding to position +4, +5, or +6 restores normal splicing, whereas mutagenesis corresponding to position -3 has no effect. Both *in silico* and *in vitro* analyses disclose a scenario that a mutation at the 3' end region of an exon causes aberrant splicing when nucleotides at positions +4, +5, and +6 are not complementary to U1 snRNA. When we identify a mutation at position -3, -2, or -1, we should see nucleotides at positions +4, +5, and +6, as we should take different therapeutic strategies for missense and splicing mutations.

SLC3A1 knockout mice model human cystinuria. *A. Sahota[1], E. Cui[1], H.J. Vernon[2], M. Yang[1], J. Chen[1], C. Osborne[1], E. Stambrook[1], S. Bledsoe[3], A.P. Evan[3], J.A. Tischfield[1]* 1) Dept. Genetics, Rutgers Univ., Piscataway, NJ; 2) Dept. Pediatrics, Johns Hopkins Univ., Baltimore, MD; 3) Dept. Anatomy & Cell Biol., Indiana Univ. Med. Sch., Indianapolis, IN.

Cystinuria is an inherited metabolic disorder caused by mutations in the diabasic amino acid transporter system rBAT/b0,+AT. Based on phenotypes in heterozygotes, cystinuria is classified as type I or non-type I. The type I form is fully recessive, resulting from mutations in the SLC3A1 gene, encoding rBAT. The non-type I form is incomplete recessive, and is caused by mutations in b0,+AT, encoded by SLC7A9. Cystine accumulation leads to stone formation, recurrent infections, and subsequent renal failure. We are using SLC3A1-/- mice to determine whether the initial sites of crystal deposition within the renal system and the mechanisms of crystal-induced renal injury are unique for each crystal type or whether different crystal types share common pathways. Urine from 4-months-old SLC3A1-/- mice was analyzed by HPLC for cystine and the dibasic amino acids. Urinary crystals were examined by light microscopy and infra red spectroscopy, and kidney and bladder sections by light microscopy. Changes in the renal expression of a set of early response genes were examined by real time PCR. At age 4 months, null mice excrete cystine (including crystals) and dibasic amino acids in the urine. At this age, there are very few cystine stones in the kidney, but there is massive stone accumulation in the bladder. There is no evidence for gene-level changes in kidneys from 1-month-old SLC3A1-/- mice compared with wild type mice. Our initial studies suggest that SLC3A1 knockout mice mimic the etiology and clinical manifestations of human cystinuria type I, but the initial site of crystal deposition and the initial pathophysiology is markedly different compared with a knockout mouse model for another stone disease, adenine phosphoribosyltransferase (APRT) deficiency. Cystinuria is a disease of obstructive uropathy, rather than intra-renal crystal deposition (APRT deficiency), suggesting that different crystal types induce different cellular and molecular responses.

Results from a Multi-national, Multi-center, Prospective, Observational Study in Patients with Late-onset Pompe disease. *J. Wokke[1], D. Escolar[2], A. Pestronk[3], P. Laforet[4]* 1) Neurology, University Hospital Utrecht, Utrecht, Netherlands; 2) Children's National Medical Center, Washington, DC; 3) Washington University, St Louis, MO; 4) Hopital de la Salpetriere, Paris, France.

Objective: The objective of this study was to better characterize the clinical presentation of patients with Late-Onset Pompe disease for the purpose of identifying the best outcome measures for the assessment of efficacy in future clinical trials. **Methods:** This was a multi-center, multinational, 12-month observational study. Eligible patients had to be both ambulatory and non-invasively ventilated. Baseline measurements included -glucosidase acid residual enzyme activity, genotyping, manual muscle testing (MMT) of 34 muscle groups, quantitative muscle testing (QMT) of seven muscle groups, functional activities and grades, pulmonary function testing (PFT), and quality of life (QOL) assessments. Pulmonary and muscle function testing was repeated at 1, 3, 6, and 12 months. A 6 minute walk test (6MWT) was introduced at the Month 12 visit. **Results:** Fifty-eight patients (22 males and 36 females), aged 24-69 years completed the study. Age at symptom onset was 28.9 11.6 years. Disease duration was 15.6 9.2 years. Almost all patients (93%) presented with proximal lower limbs muscle weakness, with mean QMT knee extensors and flexors of 39 and 48% of predicted normal, respectively. 6MWT distances were less than 80% of predicted values for 96% of tested patients. Mean FVC % at baseline was 66.8% (sd = 21.5), mean postural drop from upright to supine was -27.5% (SD = 17.6), and 22.4% of patients were using nocturnal ventilation. Significant correlations were observed between FVC %, 6MWT distance, QMT and MMT. Mean FVC% predicted and mean QMT Leg Score declined by 3.5% and 3.2% respectively over the 12-month period. **Conclusion:** In this largest cohort of late-onset Pompe Disease patients ever described, a slow progression of pulmonary or muscle weakness has been observed over 12 months. The outcome measures in this study were found to be sensitive and reliable for use in clinical trials. Relationships between clinical features and genotype will be analyzed.

An Ancient Haplotype Pinpoints *IGF1's* Role In Determining Dog Body Size. *N.B. Sutter[1], M. Gray[2], K. Chase[3], P. Quignon[1], H.G. Parker[1], S. Davis[1], E. Karlins[1], G. Johnson[4], M. Nordborg[5], C.D. Bustamante[6], R.K. Wayne[2], K.G. Lark[3], E.A. Ostrander[1]* 1) National Human Genome Res Inst, Bethesda, MD; 2) University of California, Los Angeles, CA; 3) University of Utah, Salt Lake City, UT; 4) University of Missouri, Columbia, MO; 5) University of Southern California, Los Angeles, CA; 6) Cornell University, Ithaca, NY.

Dog breeds vary 50 fold in adult mass from tiny Chihuahuas to 200 pound Mastiffs and Irish Wolfhounds. Previously, a microsatellite genome-wide scan was completed in the mid-size Portuguese Water Dog (PWD). Using 91 skeletal measurements from radiographs of 500 dogs several quantitative trait loci (QTL) for skeletal size were found. The marker FH2295 QTL is positioned within 1 Mb of the *insulin-like growth factor 1* gene (*IGF1*), a strong genetic determinant of body size in mice.

Our sequencing identified no explanatory mutations in *IGF1* exons. We sought evidence that the genes action controls body size in dogs by genotyping 75 *IGF1* SNPs in Portuguese Water Dogs, 350 dogs from 15 giant and 18 small breeds, and grey wolf, coyote, jackal, and fox samples.

We found a striking association between body size and *IGF1* haplotypes both in the PWD and in giant and small breeds ($p < 10^{-60}$). Small PWDs carry the same 13 SNP *IGF1* haplotype as dogs from all but one of the 18 small breeds. No dogs from 15 giant breeds carry this haplotype. Dogs from giant breeds primarily carry one of two divergent haplotypes. One of these haplotypes occurs in the PWD population and dogs carrying it are largest in size. The grey wolves carry haplotypes very similar to both of the large haplotypes but not the haplotype found in small dog breeds. Our data support a model in which an *IGF1* mutation that arose early in the history of dogs is responsible for much of the variation in body size in modern pure breeds. These findings aid our understanding of growth regulation and set the stage for mapping complex traits of interest to human and companion animal biologists by taking advantage of the breed structure within the canine population.

Admixture mapping identifies 8q24 as a locus explaining >39% of prostate cancer cases in younger African American men. *D. Reich[1,2], M.L. Freedman[2,3], C.A. Haiman[4], N. Patterson[2], G.J. McDonald[1,2], A. Tandon[1,2], A. Waliszewska[2,5], K. Penney[2,3], C. Montague[1,2], K. Ardlie[2,6], E.M. John[7,8], I. Oakley-Girvan[7,8], A.S. Whittemore[7,8], K.A. Cooney[9], S.A. Ingles[4], D. Altshuler[1,2,10], B.E. Henderson[4]* 1) Harvard Med School; 2) Broad Inst of Harvard & MIT; 3) Dana Farber Cancer Inst; 4) Univ Southern California; 5) Brigham & Women's Hospital; 6) Genomics Collaborative Inc; 7) Northern California Cancer Center; 8) Stanford Univ; 9) Univ Michigan; 10) Massachusetts General Hospital.

We carried out an admixture scan for prostate cancer genes in 1,597 African Americans cases and 873 controls typed at 1,365 SNPs. Admixture mapping works by screening through the genome in a population of mixed ancestry, searching for regions where cases have an unusually high rate of inheritance from the ancestral population with higher risk. Since African Americans have a ~1.6-fold higher incidence of prostate cancer, we hypothesized that a risk locus would be detectable as a rise in African ancestry compared to the average of 78%.

We identified a 3.8 Mb interval that is associated with higher African ancestry in prostate cancer cases (LOD=7.1). The region spans 125.68-129.48 Mb in Build 35 of the human genome reference sequence, and contains 9 predicted genes including *MYC*. An allele at 8q24 was also independently found by Amundadottir (2006), confirming the importance of this locus in prostate cancer. Here we also report two novel results: (i) the locus has the strongest effect in men diagnosed at age <72 ($P<0.0003$), and (ii) the previously identified allele can explain at most a small fraction of the admixture signal. We estimate that the rate of prostate cancer in younger African Americans would be reduced by 39-59% if one could treat the additional risk conferred by African chromosomes.

This study confirms that admixture mapping is a powerful and practical way of localizing genes for complex disease. Molecular identification of prostate-cancer causing variants at 8q24 has promise for prostate cancer prevention and treatment. We conclude by reporting a fine-mapping scan of ~1,700 SNPs to comprehensively search for these variants.

Variation and Selection in the UCSC Genome Browser. *D. Thomas*[1, 2], *H. Trumbower*[2], *A. Kern*[2], *D. Haussler*[1, 2], *W.J. Kent*[1, 2] 1) Biomolecular Engineering, UC Santa Cruz, Santa Cruz, CA; 2) Center for Biomolecular Science and Engineering, UC Santa Cruz, Santa Cruz, CA.

Variation and Selection data in the UCSC Genome Browser is described (http://genome.ucsc.edu). This group of tracks covers primary data from dbSNP, HapMap phase II, Perlegen, Affymetrix, and structural variants from the literature. Polymorphisms, population derived allele frequencies, linkage disequilibrium, and haplotypes are displayed visually and are available for online analyses. Results from several recent studies in natural selection have also been added to this collection. The site provides a number of tools that allow visualization and analysis of the data as well as access to download data for offline analyses.

Novel mutations in the deoxyguanosine kinase gene and viral infection predispose previously healthy children to liver failure. *J.T.C. Shieh[1], L.J.C. Wong[2], G.M. Enns[1]* 1) Dept Pediatrics, Stanford Univ, Stanford, CA; 2) Dept Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Mutations in the deoxyguanosine kinase gene (DGUOK) cause a hepatocerebral form of mitochondrial DNA depletion syndrome. Although recent reports have characterized hypotonia, nystagmus, developmental delays, and liver failure in individuals with DGUOK mutations, the clinical spectrum of this disorder and pathogenesis of acute clinical decompensation remain unclear. In this study, we show that severe liver dysfunction can manifest in otherwise normal children and we identify associated novel mutations in the deoxyguanosine kinase gene. Importantly, we illustrate that a viral infection can trigger fulminant liver failure in the context of DGUOK mutation. A previously healthy 10 month-old twin male presented with fulminant liver failure and died, and post-mortem examination demonstrated herpes simplex virus type I infection. The living twin male sibling did not have evidence of herpes simplex virus infection but demonstrated episodic elevations in liver enzymes and an abnormal liver biopsy. We hypothesized that an underlying liver abnormality predisposed these children to liver disease and sequenced DGUOK to support a mild form of mitochondrial DNA depletion syndrome. We identified novel compound heterozygous mutations of the deoxyguanosine kinase gene (encoding p.N46S/R118C) in each of the twins and verified that the parents each carried one of the mutations. Furthermore, amino acids N46 and R118 are evolutionarily conserved from C. elegans to humans. This study illustrates three key findings: 1) Novel mutations in the deoxyguanosine kinase gene can lead to liver disease without cerebral manifestations, 2) Viral infection such as herpes simplex virus can lead to fulminant liver failure and death in the setting of a predisposing genetic alteration, and 3) Liver disease or failure should prompt investigation into an underlying mitochondrial DNA depletion syndrome as a potential etiology. This study suggests that although liver dysfunction may be attributed to infection, a genetic predisposition to disease may be under-recognized.

Mouse embryonic stem cell-derived myogenic stem cells have the potential for cell-based therapy of muscular dystrophies. *B. Yang, J. Qu, X. Cao* Dept OB/GYN, Univ Iowa, Iowa City, IA.

The muscular dystrophies are inherited myogenic disorders characterized by progressive muscle wasting and weakness of variable distribution and severity, and there are no cures for these diseases. Replacement of an aberrant copy of the relevant gene through gene therapy or engraftment of normal cells into affected muscles seems to be attractive therapeutic options. Isolation of a population of myogenic lineage committed stem cells from the pluripotent embryonic stem (ES) cells would have the great potential to be used for cell-based therapy of muscular dystrophies. We have devised an in vitro differentiation method for generating myogenic stem cells (MSC) from ES cells via embryoid bodies. The MSC can be maintained in culture for more than 30 generations without obvious changes in the culture characteristics and morphology. Upon switching to muscle differentiation medium, ~90% of the MSC can form myotubes in vitro. Cell surface marker characterization revealed that the MSC are positive (>95%) for CD34 and Sca1, negative for c-Kit, and the expression levels of CD45 and Flk1 are low (~20%). Those cells are also positive for myogenic markers, Myf5 and MRF4, while the expression levels of myogenin and MyoD are low. No teratoma was observed in scid mice injected with MSC in 8 weeks while similar number of ES cells resulted in tumor formation within 2 weeks after injection. When those cells were transplanted into -SG null/scid mice via intraarterial injection through the femoral artery, sections of recipient targeted muscles exhibited 10% and 20-30% of skeletal muscle fibers expressing donor-derived protein (-SG), 3 and 9 weeks post transplantation, respectively. This is the first report that a near homogeneous population of MSC can be isolated from ES cells via in vitro differentiation. Further detailed testing of the MSC will be conducted for their ability as myogenic precursors to populate, differentiate and persist in skeletal muscle. These results will not only enrich our understanding of basic knowledge of muscle differentiation but also facilitate and promote future studies of these cells as reagents for cell-based therapies of muscular dystrophies.

Development of High Throughput Genotyping Assays for Pharmacogenomics using Multiple Technology Platforms. *M.S. Phillips, I. Mongrain, N. Gaudreault, A.M.K. Brown, Y. Renaud, P. Guelpa, A. Mallah, C. Beck, T. Van Rooij* Université de Montréal Pharmacogenomics Centre, Montréal, PQ, Canada.

Approximately half of the variability that has been observed in drug response can be explained by genetic variation that occurs in both drug target proteins and in a collection of proteins that affect drug **A**bsorption, **D**istribution, **M**etabolism and **E**limination (ADME). Our laboratory has developed a set of gene specific panels for an extensive group of targeted ADME/Tox genes; such as: CYP2D6, 2C9, 2C19, 3A4/3A5, and UGT1A1. We have developed these panels in parallel on several technologies simultaneously like the Beckman SNPstream and Sequenom MassArray platforms. This parallel development using different biochemistries and technologies has allowed us to identify known controls, to rapidly cross validate these assays against each other and to efficiently transfer them onto additional technology platforms that are more suitable for the clinic, such as, the Autogenomics Infiniti platform.

To aid in our development, we have also developed a flexible operational QA/QC software tool (SNiPer) that can capture operational information, confirm calls on known controls, perform automated clustering with confidence scores, perform Pass/Fail determination and perform haplotype assignment to patients.

Furthermore, we have developed several broad-based ADME/Tox panels on the Illumina Bead Array platform that are capable of interrogating over 200 ADME/Tox genes at once in a single assay. These panels provide a distinctive advantage and cost savings over other approaches for screening for many gene variants in ADME pathways. We are presently utilizing these ADME/Tox candidate gene panels in large pharmacogenomic research projects to identify genomic biomarkers that can be predictive for patients that might experience adverse drug reactions or variable efficacy when exposed to various medications.

Geography-dependent difference in allele frequencies of immune-related loci presumably under malarial selection pressure in Vanuatu. *M. Yasunami[1,2], M. Kikuchi[1,2], N. Okuda[1,2], T. Tsukahara[3], C. Sato[1], M. Matsuo[1], R. Ubalee[1], K.J. Lum[5], A. Kaneko[3,4], K. Hirayama[1,2]* 1) Department of Immunogenetics, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan; 2) Center of International Collaborative Research, Nagasaki University, Nagasaki, Japan; 3) Department of International Affairs and Tropical Medicine, Tokyo Women's Medical University, Tokyo, Japan; 4) Malaria Research Laboratory, Karolinska University Hospital, Stockholm, Sweden; 5) Department of Anthropology, Binghamton University, Binghamton, NY, USA.

The frequencies of genetic polymorphisms are maintained by adaptation to the environment. Like HbS mutation in African descendents, several otherwise deteriorating genetic variants seem to be even selected against pressures of life-threatening infectious agents including malaria. Within the archipelago of Vanuatu in Melanesia, there is a decreasing cline of malarial parasite incidence from North to South. We previously reported the estimation of frequencies of TNFA promoter(TNFP) alleles for the population in six islands of Vanuatu, where parasite incidence varied, and negative correlation between the frequency of TNFP-D allele and parasite incidence (Ubalee et al. 2005). TNFP-D allele conferred a significant risk in patients with severe cerebral malaria in Myanmer, and thus we concluded that this allele is under the negative selection pressure of malaria in Vanuatu population. In the present study, we further sought genetic variations which could be selected for or against malarial infection. The polymorphic microsatellite loci linked to FASL, ACP1, CR1, STAT1 TLR2, HFE and LTA were examined to estimate the allele frequencies in 95 individuals from each of six islands. Among them, alleles of TLR2-linked and LTA-linked microsatellites exhibited significant correlation to parasite incidence. The result suggested that there are some other traces of selection among these immune-related loci. We are currently identifying polymorphisms within these loci which can confer functional divergence to elucidate the underlying mechanism of natural selection.

Wilson Disease: A Pilot Study of Newborn Screening. *S. Zafari, C.A. Kroll, J.E. Brown, M.A. Pogatschnik, S.J. Minnich, M.J. Kurke, M. J. Ferber, S.H. Hahn* Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.

Background: Wilson disease (WD) is an autosomal recessive disorder of copper transport with an estimated incidence of 1 in 30,000. The disease is progressive and ultimately fatal if untreated. WD is treatable, and serious symptoms can be avoided if an early diagnosis is made. We recently reported that the newborns (NB) affected with WD had significantly lower ceruloplasmin (CP) levels in blood spots than unaffected newborns. **Method:** We investigated the feasibility of newborn screening for WD using the sandwich ELISA method to support a presymptomatic NB screening for WD. The concentration of CP was measured in the dried blood spots from 6862 blinded NB samples following routine MSMS NB screening with IRB approval. The blood spot samples with CP concentrations lower than 5.0 mg/dL were followed by ATP7b gene mutation analysis to establish an appropriate cut off. **Result:** The mean CP value was 46.9 20.7 mg/dL (range: 0.1 to >60). Birth weight (BW) ranged from 200g to 5653g and gestational weeks (GW) from 23 to 43. In NB with BW >1800g, the mean CP was 48.3 20.2 mg/dL for those >37 weeks and 33.8 20.0 mg/dL for those <37 weeks. Of these two groups, 0.35% had CP <5.0 mg/dL. In premature NB with BW <1800g (n=124), CP ranged from 0.1 mg/dL to 84.6 mg/dL with a mean of 30.5 23.4 mg/dL. Sixteen of them (12.9%) had CP <5.0 mg/dL, however, we observed an increase in CP in 9 of the 11 premature NB with additional tests over a period of 1 month. In one newborn with a CP of 4.9 mg/dL (BW=3030g), one copy of H1207R alteration was found in the ATP7b gene suggestive of a carrier. No patients were detected in 10 samples sequenced so far and others are yet to be analyzed. **Conclusion:** 93.8% of NB already reached their CP value >15mg/dL at birth. The repeated tests can significantly reduce false positive rates in premature newborns and will help avoid any unnecessary anxieties to their families and costs for confirmatory tests. Although a pilot study with larger sample number over a longer period is necessary to determine the efficiency of this method, our findings strongly support that presymptomatic newborn screening for WD is feasible.

Variable phenotype of 5p13 duplication involving NIPBL region by microarray analysis. *A.C. Tsai[1], A.S. Teebi[2], R. Klatt[2], B.A. Bejjani[3], L.G. Shaffer[3], D. Terespolsky[4]* 1) Div Clinical Gen & Metabolism, Childrens Hosp, Denver, University of Colorado; 2) Division of Clinical & Metabolic Genetics, The Hospital for Sick Children Department of Medical Genetics & Microbiology, University of Toronto; 3) Signature Genomic Laboratories, LLC, Spokane, WA; 4) The Credit Valley Hospital, Department of Laboratory Medicine, Mississauga,.

Teebi and Klatt [2005] reported a 2.5-y/o child with unilateral cleft lip and palate, hypotonia, dysmorphism, normal brain MRI and mild developmental delay(DD)with de novo 5p13 duplication by microarray analysis as a gain of three BACs containing the NIPBL region. At 4.5 y, child has mild autistic-like behaviors and attention deficits. We herein report three additional cases from two families with similar microarray findings yet variable presentations. The first patient is a 5-y/o-boy with DD, classic autistic spectrum disorder features, hypotonia and constipation. An MRI at age 3 showed increased signal in frontal white matter and along the peritrigonal region. Karyotype at 525 band and FISH for 15q11 were normal. Parental microarray assays were normal. The second patient is a 4.5 y/o male with DD, Siezure, microcephaly and dysmorphism. An MRI showed partial agenesis of the corpus callosum with left colpocephaly, decreased white matter volume and medial frontal pachygyria. Karyotype was normal at 550 band. He has profound DD: no expressive language, not walking and behavioral problems. His mother has the same duplication by microarray but has normal physical features and cognitive function. However, his 7 y/o brother with autism has not been tested. The variable phenotype in 4 individuals might be explained by dosage effect of the genes of specific function, parental origin, polygene and multifactorial threshold effect. While cautious interpretation of the microarray data is required, as many polymorphisms that previously were not understood can be uncovered, the importance of academic endeavor to describe individual phenotypes cannot be overemphasized. The clinical description of our patients also suggests that not all familial gene changes are polymorphisms. More cases are required to better understand this duplication.

An investigation into the ability of molecular modeling to explain the biochemical properties of a common mutation of the androgen receptor that allows prostate cancer tissues to grow in the absence of androgens. *J. Southwell[1,3], S. Chowdhury[1,2], B. Gottlieb[1,3,5], L.K. Beitel[1,4], R. Lumbroso[1], E. Putisima[2], M. Trifiro[1,3,4]* 1) Lady Davis Institute for Medical Genetics,; 2) Biotechnology Research Institute, National Research Council of Canada; 3) Dept of Human Genetics; 4) Dept of Medicine, McGill University; 5) Dept of Biology, John Abbott College, Montreal , Quebec, Canada.

Prostate cancer (PCa) may progress by circumventing ablation therapy due to mutations in the androgen receptor (AR) gene. The most intensively studied is the mutation T877A in the ligand binding domain (LBD) which causes the AR to become promiscuous, i.e., respond to a number of different ligands. Previous studies using X-ray crystallography have shown changes in the ligand-binding pocket that have been presumed to be the cause of this promiscuity, but have revealed very little regarding the mechanism. We have used a novel molecular modeling approach involving the receptor, as well as the coactivator TIF2 that plays a critical role in N/C terminal interactions. The T877A mutation altered the inverse relationship between exon 1 CAG repeat length and transactivation, and increased N/C terminal interactions but decreased TIF2 binding. We used modeling to compare the crystal structure of the wild type (wt) and T877A AR bound to dihydrotestosterone. This revealed both an increase in flexibility of amino acid residues in the LBD, as well as a larger solvent accessible surface for T877A compared to wt AR. The N-terminal domain FxxLF containing peptide was predicted to bind with greater affinity into the mutant than wt LBD, and the co-activator TIF2 peptide (LxxLL) to bind better with the wt receptor. The improved induced fit of the FxxLF motif containing peptide into the LBD thus appears to be due to the increased flexibility and solvent accessibility of the residues present in the peptide-binding pocket of the mutant LBD. The fact that these observations confirmed our biochemical data indicates the promise of this novel approach. We believe that such a dual modeling approach is likely to lead to advances in understanding hormone-refractory PCa.

Higher Incidence of Chromosome Duplications Identified by Array CGH Compared with FISH. *J.H. Tepperberg, I. Gadi, B. Williford, D. Fuentes, J. Whaley-Davis, C. Legacki, J. Kesler, P. Papenhausen* Cytogenetics, LabCorp, RTP, NC.

Genetic imbalances are generally associated with multiple birth defects, developmental delay, growth retardation, and dysmorphic features. The incidence of common chromosome microdeletion syndromes is estimated to be 1 in 1000-2000 while the detection rate of clinically significant subtelomere abnormalities in individuals with normal chromosomes was recently shown to be approximately 2.5% (Ravnan et. al. 2005). The relative incidence of terminal subtelomere duplications was found to be 1.12% (4/357 abnormal subtelomere cases), while interstitial duplications are considered very rare. Array based Comparative Genomic Hybridization (aCGH) is used as an adjunct to cytogenetics and FISH to detect cryptic dosage imbalance (microdeletions, duplications, insertions and unbalanced subtelomere rearrangements) associated with developmental delay and mental retardation. Array CGH analysis of 405 clinical cases submitted for aCGH (Spectral Genomics 434 BAC array) showed 5.19% (21/405) of cases with clinically significant unbalanced rearrangements. Eighteen of the 21 cases were either cryptic or extremely subtle alterations. Nine cases (23.8%) showed apparent terminal deletions, five cases (23.8%) showed interstitial duplications, four cases (19.0%) were unbalanced derivative rearrangements, and three cases (14.3%) were large unbalanced chromosome abnormalities. Although these numbers are relatively small, the percentage of interstitial duplications is significantly higher compared with what is reported in the literature. Theoretically, an unbalanced meiotic recombination event should result in a deletion and duplication. Small chromosome microduplications are thought to have a less severe phenotype compared to microdeletions and are generally not targeted by a FISH analysis. Furthermore, FISH may not be sensitive enough to detect small microduplications, even by interphase analysis. Thus, chromosome duplications may be significantly under represented in patients with unexplained MR and developmental delay. Array CGH appears to be an efficient high throughput whole genome assay to detect clinically significant chromosome microduplications.

Low serum carnitine in patients with phenylketonuria may not be due to inadequate carnitine intake. *S. Yano[1], K. Moseley[2], E. Baldwin[2], R. Koch[2]* 1) Genetics, Pediatrics Women's & Children's Hosp, LAC+USC Medical Center, Los Angeles, CA; 2) Medical Genetics, Pediatrics, Childrens Hospital Los Angeles, USC, Los Angeles, CA.

An observation of decreased serum carnitine concentration in phenylketonuria (PKU) has been reported by Bohles (1991) and others. Strict protein intake restriction is believed to be directly responsible for low serum carnitine. Iron deficiency, secondary to protein restriction, is also believed to be one of the factors leading to low serum carnitine through the reduction of carnitine synthesis which requires iron as a cofactor. Carnitine synthesis is also known to be inhibited by phenylacetic acid (PAA) which is one of the byproducts of phenylalanine (phe)in patients with PKU who have defective phenylalanine hydroxylase. Fisher et al (2000) reported that phenacetyl-carnitine was identified in urine in patients with PKU and approximately 5-10% of urine total carnitine was in the form of phenacetylcarnitine. We recently identified a group of patients with PKU (age 4-44 y), whose serum phe levels were in the recommended range (except for one), who are on a PKU medical product or large neutral amino acid supplementation, and who have low serum carnitine with normal free/acyl ratio. A high PAA is unlikely the primary cause of low serum carnitine since PAA should be low under strict diet control. Among these patients, we have identified patients with abnormal serum amino acids with a few amino acids being lower than the reference ranges: lysine, ornithine, and/or glycine have been noted to be low. Patients with PKU who have low serum carnitine and lysine are treated with a PKU Medical product which provides lysine above the dietary recommendation. Urine lysine excretion was measured and was found not to be increased in these patients. Lysine absorption may be decreased in these patients. Serum amino acids have not been routinely measured in patients with PKU as a routine metabolic evaluation. We believe that it is important to monitor serum amino acids periodically in patients with PKU since they can develop various amino acids deficiencies regardless their status of the diet treatment.

The Developmental Genome Anatomy Project (DGAP): Finding genes critical in human development. *R. Williamson[1,2], F. Alkuraya[1,2], Y. Fan[1,2], H. Ferguson[1,2], J. Gusella[1,3], D. Harris[1,4], C. Kelly[1,2], K. Kocher[1,2], A. Ligon[1,2], W. Lu[1,2], R. Maas[1,2], B. Quade[1,2], F. Quintero-Rivera[1,3], I. Saadi[1,2], C. Morton[1,2]* 1) Harvard Medical School, Boston, MA; 2) Brigham and Women's Hospital, Boston, MA; 3) Massachusetts General Hospital, Boston, MA; 4) Children's Hospital, Boston, MA.

DGAP (dgap.harvard.edu) is a collaborative effort to identify genes critical in human development from chromosomal rearrangements, utilizing a high-throughput approach of (1) patient ascertainment and collection, (2) FISH-based breakpoint localization, (3) breakpoint cloning and candidate gene identification, and (4) functional analysis in model organisms. We have mapped 151 breakpoints in 73 cases and discovered 25 disrupted genes. Twenty-five breakpoints are cloned and sequenced, and 13 knock-out mouse models evaluated. The phenotype of one case, DGAP056 [t(2;13)(p24;q21)], includes includes blepharophimosis, coloboma of upper eyelid, fundal coloboma, exotropia, profound sensorineural hearing loss, low set and posteriorly rotated ears, and mitral valve prolapse. This translocation disrupts a hypothetical gene, *FLJ21820*. *FLJ21820* is expressed in a very select number of cells in the cochlea further supporting its importance in the hearing pathway. DGAP 113 [t(1;3)(q32.1;q13.2)], has bilateral congenital cataracts, mild developmental delay, a head circumference > 95th percentile, and prominent extra-axial CSF spaces. The chromosome 1 breakpoint lies ~125 kb upstream of the *NEK7* gene. *NEK7* is highly conserved and the mouse ortholog is expressed in the murine developing brain. Studies of *Nek7*-deficient mice are ongoing. DGAP095 [t(X;2)(p11.2;q37)], exhibits seizures, developmental delay, infantile hypotonia, obesity and livedo reticularis. Diacylglycerol kinase delta (*DGKD*) is disrupted by the breakpoint on chromosome 2. DGKD is a member of a family of proteins that have been linked to the regulation of seizure susceptibility. We have created a mouse model for *DGKD* deficiency and are assessing the DGAP095 epilepsy and vascular phenotype in the mouse model.

Accelerated evolution of conserved noncoding sequences in the human genome. *S. Prabhakar*[1,2,4], *J.P. Noonan*[1,2,4], *S. Paabo*[3], *E.M. Rubin*[1,2] 1) US DOE Joint Genome Institute, Walnut Creek, CA; 2) Lawrence Berkeley National Laboratory, Berkeley, CA; 3) Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany; 4) These authors contributed equally to this work.

Changes in gene regulation likely influenced the profound phenotypic divergence of humans from other mammals, but the extent of adaptive substitution in human regulatory sequences remains unknown. Here we survey 129,405 conserved noncoding sequences (CNSs), which studies have revealed to be enriched in gene regulatory elements, for evidence of accelerated evolution in humans. We identified 1,119 conserved noncoding sequences (CNSs) with a statistically significant excess of human-specific substitutions after correcting for the local neutral substitution rate, the degree of CNS constraint in non-human lineages, unevenness of constraint within a CNS, and genome-wide relaxation of constraint in primates. These accelerated elements were disproportionately found near genes involved in neuronal cell adhesion. To assess the uniqueness of human noncoding evolution, we examined CNSs accelerated in chimpanzee and mouse. Although we observed a similar general trend towards neuronal adhesion in chimpanzee, the accelerated CNSs themselves exhibited almost no overlap with human, suggesting independent evolution towards different neuronal phenotypes in humans and chimpanzees. CNSs accelerated in mouse showed no bias toward neuronal cell adhesion. Our results indicate that widespread cis-regulatory changes in human evolution may have contributed to the rise of uniquely human features of brain development and function.

Y Chromosome and Mitochondrial DNA Analysis of a Large Dataset from Mali, Africa. *S.R. Woodward, U.A. Perego, J.E. Gomez, J. Ekins, A. Nelson, M. Nelson, J. Jensen, M. Lunt, T. Tolley, K. Ritchie, I. Masannat, S. Masek, M. Purnell, R. Hughes, N. Angerhofer, N.M. Myres* Sorenson Molecular Genealogy Foundation, Salt Lake City, UT.

The unique history of ancient Mali places it in the trans-Saharan crossroads of West Africa for the trade of slaves, gold, ivory, and salt. The genetic junction of these ancient interactions is reflected in the present-day inhabitants of Mali. An extended Y-chromosome and mtDNA analysis of 700 inhabitants of various regions of Mali is presented, with corresponding genealogical and historical information. All samples were typed at 43 Y-chromosome STR loci and sequenced for mtDNA HVR1 and HVR2, to assess the levels of admixture with known interacting historical populations and demonstrate recent events contributing to the gene pool of present-day Mali.

Admixture mapping for acute inflammatory markers in African Americans: Results from the Health and Body Composition Study. *E. Ziv[1], N. Patterson[2,3], G.J. McDonald[2,3], A. Tandon[2,3], D. Hu[1], L. Pawlikowska[1], P.Y. Kwok[1], Y. Liu[4], A. Reiner[5], W.C. Hsueh[1], J. Zmuda[6], R. Ferrell[6], R. Liu[9], T. Harris[7], S. Cummings[1,8], D. Reich[2,3]* 1) Univ California, San Francisco, San Francisco, CA; 2) Broad Inst, Boston, MA; 3) Harvard Med School, Boston, MA; 4) Wake Forest Univ, Winston-Salem, NC; 5) Univ of Washington, Seattle, WA; 6) Univ of Pittsburgh, Pittsburgh, PA; 7) National Institute on Aging, Bethesda, MD; 8) Calif Pacif Med Cntr Res Inst, San Francisco, CA; 9) Univ of Tennessee, Memphis, TN.

We used an admixture mapping approach to find genetic variants that determine serum levels of acute inflammatory markers. These markers may be important mediators of chronic diseases including atherosclerosis. Several acute inflammatory markers have different levels in African Americans and Caucasians; admixture mapping may be feasible for these phenotypes. We used samples from 1100 participants in the Health and Body Composition cohort who described their race/ethnicity as Black." The cohort included men (43%) and women who were ages 70-79 and ambulatory. We typed 1,322 ancestry informative markers to estimate locus specific ancestry and used this data to perform an admixture mapping scan for serum levels of C-reactive protein, Interleukin-6, soluble IL6 receptor (sIL6R), Tumor Necrosis Factor-a and Plasminogen Activator Inhibitory-1. Data were analyzed using ANCESTRYMAP and confirmed by ADMIXMAP and structure/MALDSOFT. We found a strong locus for sIL6R levels on chromosome 1 (LOD score=5.8 by ANCESTRYMAP, p=0.0000008 by ADMIXMAP) that exceeded the threshold for whole genome significance. The region includes the IL6R gene. The association suggests that an allele which increases sIL6R levels is more common with European ancestry and explains the admixture peak. We are currently testing candidate polymorphisms in the IL6R gene to determine which accounts for the admixture mapping peak. Our results provide empirical proof that admixture mapping can not only obtain coarse localization of a phenotypically important variant, but can also fine map it. Finding the genes that control acute inflammatory markers may help explain the relationship between inflammation and disease.

Very-high-resolution mapping of DNA copy-number alterations using high-density tiling oligonucleotide arrays.

A.E. Urban[1], J.O. Korbel[1], F. Grubert[1], C. Hart[1], R. Selzer[2], T. Richmond[2], R. Green[2], B.S. Emanuel[3], K. Kidd[1], M. Gerstein[1], S.M. Weissman[1], M. Snyder[1] 1) Yale University, New Haven, CT; 2) NimbleGen Systems, Inc., Madison, WI; 3) University of Pennsylvania, Philadelphia, PA.

Studying the emerging phenomenon of pervasive human genomic segmental aneuploidy is expected to allow for a better understanding of the basis of human phenotypic variation, in norm variants and in disease states. We have developed high-density oligonucleotide tiling microarray based High-Resolution CGH (HR-CGH). This technology allows us to detect, with sub-kilobase accuracy, the presence and extent of chromosomal aberrations ranging in size from 600 bp to several million bp, in confirmatory and ab initio studies. We first used arrays covering the beta-globin locus on Cs11 (9 bp tiling density, isothermal tiling) and all of Cs22q (85 bp tiling, isoTm). Analyzing full-complexity genomic DNA from patients with a variety of disease-causing aberrations (various heterozygous deletions, duplications, partial trisomies and partial tetrasomies) we could map breakpoints with high accuracy (typically up to 200bp resolution when confirmed by DNA sequencing following PCR) [Urban, Korbel et al., PNAS, 2006] We have extended this approach beyond the initial Cs22q experiments and have now carried out more than 120 additional hybridizations using HR-CGH arrays covering the sequence of human chromosome X (111bp or 35bp tiling, isoTm) or the ENCODE regions (38bp tiling, fixed-length oligomers) in addition to the Cs22q array. We probed these arrays with DNA from patients with developmental disorders, especially of the nervous system, as well as with DNA from healthy probands from various ethnicities (i.e. a subset of the HapMap samples as well as samples from S.W. Asia, Siberia, Pacific Islands and South America) and can observe a large number of copy number variations (13 with very high confidence, 104 with high to medium confidence and 600 with medium to lower confidence in a preliminary analysis of CsX data from 12 healthy individuals from 5 ethnicities) that are currently being experimentally verified and cataloged.

A novel mitochondrial DNA A4401G mutation is involved in hypertension in two Chinese pedigrees. *S. Wang[1], H. Zhu[1], L. Yang[2], C. Lu[1], M. Guan[2]* 1) Geriatric Cardiology, Chinese PLA General Hospital, Beijing, Beijing, China; 2) Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio.

Left ventricular hypertrophy (LVH) is one of the most important target organ damage of hypertension. Despite the involvement of multiple factors, the genetic factors including the mitochondrial genomes have been implicated to play an important role in the pathogenesis of hypertension. Recently, a systematic and extended mutational screening of mitochondrial genome has been initiated in the large clinical population of Geriatric Cardiology Clinic at the Chinese PLA General Hospital, China. In the present study, 200 sporadic Chinese subjects with left ventricular hypertension were recruited. Further genetic evaluation found that two of these Chinese families seem to be transmitted maternally. The sequence analysis of complete mitochondrial genome identified a novel A-G mutation in the junction of tRNA-Met and tRNA-Glu. This mutation appears to affect the processing of these mitochondrial tRNAs, thereby reducing the steady-state levels of these two tRNAs. As a result, it causes the defect in mitochondrial protein synthesis, thereby impairing the respiration. These genetic and biochemical data imply that the novel A4401G mutation is involved in the pathogenesis of hypertension.

Quantitative determination of gangliosides in the cerebrospinal fluid of patients with GM2 gangliosidosis using tandem mass spectrometry. *C.J. Tifft[1], J. Gu[2], J. Kurtzberg[3], R.L. Proia[4], S.J. Soldin[2]* 1) Div Genetics & Metabolism, Children's Natl Medical Center, Washington, DC; 2) Bioanalytical Core Laboratory, GCRC, Georgetown University, Washington, DC; 3) Pediatric Blood and Bone Marrow Program, Duke University Medical Center, Durham, NC; 4) Genetics of Development and Diseases Branch, NIDDK, NIH, Bethesda, MD.

Tay-Sachs (TS), caused by a deficiency of the subunit of lysosomal enzyme -hexosaminidase A, is a rapidly progressive neurodegenerative disorder for which no therapy is currently available. Massive accumulation of GM2 ganglioside in neurons of affected individuals is a hallmark of the disease. Biochemical markers of disease progression are limited in this primarily CNS disorder. We have used tandem mass spectrometry to measure ganglioside content in CSF of patients with infantile TS disease that have undergone umbilical cord blood transplantation. **Methods:** 40 l of patient or control CSF was added to 400 l of methanol containing the internal standards. The mixture was injected onto a Supelco Cosil C-18 column, washed with 20% methanol, and gangliosides were eluted using a methanol gradient. Samples were analyzed on an Applied Biosystems API-4000 System. **Results:** Sixteen CSF samples and 4 control samples were analyzed. For one of the patients, diagnosed prenatally, 9 serial samples were available for analysis. The pre-transplant sample taken at 1 month of age showed a 100-fold elevation of GM2. A progressive decline in GM2 levels was observed following transplantation although levels remained elevated and did not decrease relative to GM1 levels. **Conclusions: (1)** Tandem mass spectrometry is a reliable method for quantitating gangliosides from a small volume of CSF. **(2)** Although a decline in GM2 levels was observed following transplantation, the decline did not result in improved clinical outcome. **(3)** MS/MS can readily detect a marked elevation of GM2 ganglioside in TS disease patients prior to the onset of clinical symptoms. Modification of the technique to utilize plasma or dried blood spots may be applicable for therapeutic monitoring or newborn screening.

The difference of crossover pattern between male and female. *H. Xi, Z. Jiang, R. Deka* Dept Environmental Health, Univ Cincinnati, Cincinnati, OH.

It is well known that the recombination rate in females is much higher than in males but the underlying mechanisms have not been revealed yet. Here we present a hypothesis that the number and locations of chiasmata are important to maintain the chromosome stability during meiosis. Since eggs have a much longer stage of Prophase I, we suspect the recombinant chromosomes need more chiasmata or stable form of chiasmata to keep their stability. Therefore, the number of recombination events is much higher in females and locations of recombination sites are much stricter in females than in males. Through the analysis of several whole genome scan data, we observed the locations of recombination events in female are not uniformly distributed across the chromosome, i.e. the recombination site on the chromosomes with only one crossover seldom happens in the terminal region (which may be the unstable form). These results partially confirm our hypothesis.

Glaucoma-causing myocilin mutations require association with the Peroxisomal Targeting Signal-1 Receptor (PTS1R) to elevate intraocular pressure. *V.C. Sheffield[1,2,3], A.R. Shepard[4], N. Jacobson[4], J.C. Miller[4], I.-H. Pang[4], H.T. Steely[4], C. Searby[4], E.M. Stone[1,3], A.F. Clark[4]* 1) Howard Hughes Medical Institute, University of Iowa, Iowa City, IA; 2) Department of Pediatrics, University of Iowa, Iowa City, IA; 3) Department of Ophthalmology, University of Iowa, Iowa City, IA; 4) Glaucoma Research, Alcon Research, Ltd., Fort Worth, Tx.

Primary open angle glaucoma (POAG) is a clinically and genetically heterogenous group of optic neuropathies and a leading cause of blindness. Specific mutations in the myocilin (MYOC) gene have been shown to cause POAG with varying age-of-onset and degree of severity. Myocilin associated glaucoma is inherited as an autosomal dominant disorder and this form of glaucoma is the single leading cause of POAG accounting for up to 4% of all POAG cases. The mechanism by which myocilin mutations lead to increased intraocular pressure has not been determined, and attempts to develop animal models of POAG have been unsuccessful. Neither elimination of the myocilin protein by mutation of one or both alleles, nor overexpression of myocilin is sufficient to result in POAG. Therefore, it appears that a deleterious gain-of-function is required for pathogenesis. We present data showing that specific mutations of the myocilin protein result in a physical interaction between myocilin and the peroxisomal targeting signal type 1 receptor (PTS1R) due to mutation-induced exposure of a cryptic peroxisomal signaling site at the carboxy-terminus of the human myocilin protein. Our data show that more severe early-onset POAG mutations have a stronger association with PTS1R. We further demonstrate that the association of mutant myocilin with PST1R causes elevated intraocular pressure (IOP) in a rodent model. This is the first demonstration of a disease resulting from mutation-induced exposure of a cryptic signaling site that causes mislocalization of mutant protein to peroxisomes and the first disease-gene-based animal model of human POAG.

Two new cases of analphoid markers from novel regions, 14q32 and Yp11.2/15q fusion. *P. Papenhausen[1], I. Gadi[1], J. Tepperberg[1], P. Warburton[2], B. Pletcher[3], P. Chang[4]* 1) Dept Cytogenetics, Labcorp of America, Res Triangle Park, NC; 2) Mt Sinai Med. Center, NY, NY; 3) 90 Bergen St., Newark, NJ; 4) Jersey City Med. Center, Jersey City, NJ.

Two cases characterized by highly unusual cytogenetic findings were referred for high resolution evaluation. Case 1, a 17 y.o. male showed moderate MR(3rd grade level), strabismus, and hypomelanosis of Ito. The latter indication is often associated with mosaicism and indeed, an unsatellited supernumerary marker was found in 5 of 40 cells examined. Since C-banding was negative, a subtelomere FISH study was pursued which revealed a 14qter signal on both ends of the marker. Centomeric 14/22 FISH confirmed the analphoid nature of the apparent i(14)(qter>q32.1>neo>qter). The inbalance in this patient was therefore, tetrasomy for 14q32.1>qter in about 12% of peripheral lymphocytes. Only one apparently unpublished case of involvement of the 14qter region could be found. Case 2 involved a two week old male with a web neck, long fingers, undescended testes, hypotonia and feeding difficulties. Cytogenetics revealed only 45 chromosomes due to the fusion of the Y short arm and 15 long arm(q13qter). Q and C banding as well as alphoid Y and 15 FISH were negative. Additional FISH targeting SRY was positive while loss of the paternal SNRP confirmed Prader-Willi syndrome. An apparent neocentromere with loss of centromeric associated proteins, but presence of a kinetichore was confirmed. Subtelomeric FISH studies were also ordered, but only served to confirm Yq loss. No alphoid repeats could be detected in either marker. These cases represent two novel sites for neocentromeres, increasing the spectrum for this new class of marker.

A Comparison of Publicly Accessible Y Chromosome Databases for Ancestral and Population Studies. *U.A. Perego, J. Ekins, N.M. Myres, K. Ritchie, R. Hughes, N. Angerhofer, S.R. Woodward* Sorenson Molecular Genealogy Foundation, Salt Lake City, UT.

Y-chromosome haplotyping has been heavily utilized in academic settings for ancestral and population studies in recent years. As molecular technologies have become more affordable and publicly available, a number of online searchable genetic databases have arisen to serve the private individual. Among the largest and most comprehensive Y-chromosome databases utilized by the public for personal ancestral research are the Y Chromosome Haplotype Reference Database (www.YHRD.org), the Sorenson Molecular Genealogy Foundation Y Chromosome Database (www.SMGF.org), and the Family Tree DNA Ysearch Database (www.YSEARCH.org), each containing thousands of haplotypes correlated to genealogical, historical and geographic data. This study provides a comparison of the unique array of applications available to the end user, often at no cost, from each database. All have the common goal of making powerful genetic tools available to the public, in order to promote discovery of recent and ancient paternal connections of the human family that are significant to the individual.

Genetic Profiling of Lung Cancer. *V. Venkatraj[1], T. Bhari[1], G. Zhou[2], E.T. Castiglioni[1], J.C. Huber[2], P.W. Dunne[1], K.C. Donnelly[2]* 1) Dept VIBS, Texas A&M Univ, College Station, TX; 2) SRPH,Texas A&M Univ, College Station, TX.

Lung cancer is the leading cause of cancer mortality worldwide.DNA adducts formed as a consequence of exposure to tobacco smoke may be involved in carcinogenesis, and their quantification is used as a dosimeter to evaluate DNA repair and risk of lung cancer.In an effort to uncover the relationship between genetic changes and DNA repair we did a pilot study on primary lung tumors using two approaches. To determine the genomic copy changes in primary lung cancer evolution we employed array Comparative Genomic Hybridization (aCGH) technique (Spectral Chip TM 2600, Spectral Genomics,USA) on 10 lung primary tumors and normal tissue from adjoining areas. Second, to quantify DNA adducts we used nuclease-P1 enhanced 32P DNA post-labeling on these same samples. Our results indicated the average number of genetic aberrations were higher in smokers with lung cancer (20/tumor) compared to nonsmokers (13/tumor) with lung cancer. In addition, our data points to recurrent changes in several chromosomal regains; DNA gain of 1p13-21, 1p31-p36, 1q21-25, 8q12-24(60%) of the samples, followed by DNA gain of 1q42-43, 12q12-q24 (50%).DNA loss was observed less frequently and included 10q (40%) and 3p, 4q, 6q, 12p and 12q (30%). Some of the genes relevant to lung cancer tumoriginesis mapping to these loci include N-ras, GSTM1, L-Myc,EGFR-1,C-Myc,MDM2, Bcl-2, BAX, GSTT1,FHIT, PTEN, RARB,p16,RB and p27/Kip1. The Diagonal Radioactive Zone (DRZ) in the adjacent lung tissues from current smokers was higher than those from former smokers. Moreover, the number of DNA adducts was about 6 fold less in tumor tissue as when compared to adjoining normal tissue. These results support the conclusions that lung tumor cells in smokers have a higher frequency of chromosomal aberrations compared to lung tumor cells in nonsmokers, indicating an enhanced genomic instability. Furthermore our data points to the importance of analyzing the apparently normal adjoining tissue for genetic changes as they may reveal chromosomal changes that are relevant to early transformation events during lung cancer evolution and provide accurate quantification of DNA adducts compared to tumor samples.

Detection and characterization of insertion-deletion polymorphisms in normal human populations. *D.A. Wheeler[1,2], S.I. Yakub[1,2], Y. Ding[2], D.M. Muzny[1,2], R.A. Gibbs[1,2]* 1) Molecular and Human Genetics, Baylor Col Medicine, Houston, TX; 2) Human Genome Sequencing Center.

Insertion/deletion polymorphisms (indels) occur much less frequently than single nucleotide polymorphisms but are more likely to exhibit severe functional consequences when located in coding or regulatory sequences. The Human Gene Mutation Database catalogues over 17,000 insertions and deletions associated with 3,500 phenotypes in humans including the well known CCR5-del32 polymorphism that, as a homozygote, confers resistance to infection by R5-HIV-I infection. Inspite of this extraordinary compendium demonstrating the influence of indels on human health, relatively little effort has gone into cataloguing natural variation due to indels in human populations. We have systematically extracted data reflecting genetic diversity on a wide range of scales within each species sequenced in the Human Genome Sequencing Center. Three million whole genome shotgun reads were generated from a library of pooled genomic DNA from 8 individuals of African American origin. They were compared to the reference human genome (build 35) using BLAST and the output was examined for any read with 2 matches to the genome. Through analysis of these matches we have discovered more than 5,000 indels between 10 bases to over 1 million bases, a sampling of which have been validated by PCR. Scores of indels are involved in functional gene alterations, including single amino acid deletion, frame shift mutations and deletion of entire exons and genes. We are currently exploring the population frequencies of these indels in European, Chinese and African populations.

Gene Conversion in Palindromic Regions of the Y Chromosome. *A. Turner, U. Perego, N. Myres, R. Hughes, J. Ekins, K. Ritchie, I. Masannat, N. Angerhofer, S. Woodward* Sorenson Molecular Genealogy Foundation, Salt Lake City, UT.

Several microsatellite markers on the Y chromosome are located in the palindromic regions of the Y chromosome. The SMGF database, with over 15000 records, includes data for the multi-copy markers DYS385 a/b, DYS459 a/b, DYS464 a/b/c/d/, and YCAII a/b. These markers typically have multiple alleles in an individual. However, gene conversion may result in a single allele for one or more of those markers (homozygosity) and violate the single-step mutation model for microsatellites. We will present the distribution of alleles, and the correlation of the homozygous state in one marker with other markers, providing data on the frequency and length of gene conversion events.

KDR gene polymorphism is associated with coronary artery lesions in Kawasaki disease. *I.S. Park[1,2], E.J. Seo[2,3], J.K. Lee[2], K.J. Kim[2], J.J. Kim[2], S.J. Hong[1], Y.H. Kim[1,2], J.G. Ko[1,2]* 1) Department of Pediatrics, University of Ulsan College of Medicine and Asan M, Seoul, Korea; 2) Genome Research Center for Birth defects and Genetic disorders, Asan Medical Center, Seoul, Korea; 3) Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan M, Seoul, Korea.

Kawasaki disease (KD) is an acute febrile vasculitis of young children and is complicated by coronary artery lesions (CAL). Although an infectious agent is suspected as the cause of KD, genetic factors might influence on susceptibility to KD and the risk of the developing CAL. Forty-one common polymorphisms of 17 genes (ACE, AGTR1, CCL2, CCR5, CD14, IL1A, IL1B, IL4, IL6, IL10, KDR, MBL2, MMP3, NPPB/BNP, SERPINE1, TNF, VEGF) were genotyped in 97 Korean KD patients including 49 patients with CAL and 100 healthy controls. Allele and genotype frequencies were compared between groups using Chi square and haplotypes were estimated using the PL-EM algorithm. All genotypes were in Hardy-Weinberg equilibrium, but allele and genotype frequencies of those polymorphisms did not differ between KD patients and controls. Interestingly, we found a significant association for the 1719A/T (rs1870377) polymorphism of the KDR gene with CAL within the KD patients. The frequency of T allele was significantly lower in KD patients with CAL than in those without CAL (P=0.03, OR=0.50; 95% CI 0.28-0.91). Haplotype analysis for -319A/T (rs6824124), 1192A/G (rs2305948) and 1719A/T (rs1870377) of the KDR gene showed that TGA haplotype differed significantly between two groups. KDR gene encodes kinase insert domain receptor which is the receptor of VEGF. In previous reports, VEGF and VEGF receptor were upregulated in blood vessels in acute KD. Our findings suggest that KDR gene polymorphism may be responsible for the development of CAL in KD patients.

Autosomal dominant hidradenitis suppurativa is not linked to 1p21.1-1q25.3, in two large multigenerational Indian families. *U. Radhakrishna[1], T.Y. Mehta[2], J.V. Solanki[3], U.C. Patel[3], U. Ratnamala[1], S.K. Nath[4]* 1) Green Cross Blood Bank & Genetics Research centre, Ahmedabad India; 2) Samarpan Medical & Research Organization, Modasa, India; 3) Department of Animal Genetics & Breeding, Veterinary College, Gujarat Agriculture University, Anand, India; 4) Arthritis and Immunology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, USA.

Hidradenitis suppurativa (HS) (HS: OMIM 142690) is an aggravating chronic inflammatory skin condition characterized by swollen, painful, inflamed lesions in the axillae, groin, and other parts of the body that contain apocrine glands. It affects more females than males with an incidence ranging from 0.1/100 to 4/100. The risk of developing nonmelanoma skin cancer including buccal cancer and primary liver cancer is very high among patients with HS (Arch Dermatol 2001 137:730-4). We have studied two large Indian hidradenitis suppurativa families with autosomal dominant mode of inheritance and full penetrance. There are 84 individuals in these pedigrees including 25 affected. The age of onset is 25-35 years. All affected had typical characters of HS and some of the clinical findings included folliculitis, GI polyps, cutaneous scars, epidermoid carcinoma and polymorph function defects and hirsuitism in few affected females. Recently, a locus for autosomal dominant form of HS was (J Invest Dermatol. 2006 126:1302-6) reported on chromosome 1p21.1-1q25.3 in a large four generation Chinese pedigree, subsequently we genotyped two of our families using several polymorphic microsatellite markers closely linked to HS1 locus. Analyzing 45 individuals, all twenty markers yielded significant negative (<-2.0) at = 0. Thus the HS1 locus can be excluded as the candidate locus responsible for HS in our Indian families. We suggest that genetic heterogeneity is present in the pathogenesis of HS and that the reported HS1 locus may be ethnic specific. We are planning to perform genome-wide linkage analysis in this family to identify the responsible locus.

Molecular Investigation SCA in 28 patients suspected to SCA in Iran. *S. Saber, M. Rostami, M.M. Banoei, S.H. Nafisi, M. Houshmand* medical genetics, NIGEB, Tehran, Iran.

More than 20 types of SCA have been described.These types are given numbers(1-22,excluding the number 9). All types of SCA are characterized by a progressive incoordination of walking.They are often associated with poor coordination of hand movments,eye movments,and speech.With some exceptions,the onset of symptoms usually occurs after the age of 18.SCA is slowly progressive M.R.I and C.T of affected persons often show shrinkage or atrophy of cerebellum. Most common of SCA is SCA3(21%),SCA2 and SCA6(15%) The genetic change that causes SCA types 1,2,3,6,7,12 and 17 is called a CAG repeat expantion. We checked these types of SCA in our lab with PCR analysis across the CAG region of the SCA1, SCA2, SCA3, SCA6, and SCA7 genes to determine allele sizes. We checked 28 patients refer to our lab for detection of these types of SCA by molecular technique.13 patients were normal repeat size for these types of SCA and 6 patients were expanded repeat size(2 patients SCA3,2 patients SCA6 and 2 patients SCA2).9 patients have intermediate repeat size.After detection of normal repeat, we checked other hereditary ataxia.Then,we proved AT for 2 patients by molecular technique.

Type	Chromosome	Normal repeat size	Expanded repeat size
SCA 1	6p23	6-36	39-83
SCA 2	12p24	15-31	34-220
SCA 3	14q24.3-q32	12-40	55-86
SCA 6	19	4-18	21-33
SCA 7	3p12-p21.1	4-19	37-300

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Polymorphisms in the PTPN22 region are associated with psoriasis of early onset. *Rh.Ll. Smith[1], R.B. Warren[2], S. Eyre[1], H.S. Young[2], C.E.M. Griffiths[2], J. Worthington[1]* 1) ARC-EU, University of Manchester, Manchester, United Kingdom; 2) The Dermatology Centre, Hope Hospital, University of Manchester, Manchester.

T-cell recruitment and activation at the site of new psoriasis lesions are considered to be of primary importance in the pathogenesis of the disease. A functional polymorphism (R620W) in the haematopoietic-specific protein tyrosine phosphatase (PTPN22) gene, a negative regulator of T-cell activity, is associated with a number of autoimmune diseases including rheumatoid arthritis and Type I diabetes but not psoriasis. The purpose of this study was to investigate further the role of the PTPN22 gene region in susceptibility to chronic plaque psoriasis of onset on or before age 40yr (Type I psoriasis). Using Sequenom MassArray technology we genotyped 13 single nucleotide polymorphisms (SNPs), including R620W, in the PTPN22 region in 647 unrelated Type I psoriasis patients and 566 population based controls. Two SNPs demonstrated significant association with Type I psoriasis: - SNP rs1217414, which resides in intron 2 of PTPN22, conferred risk by carriage of two copies of the minor allele with OR = 2.39 (95% CI 1.43 - 4.11, p=0.0005); and - SNP rs3789604, located downstream of the gene- under a dominant model for the major allele (OR = 1.43; 95% CI 1.13-1.80, p = 0.0017). The rs2476601 (R620W) SNP showed no evidence of association with Type I psoriasis (p=0.67) susceptibility, thereby replicating existing literature. This study suggests that association does exist between Type I psoriasis and 2 SNPs in the region of the PTPN22 gene. SNP rs1217414 has not been identified previously as associated with antibody-associated autoimmune diseases and its exact function is unknown. SNP rs3789604, located in a predicted transcription factor binding site, has been associated with rheumatoid arthritis but only when independent of R620W. Further investigation and replication is required in other psoriasis cohorts as well as other autoimmune diseases un-associated with the R620W polymorphism.

Analysis methods for whole-genome, whole-population association and application to Metabolic Syndrome in the island of Kosrae, Micronesia. *I. Pe'er[1,2], J.K. Lowe[1,2,3], J. Salit[3], S. Purcell[2,4], M.L. Blundell[3], P.E. Bonnen[3], R.P. Lifton[5], J.L. Breslow[3], J.M. Friedmann[3,6], M. Stoffel[3], M.J. Daly[1,2,4], D.M. Altshuler[1,2,4]* 1) Broad Inst, MIT & Harvard, Cambridge, MA; 2) Massachusetts General Hospital, Boston, MA; 3) The Rockefeller University, New York, NY; 4) Harvard Medical School, Boston, MA; 5) Yale University, New Haven, CT; 6) Howard Hughes Medical Institute.

With the current ability to genotype most human variants, the approaching challenge for genetic association is, at the limit, analysis of whole genome data collected from the entire population. The magnitude of such data, the broad range of analyzable heritable traits, and the freedom from sample ascertainment issues make them very compelling. The complications arise from the collection being less controlled than traditional study design: * Sampled families have noin uniform structure, and are interconnected in a complex manner. * Hidden and known stratification may be present. * Phenotypes may be collected in different, less consistent batches. We develop methods for tackling these issues by computing between-family and within family components of association signal, and controlling remaining stratification issues empirically, thereby achieving both utilization of the available information, as well as avoidance of tainted association scores. We apply these methods to the island population of Kosrae, Micronesia, where 100k SNPs provide ample coverage of the genetic variation, and wwhere essentially the entire adult population (N>3000) have been thus genotyped and phenotyped for a collection of traits related to Metabolic Syndrome.

Sex-specific Linkage to Total Serum Immunoglobulin E in Families of Children with Asthma in Costa Rica. *BA. Raby[1,2,3], ME. Soto-Quiros[4], L. Avila[4], SL. Lake[1,3], C. Liang[1], E. Fournier[4], M. Spesny[4], JS. Sylvia[1], R. Lazarus[1,3], A. Verner[5], TJ. Hudson[5], BJ. Klanderman[1], NB. Freimer[6], EK. Silverman[1,3], ST. Weiss[1,3], JC. Celedon[1,2,3]* 1) Channing Laboratory, Brigham & Women's Hosp, Boston, MA; 2) Division of Pulmonary and Critical Care Medicine, Beth Israel Deaconess Medical Center; 3) Harvard Medical School, Boston MA; 4) Division of Pediatric Pulmonology, Hospital Nacional de Niños, San José, Costa Rica; 5) University and Genome Québec Innovation Centre, Montreal, QC; 6) Department of Psychiatry, ULCA, Los Angeles, CA.

Total serum immunoglobulin E (IgE) is a critical intermediate phenotype of asthma and allergic diseases. Although it has long been recognized that total serum IgE levels exhibit sexual dimorphism in humans (males consistently demonstrating higher total serum IgE levels than females), the basis for this difference is unknown. To identify genetic determinants of total serum IgE, a genome-wide scan of 380 short tandem repeat markers was performed in 655 members of 8 extended pedigrees of children with asthma in the Central Valley of Costa Rica. Genome-wide linkage analysis of log-transformed total serum IgE levels was performed by variance component models implemented in SOLAR. Because of prior evidence and sexual dimorphism of IgE in participating families, the analysis was repeated after stratification by sex. Only one genomic region (chromosome 7p15) showed modest evidence of linkage to IgE (LOD=1.60 at 23 cM) among all subjects. After stratification by sex, the evidence for linkage of specific genomic regions to IgE differed greatly between males and females: there was significant evidence for linkage of a novel locus on chromosome 20p12 to IgE (LOD = 3.63 at 36 cM) in males and suggestive evidence of linkage to IgE for three loci (chromosomes 3q21 [LOD = 1.93 at 146cM] and 7p21 [LOD = 2.27 at 12cM] in females, and chromosome 17q12 [LOD = 1.98 at 34cM] in males). These data are the first to provide a possible genetic basis for differences in total serum IgE between sexes. In addition, this genome-wide linkage analysis of total serum IgE is the largest ever conducted in a Hispanic population.

Mutation spectrum in X-linked retinitis pigmentosa in the Danish population. *H. Prokisch[1, 2], M.B. Hartig[1], R. Hellinger[1], T. Meitinger[1, 2], T. Rosenberg[3]* 1) GSF-Research Center, Inst of Human Genetics, Neuherberg, Germany; 2) Technical University Munich, Inst of Human Genetics, Munich, Germany; 3) Gordon Norrie Centre for Genetic Eye Disease, Hallerup, Denmark.

Retinitis pigmentosa is found with an overall prevalence of 1:4000 in the Danish and other populations (Boughman JA et al 1980; Haim et al 1993). The X-linked pattern of inheritance (xlRP) is observed in approximately 10 to 15% of RP cases 2, 3 . In the first or second decade of live affected males develop night blindness and restriction of visual fields, finally disease may lead to complete blindness. Although 5 loci have been proposed in xlRP, more than 90% of cases are caused by mutations in the RPGR and RP2 genes (Meindl et al 1996, Schwahn et al. 1998). Numerous 3` splice variants of RPGR have been described in different tissues. One transcript expressed in the retina includes exon 1 to 14 and the 3`terminal ORF15 (Vervoort et al. 2000). All mutations, which are identified so far, affect this transcript. As 50 to 80% of RPGR mutations were found in ORF15 (size ~ 1,7 kb) versus 20 to 35% of mutations in the exons 1 to 14 (size ~ 1,8kb), ORF 15 was claimed a mutational hotspot (Vervoort et al 2000, Bader et al 2003, Breuer et al 2002, Sharon et al 2003). Here we provide the molecular characterization of the 23 uncharacterized X-linked RP families and give an overview of the 30 known families in Denmark.

Lung cysts and Spontaneous Pneumothrorax: Clinical and Genetic Associations among 198 cases of Birt-Hogg-Dubé Syndrome. *J.R. Toro, S. Pautler, L. Stewart, G. Glenn, O. Toure, M. Wei, P. Choyke, B. Zbar, S. Steinberg, D. Nguyen, M. Linehan* National Cancer Institute, NIH, Bethesda, MD.

Birt-Hogg- Dubé syndrome (BHDS) (OMIM #135150) is an autosomal dominantly inherited genodermatosis that predisposes to fibrofolliculomas, kidney cancer, lung pneumatocysts and spontaneous pneumothorax. To date no study has investigated in detail the pulmonary manifestations of BHDS. In this study, we conducted the largest investigation to date of the pulmonary features, genotype-pulmonary correlation and risk factors for pneumothorax in 198 patients with BHDS. Using direct sequencing we screened patients for mutations in the BHD. BHD mutations were in all coding exons (4-14) except 4 and 8. Exon 11 had the largest number of individuals and families with BHD mutations. Screening using computerized tomography of the chest revealed pulmonary cysts in 89% (177/198) of BHDS patients. Twenty-four percent (48/198) of patients with BHDS had a history of one or more pneumothoraces. We examined the relationship of pneumothorax with categorical parameters (gender, smoking history, kidney tumors and lung cysts) and continuous parameters (the total number of cysts, total number of intraparenchymal cysts, total number of subpleural cysts, number of lobes with cysts, total lung cysts volume, largest cyst diameter and volume). The only categorical parameter that was significantly associated with pneumothorax was the presence of lung cysts ($P=0.006$). Total lung cyst volume, largest cyst diameter and volume, and every parameter related to the number of lung cysts were significantly associated ($P<0.0001$) with pneumothorax. A logistic regression analysis showed that only total number of cysts in the right parenchymal lower lobe and the total number of cysts located in the pleura in the right middle lobe were needed to classify a patient as to whether he or she is likely to have a pneumothorax or not. Kaplan-Meier analysis showed that the probability of a BHDS patient of not having the first pneumothorax by age 30 is 94%, and 75% by age 50. Our study shows a significant association between the lungs cysts (number and location) and pneumothrorax.

Genomic literacy and public attitudes toward the medical applications of genome research: a nationwide opinion survey concerning genome research in Japan. *Z. Yamagata[1], K. Muto[2], I. Ishiyama[1], A. Nagai[1], A. Tamakoshi[3], K. Mimura[4]* 1) Department of Health Sciences, University of Yamanashi, Chuo, Yamanashi, Japan; 2) Faculty of Medicine, Shinshu University, Matsumoto, Nagano, Japan; 3) National Center for Geriatrics and Gerontology, Obu City, Japan; 4) Ochanomizu University, Tokyo, Japan.

Objectives: Due to recent marked advances in genome science, studies on its medical applications have been performed. However, such medical applications have ethical, legal, and social implications (ELSI). In Japan, although there have been few large-scale surveys of public attitudes toward genome science, their attitudes toward its medical applications are unclear. Therefore, the purpose of this study was to clarify the public attitudes toward the medical applications of genome research, i.e., the relationship between genomic literacy and decision making regarding genetic medicine Participants and Methods: In 2005, an anonymous mail questionnaire survey was conducted among 4000 males and females aged 20-69 years. In the questionnaire, the attitude was classified into affective (image), cognitive (belief), and behavioral (decision making) components, and questions corresponding to each component were used. We evaluated genomic literacy based on a 3-dimensional measurement: (i) the degree of understanding of genome science terms and other science terms as terms (term understanding), (ii) the degree of understanding of genome science terms and other science terms in the given context (context understanding), and (iii) the perception of values/risks (value/risk perception). Result and Discussions: The response rate was 54.3%; 2171 participants (991 men and 1180 women) were analyzed. The participants generally had a positive attitude toward the medical applications of genome research. Participants who had a positive image, positive belief, and high level of genomic literacy made positive decisions, while those who had marked negative beliefs and a low level of genomic literacy made negative decisions. It is suggested that a deficit model of public understanding of genomic research may be generally accepted in Japan.

Global gene expression analysis links glutamatergic and GABAergic neurotransmission alterations to suicide and depression. *A. Sequeira[1], T. Kemplan[1], F. Mamdani[1], J. ffrench-Mullen[2], G. Turecki[1]* 1) Human Genetics, McGill University, Montreal, Quebec, Canada; 2) Gene Logic Inc., Gaithersburg, MD, US.

Suicide is a complex disorder with a clear genetic predisposition and often associated with psychiatric disorders. Several lines of evidence have established that molecular alterations in many different areas of the brain may be implicated in the physiopathology of suicide and depression, but typically those studies have focused on one or a few genes at a time. Microarray analysis allows the parallel monitoring of expression at the mRNA level of thousands of genes. We performed a gene expression study on total mRNA isolated from 17 cortical (Brodman areas (BA) 4, 6, 8-9, 10, 11, 20, 21, 38, 44, 45, 46, 47) and subcortical (BA24, BA29, amygdala, hippocampus, Nucleus Accumbens) brain regions from 28 suicide cases (18 with and 10 without major depression) and 13 matched controls using the Affymetrix HG-U133AB chip set. We observed the highest number of suicide specific alterations in prefrontal cortical areas in agreement with previous observations implicating this region in suicide and depression. Global ontological profiling revealed an overrepresentation in the prefrontal cortex of genes implicated in the adenosine triphosphate (ATP) metabolic pathway. Synaptic neurotransmission and G-protein coupled signaling was on the other hand globally overrepresented and lead us to the observation that Glutamatergic and GABAergic related genes were globally altered. In conclusion, this constitutes the first overview of global molecular changes in the brain of suicide victims and suggests an alteration of the ATP metabolic pathway in the prefrontal cortex and global changes in the expression of genes implicated in GABAergic and Glutamatergic neurotransmission in suicide and depression.

Fine Mapping of the *CHRNA7* Candidate Gene for Association Studies with Schizophrenia. *S.H. Stephens, S. Leonard* Psychiatry, University of Colorado Health Sciences Center, Aurora, CO.

Schizophrenia has been linked to the 15q14 locus in multiple independent studies and across ethnicities. The 15q14 linkage region was independently linked to a P50 sensory processing deficit found in most schizophrenics. The 7 nicotinic acetylcholine receptor subunit gene, *CHRNA7*, maps to this region and was selected as the best candidate gene for the P50 gating deficit based on both human and animal studies. Smoking is particularly prevalent in schizophrenics and is hypothesized to be a form of self-medication for these patients. Binding studies with 7 receptor agonists found 50% fewer receptors in the post-mortem brains of persons with schizophrenia, than those without the illness. Low levels of the 7 receptor may have downstream consequences for multiple neurotransmitter systems resulting in changes in expression normally triggered by these neurotransmitters. As smoking appears to alter gene expression in all individuals, it may actually help to normalize gene expression in schizophrenics. *CHRNA7* is now considered one of few important candidate genes for schizophrenia. Mutation screening of the *CHRNA7* coding region and intron/exon splice junctions revealed multiple synonymous variants and rare non-synonmous variants that were not associated with schizophrenia or the P50 deficit; however, this screening also revealed a large number of functional mutations in the upstream regulatory region of *CHRNA7*, particularly, the core promoter. My hypothesis is that genetic variance at the *CHRNA7* gene is associated with risk for schizophrenia and accounts for linkage to this region. To test this hypothesis I will expand on the mutation screening by fine mapping the *CHRNA7* gene using a combination of SNP and microsatellite markers to capture all genetic variance in this region. I will also use known polymorphic markers in the upstream regulatory region of the *CHRNA7* gene for single SNP association studies as well as multi-locus models for association. Results of this study will reveal whether variance in the *CHRNA7* candidate gene and/or its upstream regulatory region accounts for the linkage to schizophrenia.

Lysosomal N-acetyltransferase deficient in mucopolysaccharidosis type IIIC is encoded by the TMEM76 gene on human chromosome 8. *A. Pshezhetsky[1], V. Seyrantepe[1], S. Durand[1], N.M. Roslin[2], A. Verner[3], C.E. Beesley[4], I. Maire[5], B. Poorthuis[6], J. van de Kamp[7], O. van Diggelen[8], T.J. Hudson[2,3], T.M. Fujiwara[2], K. Morgan[2]* 1) Ste-Justine Hospital, Montreal University, Canada; 2) McGill University Health Centre, Montréal, Canada; 3) McGill University and Genome Quebec Innovation Centre, Montréal, Canada; 4) UCL Institute of Child Health, London, United Kingdom; 5) Hôpital Debrousse, Lyon, France; 6) Leiden University Medical Center, Leiden, the Netherlands; 7) Clinical Genetics Center, Utrecht, the Netherlands; 8) Erasmus University, Rotterdam, the Netherlands.

Mucopolysaccharidosis type IIIC (MPS IIIC) is an inherited recessive disorder caused by lysosomal storage of heparan sulphate. The biochemical defect in MPS IIIC is a deficiency of an enzyme required to N-acetylate the terminal glucosamine residues of heparan sulfate. Since its acetyl-CoA substrate would be rapidly degraded in the lysosome, N-acetyltransferase employs unique mechanism acting both as an enzyme and a channel and catalysing the transmembrane acetylation of heparan sulfate without transporting acetyl-CoA into the lysosome. Using linkage analysis in MPS IIIC families and a biochemical purification of lysosomal N-acetyltransferase, we identified that the enzyme is encoded by the transmembrane protein 76 (TMEM76) gene in the centromeric region of chromosome 8. The gene contains mutations in MPS IIIC patients incompatible with the normal function of the predicted protein. Functional expression of TMEM76 demonstrates that it has N-acetyltransferase enzymatic activity and lysosomal localization. Since the TMEM76 protein does not show a structural similarity to any known pro- or eukaryotic N-acetyltransferases, we think that it belongs to a new structural class of proteins capable of carrying out the transport of the activated acetyl residues across the cell membrane. Moreover, our results may define the function for a conserved family of bacterial proteins COG4299 that share homology with N-acetyltransferase-TMEM76. Our work sets the stage for DNA-based diagnosis of MPS IIIC and genotype-phenotype correlation studies and marks the end of the gene discovery phase for lysosomal storage diseases.

Identification of a novel chromosome region 13q21.3-q22.3 for susceptibility genes in familial chronic lymphocytic leukemia. *O. Toure[1], D. Ng[1], M. Wei[1, 2], D. Arthur[3], G. Marti[4], L. Fontaine[5], J.F. Fraumeni[6], L.R. Goldin[1], N. Caporaso[1], J. Toro[1]* 1) Division of Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD; 2) Program of Division of Cancer Epidemiology and Genetics, SAIC-Frederick, Inc., Frederick, MD 21702; 3) Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, NIH, DHHS, Bethesda, MD 20892; 4) Flow and Image Cytometry Laboratory, Cellular Therapy and Tissues Branch, Division of Gene and Cell Therapy, Food and Drug Administration/Center for Biologics Evaluation and Research, Bethesda, MD 20892; 5) Westat Inc., Rockville, MD, 20852; 6) Office of the Director, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, DHHS, Rockville, MD 20892.

Chronic lymphocytic leukemia (CLL) is the most prevalent form of leukemia in adults in western countries. A genome scan of CLL-prone families in 2003 found a lod score of one in band 13q22.1. To investigate this finding, we selected six CLL families consisting of 65 individuals (CLL affected n=19, unaffected n=46) for fine mapping of a 23 megabase region in 13q14.2-q22.3. The median age at diagnosis was 52 years (range 38-76). Interphase FISH revealed 13q14 deletion in 11 of 13 CLL patients. An unaffected sibling in family 2 [2-1006], who shared the at-risk haplotype, also had 13q14 deletion by FISH. Four CLL families shared a 3.8 Mb minimal region in 13q21.31-q22.3. Using direct sequencing analysis we screened 12 genes and one predicted gene for mutations. Eighty-one percent (69/85) of the polymorphisms identified did not result in a predicted amino acid change. A deep intronic polymorphism (IVS10-68_-71delGTAA) was detected in PIBF1 that co-segregated with affected and unaffected members who shared a haplotype in 13q21.31-q22.3 in one family. We did not detect nonsense or frameshift mutations in the coding region of these 13 genes. In conclusion, we identified a novel candidate region that may predispose to familial CLL.

Fabry disease in women and daughters: the key role of family history in the diagnosis, the dilemmas of treatment and prenatal diagnosis. *A. Raas-Rothschild[1], B. Mitchnick[2], A.J.J.T. Rein[3], M. Zeigler[1], G. Bach[1], R. Backenroth[4]* 1) Department of Human Genetics, Hadassah Hebrew University Medical Center, Jerusalem, Israel; 2) Day Care Hospital; 3) Division of Pediatric Cardiology; 4) Department of Nephrology.

Since 1976, nine women and 4 girls with Fabry disease have been diagnosed in the Hadassah medical center because of a positive family history. Age at diagnosis varied from birth to 60 years. Symptoms and signs were variable: onset was between age 12 and 55, and ranged from proteinuria which lead to a kidney biopsy at age 12; painful acroparesthesias which lead to psychologic treatment of a 15 year old girl; retinal vessel occlusion, hypertension and end stage renal insufficiency at age 40, and cardiomyopathy at ages 55 and 60. The family history of Fabry disease was key to the diagnosis in these women and their daughters: all were diagnosed only after a male relative. Treatment at our medical center has been started in 3 women (ages 31, 33, and 54) and two (ages 34 and 60) are in the process of starting. Mean duration of therapy is 29 + 18 (SD) months. There have been no apparent treatment related side effects, nor worsening of kidney and cardiac function; Proteinuria regressed, and there was a subjective improvement in symptomatology. Angiokeratomata appear not to have regressed. Two among three additional women (ages 29-32) have acroparesthesias during intercurrent illnesses and no other objective organic signs of Fabry disease. Among the four young girls (ages 2-7), one has been recently diagnosed as affected with attention deficit disorder, while the others are asymptomatic. We would like to present and discuss the following questions regarding female Fabry patients: When should be treatment started? What is the optimal dose of therapy? What is the best way to evaluate the efficacy of the treatment? In addition, we would like to raise some issues in prenatal diagnosis of Fabry disease.

Signature of Group II introns ORF in chick embryos? *M.A. Sabry[1], A. Jones[2], G.K. Dhoot[3]* 1) Al-Jawhara Center For Molecular Medicine, Genetics and Inherited Disorders, College of Medicine, Arabian Gulf University, Manama, Bahrain; 2) Functional Genomics and Proteomics Laboratories, School of Biosciences, University of Birmingham, UK; 3) Department of Basic Veterinary Sciences, The Royal Veterinary College, University of London, UK.

Group II introns represent a unique class of RNAs, abundant in organellar (mitochondrial and chloroblast) genomes of lower eukaryotes and higher plants. Organellar group II introns contain an ORF which encodes three proteins: a reverse transcriptase, a maturase, and an endo-nuclease, all functioning in splicing. Group II introns have not been identified in higher eukaryotes or in nuclear genomes. We followed a proteomics approach (electrophoresis/mass spectrometry) to investigate trypsin-digested protein extracts from chick embryos to identify proteins with potential significance during embryonic development. On Mascot/BLAST analysis, we identified two maturase-like, non-overlapping peptide fragments in chick embryos at stages 12 (16 somites) and 29 (6 days).Verifying these findings could shed some light onto the potential existence/expression of group II introns ORF in higher eukaryotes and provide insight into possible functional roles in these species, e.g. during development.

An excess of G over C in mutagenesis: evidence from human genetic diseases in support of methylation-insulation hypothesis for conserved amino acid evolution. *L. Xiao[1], Y.F. Yin[2], J. Zhang[1], H.L. Gao[2], K. Li[2]* 1) SNP Institute, Nanhua University, China; 2) Human Genetics, City of Hope National Medical Center, Duarte, California, CA91010, USA.

At the epigenetic point of view, the human genome contains five sequence specific nucleotides of A, C, G, T and 5-methyl C. The 5-methyl C is at about 0.25% of the sequence in adult genome and is expected to play a role in the formation of the leading type of C/T or G/A polymorphism in mammalian genomes. Recently, we recognized an excess of G>A over C>T substitutions in hemophilia B patients. Further analysis demonstrated biased point mutations between sense and antisense strands when unique changes in factor IX were counted. Data of 11 genes (based on their large number (>100) of missense mutations) retrieved from HGMD were analyzed for their point mutation spectra. Similar to factor IX, all genes selected in this study have a biased G>A over C>T unique mutations (Fig 1). The excess of G base over C base was valid when an expanded comparison between G>H (C/G/T) and C>D (A/G/T) was evaluated. Interestingly, all possible amino acid changes by substitution involving the G or C nucleotides show different consequences to protein function: a relatively tolerable or deleterious according to Blossom 62 scoring. These data prompted a hypothesis that C nucleotide is highly mutable because of the presence of 5-methyl C and neutral or tolerable C to T substitutions have been nearly saturated/exhausted during evolution. This hypothesis has two implications: First, the conserved amino acids of C, Er, G, and W have evolved by excluding C nucleotides from the first two bases in their genetic triplets and employing GDN or GDN instead; and secondly, mutations from substitutions of conserved amino acids or those with nonsynonymous cytidine are thus subjected to more severe clinical phenotypic consequences.

Pleiotropic effects of polymorphisms in MICB on human herpes virus seroposivity and schizophrenia risk. *B.H. Shirts[1], J.J. Kim[2], S. Reich[1], F.B. Dickerson[3], R.H. Yolken[3], B. Devlin[1], V.L.. Nimgaonkar[1]* 1) Dept Psychiatry & Human Gen, Univ Pittsburgh, Pittsburgh, PA; 2) Psychiatry Dept, Catholic U of Korea, Seoul, Korea; 3) Stanley Center for Developmental Neurovirology, Baltimore, MD.

We report the identification of associations of MICB SNPs with cytomegalovirus (CMV) and herpes simplex virus 1 (HSV1) seropositivity in the process of mapping for schizophrenia risk genes. Several studies have implicated exposure to herpes viruses as a risk factor for schizophrenia. We previously found associations at anonymous polymorphisms on chromosome 6q21 with schizophrenia among patients seropositive for CMV and HSV1, suggesting that such viruses confer risk in conjunction with specific host genetic variation. To localize the association further, we conducted a multi-staged study among individuals of Caucasian ancestry. We analyzed 21 SNPs spanning an 100kb genomic region in a sample of 236 schizophrenia patients and 240 unscreened controls. Based on suggestive associations we selected five SNPs at MICB to assay among two independent samples which had been serotyped for CMV and HSV1: a case-control sample recruited in Baltimore (n=272 cases, 108 controls), and a case-parent trio sample recruited in Pittsburgh (n=221). Among Baltimore control individuals there were significant associations with serum status for infectious agents: rs1051788 with HSV1 seropositivity ($p = 0.03$, OR= 2.4) and rs2523651 with CMV seropositivity ($p = 0.003$, OR = 3.8). The former association was also detectable among the parents of cases recruited in Pittsburgh ($p = 0.04$, OR = 1.4). Neither association was noted among the schizophrenia cases. With respect to schizophrenia risk, significant transmission distortion was noted at rs1051788 among the case-parent trios ($p=0.014$) regardless of antibody status. In the Baltimore cases sample we a found similar overrepresentation at rs1051788 ($p = 0.101$, OR= 1.4). Associations with schizophrenia at rs1051788 may be due to interactions between MICB alleles and HSV1 status. Our analyses suggest complex associations with schizophrenia and certain herpes viruses at the MICB gene. Further replicate studies are warranted, as are functional studies of these polymorphisms.

Juvenile Pagets disease: The second reported, oldest patient is homozygous for *TNFRSF11B* Balkan mutation (966_969delTGACinsCTT) which elevates circulating immunoreactive osteoprotegerin levels. *M.P. Whyte[1,2], P.N. Singhellakis[3], M.B. Petersen[4], S. Mumm[1,2]* 1) Ctr Metab Bone Dis & Mol Res, Shriners Hosp, St. Louis, MO; 2) Div Bone & Min Dis, Wash Univ Sch Med, St. Louis, MO; 3) Dept Endo, St. Savvas Hosp, Univ Athens, Athens, Greece; 4) Dept Genet, Inst Child Health, Aghia Sophia Children's Hosp, Athens, Greece.

Juvenile Pagets disease (JPD) is a rare, autosomal recessive osteopathy featuring accelerated bone turnover causing skeletal pain, fracture, deformity, and early-onset deafness. Most JPD patients lack osteoprotegerin (OPG) inhibition of osteoclastogenesis due to loss-of-function mutation of TNFRSF11B, the gene encoding OPG. The genetic defect is homozygous in most patients. Circulating immunoreactive OPG levels in JPD have been reported only for the Navajo mutation (selective, complete deletion of TNFRSF11B) where OPG concentrations are, as anticipated, undetectable. We update a 60-year-old Greek patient who is the second reported, oldest JPD patient. Over 4 years, serum immunoreactive OPG levels have been quantified by ELISA assay. Mutation analysis of TNFRSF11B was performed by PCR and sequencing of leukocyte DNA from the patient, his mother and sister. Since age 32 years, he has received bone antiresorptive therapy; initially synthetic human calcitonin, and then bisphosphonates of increasing potency. Despite recent normalization of biochemical markers of bone remodeling, he mentions relentless loss of sight and hearing. Mutation analysis revealed homozygosity for the same mutation, an insertion/deletion in TNFRSF11B (966_969delTGACinsCTT), recently reported for an unrelated Greek boy and Croatian man with JPD. Serum immunoreactive OPG levels and soluble RANKL concentrations were persistently elevated, consistent with a mutation that precludes biosynthesis of the carboxyterminus of OPG, including a cysteine necessary for dimerization. Hence, the circulating, mutated OPG is detectable by immunoassay, but dysfunctional in controlling osteoclastogenesis. Among JPD patients, assay of immunoreactive OPG may result in a range of levels, making laboratory diagnosis uncertain by such testing, and instead dependent on TNFRSF11B gene analysis.

The first case of inherited sub-microscopic terminal duplication 12q in non-affected mother and affected child.

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Case presentation: The patient was born to a 34-year-old mother who had one spontaneous abortion at 6-7 weeks gestation and no other pregnancies. This pregnancy was complicated by fetal hypotonia. Amniocentesis was done because of abnormal MSAFP. The chromosomal analysis was normal. The child was born at 34 weeks gestation by normal delivery. The birth weight was 5 lbs 5 oz. The newborn period was complicated by poor feeding. The child's development was delayed since birth. On exam the patient had mild dysmorphic appearance with epicanthal folds and wide nasal bridge. No other abnormalities were noted. Cytogenetic findings: Sub-telomere analysis showed an extra signal of the 12q sub-telomere probe (Vysis) on the short arm of chromosome 13 resulting in a duplication of a very small terminal portion of 12q with no other significant chromosomal abnormalities. The same abnormality was found in the patients mother, who apparently had normal development and was in good health. Discussion: To our knowledge this is the first reported case of sub-microscopic terminal duplication of 12q, where no abnormalities were initially seen on routine chromosome analysis. Interestingly, this abnormality was inherited from a parent with apparently normal development. The smallest previously reported terminal 12q duplication resulted in a mild mental retardation and no dysmorphic features (Am J Med Genet, 2004, 128A:305). A likely explanation of our findings is that this chromosomal abnormality has variable clinical presentation and may lead to mild developmental delay in some individuals, or normal development/subtle and not easily noticeable developmental abnormalities in others. Similar variability in the clinical presentation of other chromosomal abnormalities has been previously described. Another possible explanation such as UPD 12 in the affected patient is considered unlikely since UPD 12 has been previously reported in association with a normal phenotype. This work is supported in part by the NYS OMRDD.

A Study on the correlation between ER-alpha gene polymorphism and Osteoporosis in Iranian women. *F. Pouresmaeili[1], A. Roohi[1], M.J. Tehrani[2], E. Azargashb[3], J. Ghasemi[2], T. Khalili[1], Sh. Samangooee[4], B. Kazemi[5], M. Akhoondi[6], Romatology Department of Loghman Hospital* 1) Genetics & Biochemistry Dept., Shahid Beheshti University of Medical Sciences, tehran, Iran; 2) Genetics Department of Avesina Research Center, Tehran 19835-177, Iran; 3) Health Dept. , Shahid Beheshti University of Medical Sciences, tehran, Iran; 4) Romatology Clinic, Taleghani hospital, Shahid Beheshti University of Medical Sciences, tehran, Iran; 5) Cell& Molecular Biology research center, Shahid Beheshti University of Medical Sciences, tehran, Iran; 6) Avesina Research Center, Tehran 19835-177, Iran.

Osteoporosis is a common skeletal disease characterized by low bone mass and micro architectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture. Genetic factors play an important role in regulating bone mass density (BMD). While in few isolate conditions, osteoporosis can be inherited in a simple Mendelian pattern, due to single gene mutations, in the majority of cases has to be considered a multifactorial polygenic disease. The disease initiation, progression and severity in every given individual are influenced by interaction of several environmental factors with several genes. Genetically, early diagnosis of osteoporosis is important, particularly, before appearance of disease symptoms. We investigated the correlation between osteoporosis and allelic variation (polymorphism) of Estrogen receptor alpha gene in 250 individuals aged 40-60 years old . DNA was extracted from peripheral blood samples taken from all individuals under study. Analysis of PCR products and comparison of patients and controls RFLPs using restriction end nucleases PVUII and XbaI revealed that there are 3 different PVUII (PP, Pp, pp) and XbaI (XX, Xx, xx) genotypes among both groups under the study. Chai-2, Fisher test, and comparison between frequency distribution of normal-patient did not show any significant correlation between the disease appearance and observed genotypes. Therefore, we conclude that intron I polymorphism of ER-alpha has no precise effect osteoporosis and bone tissue differences in Iranian women, exclusively.

Selective reconstitution of liver cholesterol biosynthesis promotes lung maturation but does not prevent neonatal lethality in Dhcr7 null mice. *H. Yu[1], G.S. Tint[2], J. Chen[1], Y. Cai[1], G. Xu[2], S.B. Patel[1]* 1) Medicine, Medical College of Winsconsin, Milwaukee, WI 53226; 2) VA Medical Center, East Orange, NJ 07018 & UMDNJ-New Jersey Medical School, Newark, NJ. 07103-2714.

Targeted disruption of the murine 3?-hydroxysterol-?7 reductase gene (Dhcr7), an animal model of Smith-Lemli-Opitz syndrome, leads to greatly reduced cholesterol synthesis and neonatal death because of markedly impaired lung development. We recently reported partial rescue of this neonatal lethality in Dhcr7 null mice by the transgenic restoration of human DHCR7 expression in brain. To gain further insight into the role of non-brain tissue cholesterol deficiency in the pathophysiology, we tested whether the lethal phenotype could be abrogated by selective transgenic complementation with DHCR7 in the liver. We generated mice that carried a liver-specific human DHCR7 transgene whose expression was driven by the human apolipoprotein E (ApoE) promoter and its associated liver-specific enhancer. These mice were then crossed with Dhcr7+/- mutants to generate Dhcr7-/- mice bearing a human DHCR7 transgene. Robust hepatic transgene expression resulted in the significant improvement of cholesterol homeostasis in liver, lung and plasma with cholesterol concentrations increased to 80~90 % of normal levels. Significantly, the cholesterol deficiency in brain was not altered. Although late gestational lung sacculation was greatly improved, there was no parallel increase in postnatal survival in the transgenic mice. Thus, the reconstitution of Dhcr7 function selectively in liver induced a significant improvement of cholesterol homeostasis in non-brain tissues, but failed to rescue the neonatal lethality of Dhcr7 null mice. These results provide further evidence that impaired CNS development very likely plays a major role in the pathogenesis and early death of Dhcr7-/- mice.

A Balanced Accuracy Metric for Epistasis Modeling in Imbalanced Datasets using Multifactor Dimensionality Reduction. *D.R. Velez[1], B.C. White[2], A.A. Motsinger[1], W.S. Bush[1], M.D. Ritchie[1], S.M. Williams[1], J.H. Moore[2]* 1) Vanderbilt University, Nashville, TN; 2) Computational Genetics Laboratory, Dartmouth Medical School, Lebanon, NH.

Multifactor dimensionality reduction (MDR) was developed as a computational method for detecting, characterizing, and interpreting statistical patterns of epistasis. The overall goal of MDR is to change the representation space of the data using constructive induction to make interactions easier to detect using machine learning methods and statistical classifiers such as logistic regression. It is well-known that machine learning methods such as MDR may not provide robust models when the class variable (e.g. case-control status) is imbalanced or unequal and accuracy is used as the fitness measure. This is because most methods learn patterns that are relevant primarily for the larger of the two classes. The goal of this study was to evaluate three different strategies for improving the power of MDR to detect epistasis in imbalanced datasets. The first method evaluated was oversampling that samples with replacement the smaller class until the data are balanced. The second method evaluated was undersampling that randomly removes subjects from the larger class until the data are balanced. The third method evaluated used balanced accuracy [(sensitivity+specificity)/2] as the fitness metric. These three methods were compared using using simulated epistatic interactions of varying heritability (0.01, 0.025, 0.05, 0.1, 0.2, 0.3, 0.4) and minor allele frequency (0.2, 0.4) that were embedded in 100 replicate datasets of varying sample sizes (200, 400, 800, 1600). Each dataset with a given sample size was generated with different ratios of cases to controls (1:1, 1:2, 1:4). We found that the balanced accuracy metric significantly outperformed both oversampling and undersampling and fully recovered the power observed using accuracy in the balanced datasets. The new balanced accuracy metric has been included in the open-source and freely-available MDR software package for application to imbalanced case-control datasets. (Supported by NIH R01s AI59694 and LM009012, PI-Moore).

A De Novo SCN5A Mutation (W1191X) Its Relation with Brugada Syndrome. *D.J. Shin[1], E.M. Kim[2], Y.S. Bae[1], J.H. Han[1], Y.S. Jang[1,3], B.Y. Joung[3], M.H. Lee[3], S.S. Kim[3], H. Huang[4], M. Chahine[4], S.J.K. Yoon[2]* 1) Cardiovascular Genome Center, Yonsei Univ Colledge of Medicine, Seoul 120-749, Republic of Korea; 2) Research Institute of Molecular Genetics, The Catholic University of Korea, Seoul 137-701, Republic of Korea; 3) Department of Cardiology, Yonsei Cardiovascular Center, Seoul 120-749, Republic of Korea; 4) Laval Hospital Research Centre and Department of Medicine, Laval University, Quebec, Quebec, Canada.

Brugada syndrome (BS) is an inherited cardiac disorder associated with a high risk of sudden cardiac death and is caused by mutations in the SCN5A gene encoding the cardiac sodium channel alpha-subunit (Nav1.5). The aim of this study was to identify the genetic cause of familial BS and characterize its electrophysiological property of a novel SCN5A mutation (W1191X). Four families and one patient with BS were screened for mutations of the SCN5A gene by PCR and direct sequencing. Wild-type (WT) and mutant Nav1.5 channels were expressed in tsA201 cells, and the sodium currents were analyzed using the whole-cell patch-clamp technique. A novel mutation, W1191X, was identified in a family with BS. Expression of WT or mutant channel (Nav1.5/W1191X) cotransfected with the beta 1-subunit in tsA201 cells resulted in a loss of function of Nav1.5 channels. While voltage-clamp recordings of the WT channel showed a distinct acceleration of Nav1.5 activation and fast inactivation kinetics, the Nav1.5/W1191X mutant failed to generate any currents. Co-expression of both WT and mutant channels resulted in a 50% reduction in sodium current. Our results revealed that the W1191X mutation is associated with BS that resulted in the loss of function of the cardiac sodium channel.

Morbid obesity is associated with multiple obesity genes: PPARG, UCP2, GNB3, and ESR1. *W.H. Pan[1, 2], H.H. Chen[2], C.S.J. Fann[1], W.J. Lee[3]* 1) Institute of Biomedical Sciences, Academia Sinica, Taiwan; 2) Department of Biochemical Science and Technology, Institute of Microbiology and Biochemistry, College of Life Science, National Taiwan University, Taiwan; 3) Min-Sheng Hospital, Taiwan.

The prevalence of obesity is increasing rapidly worldwide. It is urgent to uncover genes which interact with environmental factors and contribute to the development of obesity in order to overcome the problem. Previous studies showed heterogeneous findings on potential obesity loci, using varied obesity phenotypes across multiple populations. Yet there are relatively few systematic studies in Chinese to screen for obesity genes. Our attempt is the first of its kinds in Han Chinese to search for major obesity genes, using morbid obesity as the phenotype(BMI>=40), which is rare (around 0.064%)in Taiwan. We applied a two-staged association study design with a total of 315 unrelated cases and 315 controls. All subjects participated in our study voluntarily. Signed informed consents were obtained. The inclusion criteria of the case were:(1)age in the range of 20-59(2)BMI>=40, (3)ancestral origin of Han Chinese from either side of parents. Each case was matched with a control (BMI<=24) by gender, age, educational level and residential location. In 1st stage of the study, a total of 94 cases and 94 matched controls were genotyped for 133 tag SNPs http://www.hapmap.org) or previously identified SNPs on 33 candidate genes. Eighteen genes have been linked or associated with obesity in at least 5 positive studies. The SNPs of another 15 genes have been significantly associated with obesity in Asians. A total of 17 SNPs on 11 genes showed potential associations with morbid obesity in the 1st stage. Subsequently, more subjects (221 cases and 221 matched controls) were genotyped for these 17 SNPs. With data from all cases and controls, we found 5 SNPs (in PPARG, UCP2, GNB3, and ESR1) were significantly associated with morbid obesity. Three SNPs increase risk of morbid obesity in recessive manner and the other 2 dominant.Our data suggest that morbid obesity may be resulted from joint effects of multiple obesity genes.

Improved Beta-thalassemia genotyping by means of reverse-hybridization teststrips tailored to population-specific mutations. *H. Puehringer[1], H. Najmabadi[2], W. Krugluger[3], H.-Y. Law[4], V. Viprakasit[5], C. Oberkanins[1]* 1) ViennaLab, Labordiagnostika, Vienna, Austria; 2) Genetics Research Center, Social Welfare and Rehabilitation Sciences University, Tehran, Iran; 3) Dept. Clinical Chemistry, Municipal Hospital Rudolfstiftung, Vienna, Austria; 4) Genetics Service, KK Women's and Children's Hospital, Singapore; 5) Dept. Paediatrics, Mahidol University, Bangkok, Thailand.

Beta-thalassemia is among the most common inherited diseases throughout Southeast Asia, India, the Middle East, parts of Africa and the Mediterranean area. Mutations in the beta-globin gene are leading to structural abnormalities (e.g. Hb S, Hb E, Hb C), reduced synthesis or complete absence of the beta-globin chains and consequently to deleterious effects of haemoglobin imbalances. In each population at risk beta-thalassemia results from a limited number of common mutations and a larger, more variable number of rare mutations. We have improved an existing reverse-hybridization assay (Beta-Globin StripAssay), originally designed for Mediterranean countries, to make it applicable for a wider range of ethnic groups. Three separate test strips, specific for the most prevalent mutations in Southeast Asia, the Middle East plus India and the Mediterranean region, have been designed. Each test strip comprises 22 mutations and represents an average allele coverage of 91.4 %, 91.7 % and 95 %, respectively. Comprehensive genotyping of beta-thalassemia is performed by a single multiplex DNA amplification reaction and subsequent hybridization to the adequate test strip. The entire procedure from blood sampling to the identification of mutations requires less than 6 hours, and hybridization/detection may be carried out manually or essentially automated using existing instrumentation (e.g. TECAN profiBlot). The test is simple and convenient, and requires very small amounts of samples, which is of particular importance for prenatal diagnosis. The broad range of beta-thalassemia mutations covered by the StripAssay should make it a globally useful diagnostic tool.

Possible role of Infection in the etiology of Endometriosis. *K. Vijayalakshmi[1], M. Sireesha[2], P. Shivani[3], Y.R. Ahuja[1], H. Qurratulain[2,3]* 1) Dept of Genetics, Vasavi Hospital, Lakdi-ka-pul, Hyderabad -500 004, Andhra Pradesh, India; 2) Dept of Genetics, Bhagwan Mahavir Medical Research Centre, AC Guards, Hyderabad-500 004, Andhra Pradesh, India; 3) Dept of Genetics & Molecular Medicine, Kamineni Hospital, LB Nagar, Hyderabad- 500 068, Andhra Pradesh, India.

Endometriosis is a common, chronic, benign, gynecological disorder associated with pelvic pain and infertility. Its main characteristic is the presence of endometrial tissue outside the uterus. Even though it is not life-threatening, it is associated with considerable morbidity and suffering. The prevalence of pelvic endometriosis ranges between 6% and 10% women during their reproductive years. Endometriosis is one of the most common gynaecological disorders, but its aetiology and pathogenesis remain obscure. Hormonal, genetic and environmental factors are considered to play a role in its etiology. The aim of the present study was to identify novel genes with differential display Reverse Transcriptase Polymerase chain reaction (DD-RT-PCR) between eutopic and ectopic endometrium. Eutopic and ectopic tissue from 3 patients was collected for RNA isolation and cDNA was prepared using reverse transcriptase. Differential display RT PCR was carried out twice using two sets of 7 different arbitrary primers. Ectopic endometriotic tissue of one case showed a unique band of 350bp after DD-RT-PCR using the second set. The band was cut out, eluted, re-amplified with individual primers and automatically sequenced. The sequence when subjected to BLAST search revealed 100% homology of 157 bp with Shigella dysenteriae species. The identification of Shigella bacteria in the present study suggests that infection may play an important role in the etio-pathogenesis of endometriosis. This has not been reported in the world literature as yet. It could be postulated that the infection like Shigella may be the trigger that sets into action, the immunological changes in the pelvic peritoneum resulting in the phenotype of endometriosis. Further work in this area is required to establish the role of infection in the etiology of endometriosis.

The attitude of Chinese females on prenatal screening of fragile X syndrome. *Y-Z. Zhang[1], N. Zhong[1,2]* 1) Peking University Center of Medical Genetics, Beijing, China; 2) New York State Institute for Basic Research.

Fragile X syndrome (FRX) is the most common cause of inherited mental retardation. The prevalence of FRX is estimated at 1 in 4000 males and 1 in 8000 females. Our earlier study has determined that the frequency of FRX in China is similar to that in western countries. We found that FRX accounts for 2.8-3.2% of Chinese mental retardations. A recent national survey conducted in China determined that there are about 13 millions of mental retardation among the children of 0-6 years old, which may give a figure that there exist, at least currently, about 500,000 fragile X families in China. Because there is no causative treatment presently and the major effort for preventing this disease is prenatal diagnosis, we have performed a preliminary study on the prenatal attitude among 72 Chinese women who have never heard about FRX before participating. The 72 participants were grouped into: married with child(ren) (6/72), married with no child (40/72), and unmarried single (26/72). A one-hour short course of education was provided to these females for introducing the basic knowledge about FRX, which includes clinical features of the FRX, genetic defect of FRX, current status of diagnosing and treatment of FRX, and prenatal diagnosis of FRX, etc. After the short course, a survey with 15 questions about the FRX and prenatal attitude was conducted. About 80% of the 15 questions were correctly answered among three groups, suggesting that a short course of education provided to non-knowledgeable population may increase the awareness on FRX. Seventy-one percent (51/72) women preferred to have prenatal screening for FRX, even without a family history. Ninety-six percent (69/72) of women chose to terminate pregnancy if a positive FRX was found in the fetus. The strong attitude of prenatal screening of FRX in the Chinese women indicated the acceptance of applying prenatal screening for severe birth defect, for which there is no effective treatment or prevention available.

Redefining the null hypothesis for Hardy Weinberg equilibrium. *K. Ryckman[1,2], L. Jiang[1], J. Haines[1], S. Williams[1,2]* 1) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN; 2) Department of Medicine, Vanderbilt University Medical Center, Nashville, TN.

Purpose: Current approaches for testing deviations from Hardy-Weinberg equilibrium (HWE) in case-control studies usually test the null hypothesis of genotype frequencies in cases against the case allele frequency and the same in controls. Using this method violates the assumption of a random mating population as it assumes that cases mate randomly with cases and controls with controls. We have therefore compared the typical way of HWE testing to what we propose is a more accurate and informative method. Methods: Our method uses an estimate of the population allele frequency that is a combination of both case and control allele frequencies, p_T, to test for deviations from HWE. The proportion of case and control allele frequencies used in the calculation of p_T is based on the prevalence of the disease in the population. A chi-square test is then used to detect deviations from HWE. This method was applied to simulated case-control data, as well as a study examining age-related macular degeneration (AMD). Results: Under dominant, recessive and additive models our method of calculating HWE is more sensitive to detecting deviations than the commonly used method of testing for HWE. Our simulations show that under most genetic models using p_T to calculate HWE can be just as powerful for detecting disease associated SNPs as standard allelic and genotypic chi-square association tests. The AMD data shows two SNPs (rs2019724 and rs6428379) that were previously significantly associated with AMD, demonstrate a similarly strong deviation from HWE in cases when using p_T (P0.001 for both SNPs). However, these SNPs did not deviate significantly (rs2019724 P=0.8046, rs6428379 P=0.8820) in the cases when using the traditional method of testing for HWE. Conclusions: These observations suggest that using p_T rather than case or control allele frequencies alone is a better technique for assessing deviations from HWE. In addition, this method can be a powerful tool for identifying potential disease pre-disposing genes.

Evidence for interacting susceptibility genes on chromosome 1p and 4q as well as on 2q and 6q in 108 families with bipolar affective disorder. *J. Schumacher[1], R. Abou Jamra[1], R. Fürst[2], R. Kaneva[3], A. Flaquer[2], TG. Schulze[4], G. Orozco Diaz[5], V. Milanova[6], S. Cichon[7], P. Propping[1], M. Rietschel[4], TF. Wienker[2], MM. Nöthen[7]* 1) Institute of Human Genetics, University of Bonn, Germany; 2) Institute for Medical Biometry, Informatics and Epidemiology, University of Bonn, Germany; 3) Laboratory of Molecular Pathology, Medical University, Sofia, Bulgaria; 4) Central Institute of Mental Health, Division Genetic Epidemiology in Psychiatry, Mannheim, Germany; 5) Civil Hospital Carlos Haya, Málaga, Spain; 6) Department of Psychiatry, Medical University, Sofia, Bulgaria; 7) Department of Genomics, Life & Brain Center, University of Bonn, Germany.

Linkage evidence between bipolar affective disorder (BPAD) and loci on chromosomes 1p, 2q, 4q, and 6q was observed in four European samples consisting of 108 families with German, Spanish, Bulgarian, and Roma descent. Here we report on a systematic interaction analysis using all 4 regions under a broad and narrow disease definition. In each region, non-parametric linkage analysis was performed after conditioning on linkage evidence to the other three regions. On chromosome 2q we observed a NPL-Score of 5.93 (increase of the initial NPL finding) under a broad disease definition when conditioning on linkage signals to chromosome 6q. Vice versa, we found a NPL-Score of 5.08 on 6q when conditioned on the initial linkage data to chromosome 2q. On chromosome 1p, a NPL-Score of 3.12 was observed using a narrow disease definition and conditioned on linkage findings to chromosome 4q, and a NPL-Score of 2.41 on chromosome 4q was found when conditioned on linkage signals to chromosome 1q. Our results point to an interaction between chromosomal loci on 1p and 4q, and between 2p and 6q. Susceptibility genes located in these regions may have interfering regulative effects or interact together within the same biological pathway.

Epigenetics of the retinoic acid paradox. *S. Rossetti, M.Q. Ren, G. Bistulfi, N. Sacchi* Cancer Genetics, Roswell Park Cancer Institute , Buffalo, NY.

Vitamin A through its bioactive derivative retinoic acid (RA) normally inhibits human cells' growth. In contrast, RA, can promote the growth of human cancer cells. Specifically, RA was shown to induce cancer cell growth both in vitro and in vivo. The molecular basis of the RA-paradox is obscure. Normally, RA inhibits cell growth by modulating the transcription of cellular genes carrying regulatory regions characterized by distinct sequence motifs, either the RA-responsive elements or the GC -boxes. RA- mediated transcriptional regulation involves dynamic epigenetic changes at the chromatin associated with these cellular genes. We found that an impaired integration of RA signal through the RA receptor alpha (RARA) triggers a domino repressive epigenetic effect in human cells, which leads to concerted transcriptional silencing of RA-responsive tumor suppressor genes. Unexpectedly, in conjunction with an aberrant epigenotype, cells become susceptible of being growth promoted by RA. Apparently, the epigenotype plays a major role in determining the biological cell response to RA.

A prospective study of two major age-related macular degeneration susceptibility alleles and interactions with modifiable risk factors. *D.A. Schaumberg[1,3], S.E. Hankinson[2,5], Q. Guo[2,4], E. Rimm[2,5,6], D.J. Hunter[2,4,5,6]* 1) Div Preventive Med, Brigham & Women's Hosp, Harvard Medical School; 2) Channing Lab, Brigham & Women's Hosp, Harvard Medical School; 3) Schepens Eye Research Inst, Dept Ophthalmology, Harvard Medical School; 4) Program in Molecular & Genetic Epidemiology, Harvard Sch Public Health; 5) Dept Epidemiology, Harvard Sch Public Health; 6) Dept Nutrition, Harvard Sch Public Health, Boston, MA.

Purpose: Common variants in complement factor H (CFH) and LOC387715 are associated with age-related macular degeneration (AMD) in prevalent case-control studies. We aimed to delineate the magnitude of susceptibility to AMD due to these variants and their interactions with modifiable risk factors in two prospective cohorts. **Methods**: Cases who developed AMD (N=457) were compared with 1071 age- and sex-matched controls in a prospective nested case-control study of participants in the Nurses Health Study and the Health Professionals Follow-up Study. We determined the incidence rate ratios (IRR) and 95% confidence intervals (CI) for AMD associated with the genotype at each locus and examined interactions between the genes and with modifiable risk factors for AMD. **Results**: Cohort participants with one and two copies of the Y402H variant of CFH were 1.98 (CI= 1.64 to 2.40) and 3.92 (CI=2.69 to 5.76) times more likely to develop AMD; whereas the IRR (CI) for one and two copies of LOC387715 A69S were 2.38 (1.92 to 2.96) and 5.66 (3.69 to 8.76), respectively. The estimated fraction of AMD cases in the population attributable to these two variants was 63% (58% to 68%). Subjects homozygous for both risk alleles had a 50-fold increased risk of AMD (CI=11 to 237) compared to those with no risk alleles, and there was evidence that cigarette smoking and obesity multiplied the risks associated with these variants. **Conclusion**: With these data, AMD emerges as a paradigmatic example of a common disease caused by a complex interaction of an underlying genetic predisposition conferred by common variants, and exposure to modifiable risk factors. These observations highlight the need for continued attention to risk modification strategies to reduce the public health impact of AMD.

Sex-specific heritability and linkage of 1,373 traits in French-Canadian population. *O. Seda[1], J. Tremblay[1], D. Gaudet[2], P. Brunelle[1], A. Gurau[1], E. Merlo[3], L. Pilote[4], T. Kotchen[5], A.W. Cowley[5], P. Hamet[1]* 1) Research Ctre, CHUM Research Ctre, Montreal, QC, Canada; 2) Complexe Hospitalier de la Sagamie, Chicoutimi, QC; 3) École Polytechnique de Montreal, Montreal, QC; 4) The Montreal General Hospital, Montreal, QC; 5) Medical College of Wisconsin, Milwaukee, WI.

Sex is one of the influential factors affecting the genetic architecture of most complex traits, yet details of its effects received only limited attention so far. We assessed the sex-specific heritability and linkage of 1,373 direct and derived anthropometric, metabolic, hemodynamic and humoral phenotypes in 120 French-Canadian families (n = 810 subjects) from the Saguenay-Lac-St-Jean (SLSJ) region of Quebec, Canada. Multipoint linkage analysis was performed with a variance components approach implemented in SOLAR, version 2.1.1., in three settings: all, males and females. The genetic information was represented by 437 microsatellite markers uniformly distributed throughout the whole genome. To asses the significance of the sex-specific linkage LOD score difference, we computed an empirical p-value based on 10,000 permutations for each locus with a LOD difference between sexes >2. Using linear regression, we found 45.7% phenotypes to be age- and sex-independent, 16.0% phenotypes to be age-dependent only, 19.2% phenotypes to be sex-dependent only, and 19.2% phenotypes to be both age- and sex-dependent at $p < 3.65 \times 10^{-5}$. We have identified over 25 loci showing highly significant linkage x sex interaction. Furthermore, comparative genomic analysis of sex-specific QTLs revealed several loci significantly linked to similar or even identical phenotypes in both humans and rodent models. We identified female-specific linkage of diastolic blood pressure to the chromosome 2 locus (173 cM LODall = 1.5, LODmen = 0.01, LODwomen = 2.37) syntenic to the rat chromosome 3 region with a peak female-specific linkage of mean arterial pressure in hypertriglyceridemic rats. We propose that multiple geoethnic human cohorts of men and women as well as comparative genomics using non-human models of relevant pathophysiological states are necessary to gain insight into sex-driven aspects of genetic architecture of complex traits.

Multiple Sclerosis North American Pregnancy Programme (MS-NAPP). *A. Sadovnick, E. Dwosh, C. Guimond*
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Multiple sclerosis (MS) is one of the most common neurological disorders, other than trauma, affecting young adults, especially during the period of their lives when reproduction is an option. Females are affected approximately twice as often as males. Until the advent of laboratory tests for MS, in particular magnetic resonance imaging (MRI), it was not uncommon for the lag time (time from the onset to diagnosis of MS) to be years or even decades. Thus, in many situations, women had pregnancies after the onset of MS but without knowing that they had the disease. The disadvantage (or possibly an advantage) stemmed from the fact that informed decisions about childbearing did not have to be made. Reproductive issues for males with MS were either never or very rarely addressed. The objectives if MS-NAPP are to: 1. Develop an interactive website for use by health care professions in the reproductive counseling of males and females with MS. Topics include recurrence risks, interaction of pregnancy and MS with respect to disease course and pregnancy management and outcome, long-term effects on the developing child (e.g. immunological), and psychosocial issues. 2. Through the use of questionnaires, to determine the reproductive patterns currently followed by MS patients since the clinical use of disease modifying therapies (DMTs) such as interferons. 3. Develop a prospective, pregnancy register to determine the safety of the DMTs and symptom-specific therapies such as prednisone and hence identify needs for wash-out as well as safety for reintroduction of therapy during gestation and breastfeeding, identify any long-term impacts on children born to MS parents (e.g. problems with the immune system, child development in general. etc.). The long-term goal is to develop the first ever evidence-based protocol for reproduction and MS. Data from items 1 and 2 will be presented as the ASHG 2006 meeeeting. Funding for MS-NAPP is through Berlex Canada, Teva Neuroscience, and the Edward Bronfman Family Foundation. Intellectual and technical support has been provided by the National MS Society, the MS Society of Canada, and the Consortium of MS Clinics.

Global Impact of a Web-based Birth Defects Information System. *W. Wertelecki* Dept Med Genetics, Univ South Alabama, Mobile, AL.

IBIS, an International Birth Defects (BD) Information System, is a web-based resource that evolved from systems developed for Ukraine and Alabama (http://www.ibis-birthdefects.org). BD information on most websites provide information of varied quality, and limited in scope. IBIS was designed to facilitate access to a broad spectrum of BD information, selected for its quality and inclusive of non-English materials. The majority of information in Ukraine had to be developed by Ukrainian IBIS partners. IBIS is organized along information channels, mainly about specific disorders, topics concerned with child development and support sources. A companion website (Pandora Word Box, www.consultsos.com/pandora/intro.htm) was developed to complement IBIS with humanistic vistas, inclusive of bioethics.

Visitors to IBIS quickly expanded from Ukraine and Alabama to include a large array across the globe. In the past three years, IBIS has welcomed ~1.26 million visitors and Pandora ~1 million. Ukrainian visitors to IBIS rank ninth, and those from neighboring Poland and Russia rank twenty-second and twenty-fifth. Mostly English-speaking countries outrank Ukraine in this regard. Visitors to the Pandora web site offer a frame of reference because it was not promoted in Ukraine, as was IBIS. Visitors to Pandora from Poland and Russia rank thirteenth and thirty-eighth while Ukraine ranks forty-ninth. We conclude that IBIS is a major BD information resource in Ukraine. Although English is a medical "lingua franca", BD information in native languages is needed by patients and their families. In our view, a Spanish IBIS version, is demonstrably needed.

Association of ENPP1 Variants with Quantitative Measure of Glucose Homeostasis and Adiposity: The IRAS Family Study. *N. Palmer[1], A. Lehtinen[1], J. Campbell[1], J. Norris[2], M. Bryer-Ash[3], S. Haffner[4], C. Langefeld[1], D. Bowden[1]* 1) Wake Forest University, Winston-Salem, NC; 2) University of Colorado, Denver, CO; 3) University of California, Los Angeles, CA; 4) University of Texas, San Antonio, TX.

Ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) is a candidate for type 2 diabetes (T2DM). It is widely expressed in insulin-sensitive tissues and has elevated activity in T2DM. ENPP1 interacts with the insulin receptor to impair insulin signaling. Six SNPs, with prior evidence of association, were genotyped in 1102 Hispanic and African Americans in the IRAS Family Study. SNPs were tested for association with quantitative measures of glucose homeostasis (insulin sensitivity; S_i, acute insulin response, fasting insulin and fasting glucose) and adiposity (body mass index; BMI, waist circumference and waist to hip ratio, visceral and subcutaneous adipose tissue) using variance component analysis in SOLAR. In Hispanic Americans, ENPP1 was contained in two LD blocks. Two SNPs within the block spanning the coding region were associated with S_i ($P<0.007$) while flanking SNPs trended toward association. The SNP most strongly associated with S_i ($P<0.007$) was a coding mutation (K121Q). This SNP has been shown to be associated with quantitative measures of insulin resistance (fasting insulin, fasting glucose, and S_i). Increasing evidence suggests that the same mutation is also associated with obesity phenotypes; however, our data do not support these findings in Hispanic Americans. In African Americans there was a single LD block which contained all six SNPs. There was no association with measures of glucose homeostasis; however, there was modest evidence for association with BMI and waist ($P<0.04$). In conclusion, ENPP1 SNPs are significantly associated with S_i, in a well-characterized Hispanic cohort which is consistent with the biological effect of this gene in vivo. This study is unique because we assess a mechanism of contribution of this gene to diabetes via quantitative measures of glucose homeostasis. Based on our findings, further studies are warranted to determine the validity of ethnic specific associations.

Origin of complex human mutations. *J. Zhang, L.D. Liu, Y.F. Yin, H.L. Gao, L. Xiao, K. Li* SNP Institute, Nanhua Univ, Hengyang, China 42100.

Indels are a unique type of infrequent mutations, and about 20% of which are del2_ins1 microindels. Upon examination of the preferential del2_ins1 microindels microindels reported in the HGMD, Lac I gene in Big Blue mice, and the IRAC p53 human somatic databases, we revealed that: i). ratios of del2_ins1 over del1_ins2 are between 2-5, similar to the ratio of deletion over insertion; and ii). del2_ins1 microindels indels have 30-40% CG>TA in the inserted bases, inherited the characteristic figureprint of single base substitution. These pieces of evidence are consistent with the simple speculation that the preferential del2_ins1 microindel may derive from the two leading types of mutations: single base substitution and deletion. Interestingly, an opposite supporting clue was identified by analyzing the del1_ins2 microindel. As expected, 8 of 14 such microindels in HGMD may attribute to the single nucleotide run (tab 1). Thus, the first two leading types of simple mutations of single base substitution and single base deletion, as well as the third leading mutation type of single base insertion play important roles in the genesis of complex mutations.

Mut Id	cx942100	cx993297	cx022758	cx992105	cx992099	cx983277	cx992110	cx992103
5'seq	T	C	T	C	C	A	G	C
inserted seq	TT	AT	AG	TG	AC	AA	AA	TT
deteded seq	G	C	T	A	G	G	G	C
3'seq	T	T	G	G	C	C	A	T

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A 1-2-3 principle as the standard for distinguish of rare single nucleotide polymorphism from causal mutation.
Y.F. Yin, L.D. Liu, C.L. Zhou, K. Li, H.L. Gao SNP Institute, Nanhua University, Hengyang, China 42100.

Correct identification of the real causal mutation is required for prenatal screening. Using hemophilia B as a model, we suggested a 1-2-3 principle for distinguishing rare polymorphisms from mutations. Multiple genetic changes are classified into three subgroups. A 1-2-3 principle has been applied to the analyses of 30 patients carrying multiple nucleotide changes in factor IX. Nucleotide changes are annotated as the causal mutation when they fall into the subgroup I: nonsense mutation, frameshift deletion or insertion. For mutations in subgroup II (mutant alone reported in literature, or a highly conserved amino acid substitution), both criteria are required or one criterion plus another criterion from subgroup III are required to annotate a mutation. However, at least three criteria are needed to separate causal mutations from polymorphisms when the multiple nucleotide changes all fall into the subgroup III. Subgroup III includes: not a CG to TA transition / mutant aa not used by other species / physiochemical different aa / residing in important domains. Of the 30 hemophilia B patients with multiple mutations two (potentially three) have double causative mutations. Twenty-seven polymorphisms have been identified, which are consistent with the rare polymorphism database (www.kcl.ac.uk/ip/petergreen/haemBdatabase.html) except we identified two novel rare polymorphisms. As the incidence of hemophilia B is about 1/40,000, a real double mutation is estimated in every forty-thousand patients. Results obtained based on the 1-2-3 principle stand true statistically and the 1-2-3 principle may have applications in the analyses of other genetics diseases with low incidence. The two novel rare genetic variants are to be confirmed by DNA pool.

Molecular characterization of lactase gene regulation in African populations. *A. Ranciaro[1,2], F.A. Reed[1], J. Hirbo[1], K. Powell[1], M. Osman[3], S. Omar[4], M. Ibrahim[3], S.A. Tishkoff[1]* 1) biology, university of university, college park, MD; 2) Dept. of Biol., Univ. of Ferrara, Ferrara, Italy;; 3) Dept. of Molecular Biology, Inst. of Endemic Diseases, Univ. of Khartoum, Khartoum, Sudan; 4) KEMRI, CBRD, Nairobi, Kenya.

In most human populations, the ability to digest lactose, the sugar present in milk, declines rapidly after weaning because of decreasing levels of the enzyme lactase (lactase-phlorine hydrolase, LPH) in the small intestine. However, there are individuals who maintain the ability to digest milk and other dairy products into adulthood due to a genetic adaptation. The highest frequencies of LP are in N. European and certain African and Arabian pastoralist populations and lowest frequencies are in Native Americans, Pacific islanders, Sub-Saharan Africans(with the exception of East African pastoralists) and Southeast Asians. This pattern suggests that selection has played a major role in determining the frequencies of LP in different human populations since the development of pastoralism ~9,000 years ago. In order to identify genetic mutations associated with LP, we collected phenotype data from 470 individuals from Tanzania, Kenya, and the Sudan. We resequenced 2 kb of the promoter region of the gene coding for LPH (LCT), as well as introns 9 (1.7 kb) and 13 (3.3 kb) of the MCM6 gene, which had previously been identified as regions with SNPs associated with LP in Europeans, in 150 African individuals at the extremes of the phenotype distribution. Two novels SNPs were identified that showed a significant phenotypic association with the LP trait, one common in Tanzanian and Kenyan pastoralist populations, and the other common in the Beja population from the Sudan. Resequencing of these regions in a panel of great apes indicated that the alleles associated with LP are derived. We then screened a larger panel of African populations, as well as Middle Eastern populations, to determine the geographic distribution of these alleles. We have characterized patterns of nucleotide diversity and genetic signatures of natural selection in these regions in order to reconstruct the evolutionary history of LCT regulatory regions.

Association of warfarin dose with genes involved in its action and metabolism. *M. Wadelius[1], L.Y. Chen[2], N. Eriksson[3], S. Bumpstead[2], J. Ghori[2], C. Wadelius[4], D.R. Bentley[2], R. McGinnis[2], P. Deloukas[2]* 1) Dept Medical Sciences, Clinical Pharmacology, University Hospital, Uppsala, Sweden; 2) The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, UK; 3) UCR - Uppsala Clinical Research Center, Uppsala Science Park, Uppsala, Sweden; 4) Department of Genetics and Pathology, Medical Genetics, Rudbeck Laboratory, Uppsala, Sweden.

Warfarin is a commonly prescribed anticoagulant that is difficult to use because of the twenty-fold variation in dose required to achieve a therapeutic effect. Of all used drugs, warfarin probably has the highest risk of severe side-effects in the form of bleeding. We genotyped 201 Swedish warfarin-prescribed patients for polymorphisms in 29 genes involved in the action and biotransformation of warfarin, and tested them for association with warfarin dose requirement. Genotyping was performed with the Homogeneous Mass Extend assay (Sequenom). In our study, single polymorphisms in or flanking eleven genes and haplotypes of twelve genes were associated with dose ($p<0.05$). VKORC1, CYP2C9, CYP2C18, CYP2C19 were significant after experiment-wise correction for multiple testing ($p<0.000175$), while PROC and APOE were significant after within-gene correction. We found that the association of CYP2C18 and CYP2C19 was fully explained by linkage disequilibrium with CYP2C9*2 and/or *3. A multiple regression model with VKORC1, CYP2C9, PROC and the non-genetic predictors age, bodyweight, drug interactions, and indication for treatment jointly accounted for 62% of the warfarin dose variance. Weaker associations observed for other genes could explain up to ~10% additional dose variance, but require testing and validation in an independent and larger data set. We are therefore genotyping 1500 additional Swedish warfarin-prescribed patients for tagSNPs and functional SNPs in the 29 genes. Translation of warfarin pharmacogenetics into guidelines for prescription will be likely to have a major impact on the safety and efficacy of warfarin.

No effect of 'autoimmunity' genes on the clinical outcome of Multiple Sclerosis. *S.V. Ramagopalan[1,2], G.C. DeLuca[1,2], B.M. Herrera[1,2], M.R. Lincoln[1,2], S. Orton[1,2], M.W. Chao[1,2], A.D. Sadovnick[3], G.C. Ebers[1,2]* 1) Department of Clinical Neurology, University of Oxford, Oxford, United Kingdom; 2) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom; 3) Faculty of Medicine, Division of Neurology, University of British Columbia, Vancouver, Canada.

There can be few diseases with as much variation in outcome as multiple sclerosis (MS). There is substantial support for a significant inherited component in MS; evidence also suggests that genetic factors may affect the phenotypic expression of the disease. In sibling pairs with MS the rate of acquisition of disability is similar and significant concordance for disease course exists. Further supporting a role for disease modifying genes in MS, monozygotic twins concordant for MS have a more similar disease course than dizygotic twins (unpublished data). Despite the many unique clinical and serological features associated with specific autoimmune diseases, there have been numerous reports that suggest that different autoimmune diseases share susceptibility loci. If this is true, one or more of the known autoimmune genes (major histocompatibility complex class II transactivator (MHC2TA), cytotoxic T-lymphocyte-associated protein 4 (CTLA4), protein tyrosine phosphatase, non-receptor type 22 (PTPN22) and caspase recruitment domain family, member 15 (CARD15)) may affect the clinical outcome of the putative autoimmune disease MS. We therefore investigated the effect of MHC2TA, CTLA4, PTPN22 and CARD15 on MS disease severity. We genotyped a dense panel of single nucleotide polymorphisms (SNPs) using the MassEXTEND protocol (Sequenom Inc., San Diego, CA). Examination of SNPs for each gene and their corresponding haplotypes did not show any association with outcome of the disease.

Detection of interactions: are we using correct approach? *L. Padyukov[1], H. Källberg[2], L. Klareskog[1], L. Alfredsson[2]*
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Current analysis of gene-gene and gene-environment interactions in complex diseases is based on the assumption that each pair of risk factors is independent and deviation from independence for the patient group but not for the control group could be interpreted as an interaction. However, this analysis does not directly compare influence of risks between cases and controls. We propose that an examination of the disease odds for each specific combination of risk factors in comparison with a reference odds in which both risk factors are absent, provides a more accurate basis for studying interaction and that interaction between two factors should be defined as a deviation from additivity of absolute effects. In this case, quantification of the amount of interaction can be made by means of the attributable proportion due to interaction. Using a large Swedish population-based material for investigation of RA (816 cases with anti-CCP positive RA and 793 controls) we have investigated interaction between two well-established genetic risk factors of RA: shared epitope (SE) alleles of MHC2-DRB1 gene and A allele of PTPN22 gene. The Chi-square test for the entire RA patient group did not show significant association. However, using cases and controls without SE alleles and A allele of PTPN22 as a reference group demonstrates high odds for individuals with SE without A PTPN22 alleles (5.1 95%CI 3.7-6.8), and with both variations (10.1 95%CI 7.0-14.8), while no difference in group with A PTPN22 allele without SE alleles was found (1.2 95%CI 0.7-2.0). Attributable proportion due to interaction confirms the significant deviation from additivity of effect in double positive group (0.5 95%CI 0.3-0.7) hence clearly demonstrating genetic on susceptibility to RA. Since so dramatically different conclusions could be drawn from different types of statistical analyses, one has to be aware of the methodology used to reveal genetic interactions. We here present a way to detect these interactions with better sensitivity, which also provides a simple way for biological interpretation of the data.

The complex PRKCA genome region on 17q shows association to Multiple Sclerosis in independent populations.

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Complex diseases such as multiple sclerosis (MS) likely result from problems in networks of interactions between several genes and environmental factors. HLA-DRB1*15 is the only consistent locus observed in most populations, but numerous genome screens also provide evidence for a MS locus on 17q. Association screen of the critical linkage region on 17q24 in Finnish MS families identified several SNPs within the PRKCA gene region. This association was also replicated in Canadian MS pedigrees. Two allelic variants of PRKCA were found to be over-represented in Finnish or Canadian MS cases (odds ratios: 1.34 and 1.64). An analysis of 200 MS trio families from Sweden, Denmark and Norway suggested a role for the Finnish risk haplotype in MS susceptibility also in other Scandinavian populations. Transcript levels of PRKCA correlated with the copy number of the risk alleles in 20 Finnish and 11 CEPH individuals of European origin in an initial expression analysis. A recent sequencing effort of chromosome 17 revealed this chromosome to be highly enriched with segmental duplications, which may predispose to copy-number or other structural variations. We have previously shown that the critical MS region is flanked by duplicated sequences. Detailed analysis of these duplicons identified a set of retrotransposable sequence elements, which are clustered in 12 duplicated sequence domains within the 17q. An ongoing analysis suggests variation in the copy-number of these retrotransposable elements, which may co-segregate with the PRKCA risk haplotype.

Prenatal diagnosis of Smith-Lemli-Opitz syndrome (SLOS): Identification of a novel, *de novo* mutation of the *DHCR7* gene. *J.S. Waye[1,2], B. Eng[2], M.A. Potter[1,2], M.J.M. Nowaczyk[1,2,3], D. McFadden[4], S. Langlois[5]* 1) Dept Pathology & Molecular Med, McMaster Univ, Hamilton, ON, Canada; 2) Genetic Services, Hamilton Health Sciences, Hamilton, ON, Canada; 3) Dept Pediatrics, McMaster Univ, Hamilton, ON, Canada; 4) Dept Pathology, Children's & Women's Health Care Ctr, Vancouver, BC, Canada; 5) Dept Medical Genetics, Univ of British Columbia, Vancouver, BC, Canada.

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive disorder of cholesterol biosynthesis resulting from deficient 7-dehydrocholesterol reductase. We report a prenatal SLOS case in a 35-year-old G2P1 woman with a positive MSS for Down syndrome (estriol, AFP). Cytogenetic analysis of cultured amniocytes indicated a normal 46, XX karyotype, and detailed ultrasound examination revealed severe IUGR and multiple congenital anomalies. The pregnancy was terminated and autopsy examination showed a female fetus with a cardiac defect, renal hypoplasia, hypoplastic and unilobar lungs, dysgenesis of the corpus collosum, equinovarus deformity of the feet, bilateral 2-3 toe syndactyly, and uterus didelphys with septate vagina. Elevated 7-dehydrocholesterol levels in the fetal blood and amniotic fluid confirmed the diagnosis of SLOS. Nucleotide sequence analysis of the *DHCR7* coding sequences revealed two mutations. The fetus was heterozygous for the common splice acceptor mutation of IVS8 (c.964-1G>C), and heterozygous for a silent mutation at codon 137 (p.Ala137Ala, c.411A>G). The latter mutation alters the conserved, penultimate nucleotide of exon 5 and may adversely affect splicing. The mother was heterozygous for the c.964-1G>C mutation, but neither parent carried the novel c.411A>G mutation. Paternity was confirmed using a panel of 15 STR markers. We propose that the c.411A>G mutation occurred *de novo* during spermatogenesis, or that the father is a germline mosaic. Prenatal testing was conducted for the subsequent pregnancy, and the fetus was shown to be heterozygous for the maternal c.964-1G>C mutation and negative for the c.411A>G mutation. Haplotyping confirmed that the fetus inherited a different paternal chromosome than the previous, affected fetus.

Variant of transcription factor 7-like 2 gene and the risk of type 2 diabetes mellitus in large cohorts of U.S. women and men. *C. Zhang[1], L. Qi[1], DJ. Hunter[1, 2, 3], JB. Meigs[4], JE. Manson[2, 3, 5], RM. Van Dam[1], FB. Hu[1, 2, 3]* 1) Nutrition, Harvard Sch Public Health, Boston, MA; 2) Channing Laboratory, Department of Medicine, Brigham and Womens Hospital and Harvard Medical School, Boston, MA; 3) Department of Epidemiology, Harvard School of Public Health, Boston, MA; 4) General Internal Medicine and Clinical Epidemiology Units, General Medicine Division, Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts; 5) Division of Preventive Medicine, Department of Medicine, Brigham and Womens Hospital and Harvard Medical School, Boston, Massachusetts.

Emerging evidence indicates that variation in the transcription factor 7-like 2 gene (TCF7L2) may play a role in the pathogenesis of type 2 diabetes mellitus (T2DM). In a prospective, nested case-control study (total n=3,520) within the Nurses' Health Study (687 type 2 diabetes cases and 1051 controls) and the Health Professionals Follow-up Study (886 cases and 896 controls), we examined the association of a common variant of the TCF7L2 gene (rs12255372 (T/G)) with T2DM risk among Caucasians. Frequencies of the T allele were significantly higher among cases than controls; each copy of the T allele was associated with a 1.32 (P=0.0002) and 1.53-fold (P<0.0001) increased T2DM risk in women and men, respectively. The odds ratios (95% CI) associated with homozygous carriers of the T allele were 1.86 (1.30, 2.67) and 2.15 (1.48, 3.13) in women and men, respectively. Population attributable risks for diabetes associated with the T allele were 14.8% and 22.3% for women and men, respectively. In a meta-analysis of 3347 cases and 3947 controls, each copy of the T allele was associated with a 1.48-fold increased risk (P<10-16). Our findings confirm that the TCF7L2 gene represents an important locus for predicting inherited susceptibility to T2DM.

Semen analysis of patients with TGCT; correlation with Y chromosome gr/gr deletion. *E. Rapley[1], J. Coffey[1], R. Linger[1], D. Dudakia[1], J. Pugh[1], K. Lindsay[2], M. Stratton[1], R. Huddart[3], E. Rapley[1]* 1) Testicular Cancer Genetics Team, Section Cancer Genetics, Institute of Cancer Research,UK; 2) Andrology Unit, Hamersmith Hospital NHS Trust, UK; 3) Section of Academic Radiotherapy, Institute of Cancer Research, UK.

The gr/gr deletion of the Y chromosome is associated with a 2 fold increased risk of testicular germ cell tumour (TGCT). The deletion is also associated with infertility, itself a risk factor for TGCT. We report the provisional findings of a study examining the relationship between the 'gr/gr' deletion, TGCT and fertility. Research participants were divided into 5 groups; gp1, TGCT case with a family history of disease; gp2, TGCT case without a family history of disease; gp3 & gp4, unaffected relatives of gp1 & gp2, respectively; and gp5, healthy male controls. To date 209 research participants have provided semen samples, 204 of which have been examined for gr/gr deletions. 'gr/gr' deletions were identified in 5 patients, 1 in gp1, 3 in gp2 & 1 in gp4 (son of TGCT patient in gp2). No gr/gr deletions were detected in male controls. Semen analysis (according to WHO 1999) of the gr/gr deletion cases revealed a full range of fertility parameters. Two patients (gps 1&4) had normal semen analysis, 2 patients (gp2) were oligospermic (< 20 million per ml) and one patient (gp2) was azoospermic. The three patients with abnormal semen analysis, had fathered children. Control male patients had a higher frequency of normal semen analyses (80.4%) than TGCT patients (40.6%;gp1 & 41.0%;gp2); some part of this difference may be treatment related. 'gr/gr' deletion was more common in TGCT patients (3.3% vs 0% in controls), as previously reported, but numbers are too small for conclusive p values. The provisional data suggests that TGCT patients have reduced fertility, as measured by the difference in frequency of normal semen parameters between TGCT cases and controls. While 'gr/gr' deletion is associated with an increase risk of TGCT, there was no correlation between fertility status and the presence of 'gr/gr' deletion in this series and despite abnormal semen analysis, several individuals had fathered children.

Genome-wide *In Silico* Prediction of USF1 Binding Sites and Target Genes. *T. Wang, J.J. Connelly, S.G. Gregory, E.R. Hauser* Center for Human Genetics, Duke University Medical Center, Durham, NC.

Transcription factors (TFs) play an important role in transcriptional regulation by interacting with transcription factor binding sites (TFBSs) to control gene expression in temporal, cell type, or environmental specific ways. Upstream stimulatory factor 1 (USF1) is a key regulator of the stress and immune responses, the cell cycle and cell proliferation. Several publications, and our own unpublished data, have identified single nucleotide polymorphisms (SNPs) in USF1, and several other TFs, which are significantly associated with coronary artery disease (CAD) (Pajukanta *et al*., 2004; Komulainen *et al*., 2006). We therefore hypothesize that identifying the target genes of TFs, such as USF1, will improve our understanding of genetic effects in CAD risk. Experimental approaches to *de novo* localization of TFBS include ChIP-chip microarrays (Rada-Iglesias *et al*., 2005). This method, though successful, is time-consuming when being applied on a genome-wide scale. Thus it would be advantageous to develop an *in silico* computational method to independently predict TF binding sites. Using USF1 as our model, our goals were to develop, evaluate and optimize methods for USF1 binding site prediction, and make genome-wide predictions of potential TFBSs and target genes. We have developed a flexible and efficient core binding site search method to identify all potential TFBSs for USF1, and also gathered several important features of each potential TFBS. Utilizing all features, we developed several prediction methods based on statistical classification models. We used published USF1 ChIP-chip results from ENCODE regions to evaluate and optimize our prediction models. The final prediction model was able to achieve optimal sensitivity, specificity and discovery rate. Then we applied this method to make genome-wide predictions. The results of this study will help identify and prioritize CAD candidate genes, prioritize SNPs within and around candidate genes and allow targeted evaluation of gene-gene interactions.

Extended mutation search in patients with L1 syndrome -update of the L1 mutation database-. *Y.J. Vos[1], K.K. Bos[1], E. Verlind[1], A. Vrieling-Kooistra[1], A.M. ten Berge[1], B.E. Hiemstra[1], I. Stolte-Dijkstra[1], W.S. Kerstjens-Frederikse[1], N.B.B. Knops[2], R.M.W. Hofstra[1]* 1) Department of Genetics, University Medical Center, Groningen, Netherlands; 2) Wilhelmina Children's Hospital, Utrecht, Netherlands.

L1 syndrome comprises four neurological syndromes that all have been found associated with mutations in the *L1CAM* gene. They include 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). L1 syndrome is an X-linked recessive disorder with an incidence of one in every 30,000 males. In our laboratory an efficient screening method has been developed and the DNA of more than 250 patients has been analysed, resulting in the identification of 48 different mutations in 50 patients. Among the newly identified mutations was one deletion of the entire gene in a patient with X-linked Hydrocephalus and Nephrogenic Diabetes Insipidus. The deletion includes besides the *L1CAM* gene also the *AVPR2* gene.

All new mutations are added to the L1CAM database which can be found at our website. It includes almost 200 different pathogenic mutations, scattered over the entire gene. The database was taken over from Prof dr. G. van Camp (University of Antwerpen). It can be found at the L1CAM web page at the website of the University of Groningen: http://www.rug.nl/umcg/faculteit/disciplinegroepen/medischegenetica/hereditarydiseases/ l1cam/index.

In only 20% of our patients a mutation was found. So we extended the mutation analysis of the coding region of the *L1CAM* gene with a screen of the most important regulatory sequences namely the promoter region, the Homeodomain and Paired Domain binding site (HPD site) and the Neuron Restrictive Silencer Element region (NRSE region). Screening of 140 patients did not result in additionally mutations.

Neurotrophins, their receptors and EGR1: peculiarities of expression in human lung cancer and normal lung tissue. *E.L. Voloshenyuk, L.V. Dergunova, N.M. Raevskaya, S.A. Limborska* Institute of Molecular Genetics RAS, Moscow, Russian Federation.

Neurotrophins (NTs) are a family of related polypeptide growth factors, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), NT3, NT4/5, and NT6. These molecules bind two types of cell surface receptors characterized by different binding affinities and including a low affinity glycoprotein receptor p75 and high-affinity receptors recognized by a tyrosine-specific protein kinase (Trk) activity. NTs play a role in the modulation of certain human malignancies, including those of neurogenic and ectodermal origin. They are also involved in stimulation of clonal growth on human lung cancer cells in vitro via NT receptors. mRNA expression of NGF, BDNF, NT3, their related high- (TrkA, TrkB, TrkC) and low-affinity (p75) receptors, and also NGF-induced gene EGR1 was studied by RT-PCR in 30 double surgical specimens (histologically diagnosed human lung cancer and appropriate adjacent tissue) and in 10 specimens of normal lung tissue. Tumor specimens set included 25 squamous cell lung cancer samples and 5 adenocarcinoma samples. The expression of GAPDH mRNA was used as the internal control. In normal tissues we have observed differential expression pattern of all NTs examined. Varying levels of NGF expression was demonstrated in double specimens. Interestingly, half of the double specimens studied have shown no difference in NGF expression between cancer and adjacent tissue. In the majority of the double specimens we have detected low levels of NT3 and BDNF expression for both cancer and adjacent to tissue. No expression of TrkA, TrkB, p75 was found in double specimens and normal tissues. However, we have observed differential expression patterns of TrkC in normal tissues. TrkC expression was not detected in the majority of the double specimens, whereas in 32% cases we have detected high levels of TrkC expression in adjacent tissue compared with cancer. High levels of EGR1 expression was detected in normal tissues. In the majority of double specimens (74%) no EGR1 expression was revealed in cancer tissue compared to its high expression level in adjacent tissue.

hMSH2 Mutation Identified in a Family with Muir-Torre Syndrome: Genetic Counseling Perspective. *Z. Wang*[1, 2], *M. Flynn*[1, 2], *M.A. Whalen*[1, 2], *J.M. Milunsky*[1, 2, 3] 1) Ctr Human Genetics, Boston Univ Sch Medicine, Boston, MA; 2) Department of Pediatrics, Boston University School of Medicine, Boston, MA; 3) Department of Genetics and Genomics, Boston University School of Medicine, Boston, MA.

Muir-Torre syndrome (MTS) is an autosomal dominant disorder characterized by sebaceous adenomas and carcinomas, and multiple keratoacanthomas in conjunction with tumors typical of hereditary non-polyposis colorectal cancer (HNPCC) spectrum. hMSH2 and hMLH1 mutations have been described in families with MTS, with hMSH2 mutations predominating. We identified a well-described splicing mutation in intron 5 of the hMSH2 gene (c.942+3 AT) in a 56-year-old male (proband), who was diagnosed with sebaceous carcinoma at age 49 and colon adenocarcinoma at age 52. One of his brothers was diagnosed with colon adenocarcinoma at age 54, and another brother died at age 15 from a glioblastoma. The probands mother was diagnosed with breast cancer in her 50s. She also has a history of sebaceous tumors and colon polyps. His maternal aunt (age 72) has uterine cancer. His maternal grandmother was diagnosed with breast cancer in her late 70s. After a mutation was identified in the proband, subsequent genetic counseling and testing identified the familial hMSH2 mutation in one of the probands daughters, his brother, and two of his brothers children. The individuals with the familial mutation have been started on surveillance protocols, including colonoscopy, EGD/upper GI series, and dermatology evaluations. As glioblastoma is a cancer that has been reported to be associated with hMSH2 mutations, we discussed the benefits and ethical considerations of molecular testing for the minor children in this family. Due to the early brain tumor in the family along with the availability of screening options, this family has decided to proceed with molecular testing in the minors. Early recognition of MTS will allow more optimal anticipatory guidance for those mutation positive family members and free those mutation negative family members from years of costly and unpleasant screening protocols.

Polymorphisms in methotrexate efflux transporters are associated with methotrexate effects in psoriasis. *R.B. Warren[1,3], R. Smith[1], E. Campalani[2], C.H. Smith[2], J.N.W.N. Barker[2], C.E.M. Griffiths[3], J. Worthington[1]* 1) ARC-EU, University Of Manchester, Manchester, United Kingdom; 2) St Johns Institute of Dermatology, St Thomas Hospital, London, United Kingdom; 3) The Dermatology Centre, University of Manchester, Hope Hospital, Manchester, United Kingdom.

Methotrexate remains a first-line systemic therapy for severe psoriasis but unpredictable efficacy and toxicity limits its use. Previous pharmacogenetic studies have focused on isolated single nucleotide polymorphisms (SNPs), within the folate and purine pathways. Efflux of methotrexate from cells has the potential to reduce efficacy, therefore we hypothesised that SNPs in the ATP-binding cassette (ABC) family of efflux transporters, ABCC1 and ABCG2, are associated with clinical response to methotrexate. DNA was collected from 374 psoriasis patients defined as either responder or non-responder to methotrexate therapy. Haplotype tagging SNPs (r2 >0.8) for ABCC1 (n=42) and ABCG2 (n=12) with a minor allele frequency of >5% were selected from the HAPMAP phase II data, giving 73.3% and 74.1% gene coverage respectively. Genotyping was undertaken using the MassArray spectrometry (Sequenom). SNP rs17731538 (ABCG2) was significantly (p=0.02) associated with response to methotrexate and development of any adverse event (p=0.01); carriage of the major allele confers an OR = 1.84 (95%CI 0.98 -3.4, p=0.02) and 1.2 (95%CI 0.7-2.1, p=0.2) respectively. This SNP had a strong (p=0.0005) association with development of significant gastrointestinal adverse events. SNP rs2238476 (ABCC1) was significantly (p=0.02) associated with response to methotrexate and development of an adverse event (p=0.01); carriage of the major allele confers an OR =2 (95%CI 0.79-4.9,p=0.07) and 2.16 (95%CI 0.9-5.3 p=0.03) respectively. Two further SNPs rs246240 (ABCC1) and rs3784862 (ABCC1) were also associated with the development of an adverse event, p= 0.01 and p=0.005; conferring an OR 1.6 (95%CI 0.97-2.8, p=0.03) and 1.8 (95%CI 1.1-3.2, p=0.01) respectively. These data indicate that SNPs in the efflux transporters may prove important when identifying psoriasis patients suitable for methotrexate therapy and this should be validated in a prospective trial.

Novel acetylcholine receptor duplication mutations in three Iranian families with congenital myasthenic syndrome. *P. Soltanzadeh[1, 4], P. Richard[1], J. Müller[2], K. Kahrizi[3], A. Ghorbani[4], A. Abicht[2], Y. Shafeghati[3], H. Najmabadi[3], J.A. Urtizberea[5], A. Soltanzadeh[4], H. Lochmüller[2], D. Hantaï[1]* 1) INSERM U582/Institute of Myology & Functional Unit of Cardiogenetics & Myogenetics, Pitié-Salpêtrière Hospital, Pierre & Marie Curie University, Paris, France; 2) Molecular Myology Lab, Friedrich-Baur-Institute, Department of Neurology, Ludwig-Maximilians-University, Munich, Germany; 3) Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran; 4) Department of Neurology, Tehran University of Medical Sciences, Tehran, Iran; 5) AP-HP, Hôpital Marin, Hendaye, France.

Most congenital myasthenic syndrome (CMS) mutations affect the subunit gene of the acetylcholine receptor (*CHRNE*). Only one *CHRNE* mutation has already been documented in Iranian CMS patients. In three Muslim Iranian families, two from the northeastern province of Semnan and the third from the western province of Hamadan, CMS presented with early-onset ptosis and a slowly progressive generalized weakness. In the first family, without known consanguinity, one of the two affected siblings was analyzed. In the second family which had consanguinity, there was only one affected girl. In the third family from the western province of Hamadan, consanguineous marriage led to two affected siblings. *CHRNE* screening was performed by sequencing of the entire coding sequence and the promoter region. A homozygous frameshift mutation 98_101dupTCAA in exon 2 was found in the first two families. This duplication is predicted to introduce a shift in the reading frame leading to a null allele. A homozygous duplication of 14 nucleotides [GGCTCCGCAGCTCT] at position 444 of the *CHRNE* cDNA including the end of intron 5 and the first nucleotides of exon 6 was identified in the third family. This duplication probably generates a new splice acceptor site in mRNA. This study shows that *CHRNE* mutations exist in Persian population and *CHRNE* screening has to be considered in Iranian patients with a myasthenic phenotype. The possibility of a founder mutation in Semnan province has to be investigated by haplotyping analysis.

The GenDisCAN project (Gene Discovery for Complex traits in Asian population of Northeast) - study design and interim results -. *J. Sung[1,6], J-S. Seo[2,6], H-R. Kim[3], J-I. Kim[4], S-I. Cho[5], H-S. Park[2], M. Lee[6,7]* 1) Dept Preventive Medicine, Kangwon Natl Univ Sch Medicine, Chuncheon, Kangwon, Korea; 2) Department of Biochemistry and Molecular Biology, Seoul National University College of Medicine; 3) Department of Biochemistry and Molecular Biology, Ewha Womens University Medical College; 4) Department of Biochemistry and Molecular Biology, Hallym University Medical College; 5) Department of Biostatistics and Epidemiology Seoul National University School of Public Health; 6) ILCHUN Molecular Medicine Institute, Medical Research Center, Seoul National University; 7) Macrogen Inc.

The GenDisCAN (Gene Discovery for Complex traits in Asians of Northeast), started in 2002, is one of few non-Caucasian studies, which are devoted to the genetics of complex diseases. The study successfully included 1,518 individuals from 196 families, in three isolated rural provinces of Mongolia, and now recruiting 2,500 individuals this year. One huge family consists of 700 relatives. Measurements of detailed anthrometrics were taken in addition to basic clinical tests and questionnaires. A biorepository was constructed to store sera, plasma, and DNA extracts. We finished genotyping 400 microsatellite markers. A make-up genotyping is undergoing for mistyped markers (>1% genotype errors) and markers with low information contents (heterozygosity < 0.6). Identity by descent (IBD) matrix are being calculated. Heritabilities were estimated and genome-wide linkage analyses were tried (using 196 persons). Heritabilities were estimated to be 0.55 (height) 0.40 (weight), 0.49 (BMI), 0.44 (intraocular pressure, right), 0.42 (intraocular pressure, left), 0.40 (bone density, radius), 0.33 (bone density, heel). Some preliminary QTL analyses (by SOLAR) showed novel loci for LDL cholesterol (LOD score 2.6 on 3q), bone density of radius (LOD 2.7 on 7q), and forehead width (LOD 3.6 on 8q). Our study was designed and ethically permitted to incorporate technical progresses and is open to collaborations to hurdle the complexities of complex human diseases. Keywords: gene discovery study, population isolate, Quantitative Trait Loci (QTL), complex diseases/traits, Mongolian, large extended family.

Preimplantation genetic diagnosis (PGD) for Chronic Granulomatous Disease (CGD, gp91-phox) coupled to HLA matching and with subsequent successful pregnancy. *C. Pangalos[1], C. Konialis[1], G. Kokkali[2], A. Biricik[3], F. Fiorentino[3], C. Pantos[2]* 1) Dept. of Molecular Genetics and Preimplantation Diagnosis, Diagnostic Genetic Center, Athens, Greece; 2) Center for Human Reproduction, Genesis Maternity Hospital, Athens, Greece; 3) Genoma Molecular Genetics Laboratory, Rome, Italy.

Preimplantation genetic diagnosis (PGD) with HLA matching has only recently been recognized as an invaluable approach for couples wishing to produce a healthy offspring, which in turn will act as donor of cord blood hemopoietic stem cells for transplantation into seriously affected siblings. Due to the severe limitations of the whole procedure, only a very small number of cases have resulted in successful transplantation and therapy of the affected sibling, albeit demonstrating the usefulness of the approach. The present case involved a Greek couple with a 3-year old affected male child, harboring the R73X C>T mutation in the CYBB/gp91-phox gene and a phenotype characterized by recurrent infections. A flexible, indirect HLA typing protocol, based on single-cell multiplex PCR analysis of polymorphic STR markers within the human HLA complex, was optimized for the simultaneous amplification of the informative STR markers together with the gene region containing the mutation and the sexing marker amelogenin. Detection of the mutation was performed by minisequencing. Following an adapted IVF protocol, a total of 37 embryos were biopsied on day 3, resulting in an equal number of blastomeres. A successful result for all STR markers and for the mutation detection was obtained for 36 (out of the 37) samples and 6 embryos were found to be unaffected and HLA-matched. 2 of these embryos were transferred and a singleton pregnancy was achieved. The results of the PGD were confirmed following standard prenatal diagnosis from a CVS sample. Birth is expected within the next few weeks, at which time cord blood haemopoietic stem cells from the newborn will be obtained for transplantation into the affected sibling, demonstrating the valuable application of this rather complex approach in similar cases of X-linked chronic granulomatous disease.

Obesity, reduced fertility, and disrupted cicadian rhythm in mice with a targeted mutation in the Prader-Willi syndrome gene *MAGEL2*. *R. Wevrick[1], S.V. Koslov[2], J.M. Bischof[1], A.A. Tennese[1], J. W. Bogenpohl[3], R. van Gelder[4], E.D. Herzog[3], C.L. Stewart[2]* 1) Dept Med. Genetics, Univ Alberta, Edmonton, AB, Canada; 2) Lab. of Cancer and Dev. Biol., NCI, Frederick, MD; 3) Dept of Biology, Wash. Univ, St. Louis, MO; 4) Dept of Ophthalmology and Visual Sciences, Wash. Univ School of Med, St. Louis, MO.

Prader-Willi syndrome is a complex disorder resulting from the inactivation of paternally expressed, imprinted genes on chromosome 15q11-q13. Findings include neonatal failure to thrive, developmental delay, sleep apneas, pain insensitivity, excessive daytime sleepiness, neuroendocrine abnormalities, hypogonadotrophic hypogonadism, and insatiable appetite leading to morbid obesity, indicative of hypothalamic dysfunction affecting sleep, circadian rhythm, appetite, and fertility. We are examining the effects of loss of PWS genes individually, and have generated mice with targeted mutations of the PWS imprinted candidate genes*necdin* and *Magel2*. We now show that *Magel2*-null mice recapitulate fundamental aspects of hypothalamic deficiency in PWS. MAGEL2 shares a MAGE protein homology domain with necdin and may, like necdin, promote neuronal migration and differentiation during development.*Magel2* is highly expressed the hypothalamus and in a circadian manner in the suprachiasmatic nucleus. *Magel2*-null mice have reduced total activity and a less coherent circadian rhythm than control littermates, although they do entrain to a light:dark cycle with a normal period, suggesting a defect in circadian output from the suprachiasmatic nucleus. *Magel2*-null mice weigh less at weaning, have increased weight gain after weaning that is exacerbated by a moderately high-fat diet, and have reduced fertility compared to control. These results strongly implicate loss of *MAGEL2* in the hypothalamic deficiency that profoundly affects appetite, sleep, and reproduction in people with PWS. Previous studies in gene-targeted mice have shown that loss of necdin causes defects in neonatal respiration, sympathetic nervous system function, and pain sensitivity. We propose that the combined loss of necdin and MAGEL2 plays a major role in the complex phenotype of PWS.

Genes of the extracellular matrix contribute to the development of intracranial aneurysms. *Y.M. Ruigrok[1,2], G.J.E. Rinkel[2], R. van 't Slot[1], M. Wolfs[1], S. Tang[1], C. Wijmenga[1]* 1) Complex Genetics Section, Department of Biomedical Genetics, University Medical Center Utrecht; 2) Department of Neurology, University Medical Center Utrecht.

Intracranial aneurysms probably develop due to the interaction of several genes and environmental factors. Previously, we hypothesized that a disruption of the extracellular matrix (ECM) of the arterial wall is a likely factor in the pathogenesis of intracranial aneurysms. We analyzed 44 potential candidate genes involved in the maintenance of the integrity of the ECM in 382 Dutch Caucasian patients with intracranial aneurysms and 609 Dutch Caucasian controls for 384 tag single nucleotide polymorphisms (SNPs) using the GoldenGate assay on an Illumina BeadStation 500 GX. We identified SNPs that were associated with intracranial aneurysms ($p<0.01$) in six of these 44 genes: serpine1 (PAI1, p=0.0008), transforming growth factor, beta induced (TGFBI, p=0.0026), perlecan (HSPG2, p=0.0044), fibronectin (FN1, p=0.0069), fibrillin 2 (FBN2, p=0.0077) and collagen 4A1 (COL4A1, p=0.0087). In a second independent cohort of 310 Dutch Caucasian intracranial aneurysm patients and 336 Dutch Caucasian controls, the association for the HSPG2 gene (combined odds ratio (OR) 1.33, 95% confidence interval (CI) 1.13-1.57, p=6x10-4) was replicated. The population attributable risk (PAR) for this SNP is 19%. Combining the two cohorts still showed association for the PAI1 (combined OR 1.27, 95% CI 1.07-1.50, p=0.004, PAR 6%), FBN2 (combined OR 1.37, 95% CI 1.07-1.75, p=0.01, PAR 3%) and COL4A1 (combined OR 1.22, 95% CI 1.05-1.42, p=0.007, PAR 7%) genes. Our findings indicate that variation in genes involved in the maintenance of the integrity of the ECM of the arterial wall plays a role in susceptibility to intracranial aneurysms. These findings further support our hypothesis that diminished maintenance of the ECM of the arterial wall is important in the development of intracranial aneurysms.

An atypical presentation of systemic epidermal nevus syndrome in childhood. *N. Shur[1], R.W. Marion[1, 2], J. Samavich[1], J. Schaffer[3], A. Roe[2]* 1) Department of Pediatrics, Division of Genetics, Montefiore Medical Center, Bronx, NY; 2) Department of Obstetrics, Division of Genetics, Albert Einstein College of Medicine, Bronx, NY; 3) Department of Dermatology, New York University, Manhattan, NY.

Background: Epidermal nevus syndromes (ENS) are rare neurocutaneous disorders characterized by extensive nevi on the skin, and variable neurological, ocular, and skeletal abnormalities. ENs are postulated to arise from post-zygotic mutations and reflect cutaneous mosaicism. Mosaicism in extracutaneous tissue causes systemic disruptions termed organ nevi. The diagnosis of ENS depends on the presence of characteristic nevi distributed along the Blaschko lines. We describe an atypical presentation of systemic ENS with lesions not evident at birth and did not appear to follow lines of Blaschko. Case report: We evaluated a 3 1/2-year-old African-American boy with global developmental delay. The full-term product of an unremarkable pregnancy and delivery, the only initial perinatal abnormality was a mild eczematous-appearing rash. At 2 y.o dark, velvety, papillomatous lesions appeared and, over the next year, became increasingly extensive, involving his entire trunk and crossing the midline. On exam, he had microcephaly, a sloping forehead and high hairline, brachydactyly of his fingers, and prominent hypertonicity. Initial genetic testing for high resolution chromosomes, FISH for subtelomeric probes, and Fragile X was negative. Further testing ruled out neurocutaneous syndromes. including SjÖgren-Larsson syndrome, and infantile Gaucher disease. Repeat dermatological evaluation and biopsy led to the diagnosis of ENS. Discussion: In most cases, the diagnosis of ENS is straightforward with nevi typically appearing at birth and following lines of Blaschko. However, our patient demonstrates that occasionally, EN may be so extensive that the linear bands become confluent. In addition, profound neurological problems may become evident prior to the skin involvement. Our case highlights the importance of considering an atypical presentation of ENS in patients with variable neurocutaneous manifestations.

A rapid and flexible multiplexed SNP based procedure to screen for DNA variation in the mitochondrial genome.

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Mutations in the mitochondrial genome have been increasingly associated with several cancers and individuals with certain mitochondrial polymorphisms have increased susceptibility to mitochondrial diseases such as Parkinsons disease and Lebers hereditary optic neuropathy. Rapid, efficient, low cost and large scale identification of variations in the mitochondrial genome that better predict likelihood of disease would be extremely valuable. This is particularly important for prostate and renal cancers where screening tools for early detection do not exist or are crude.

We have developed a single nucleotide polymorphism (SNP) genotyping technology to assay for known mitochondrial variations in a high throughput, multiplexed and flexible manner. Utilizing the Beckman SNPStream single base extension genotyping technology we have taken a novel approach which provides the ability to specifically, sensitively and at low cost assay variations in the mitochondrial genome. We are initially screening nearly 300 prostate or renal cancer patients. Developing this capability to generate panels of mitochondrial genetic markers associated with prostate and renal cancer in particular will provide a powerful technique to supply accurate information crucial for determining susceptibility to mitochondrial disease, treatment options including early interventions and patient outcomes.

Abnormal Magnesium Balance in *Trpm6* and *Trpm7* mutant mice. *R.Y. Walder[1], L.V. Ryazanova[4], B. Yang[2], M.P. Andrews[1, 6], X. Cao[2], M.V. Dorovkov[4], L. Rondon[5], A. Mazur[5], J.B. Stokes[3], V.C. Sheffield[1, 6], A.G. Ryazanov[4]* 1) Dept Pediatrics, Univ Iowa, Iowa City, IA; 2) Dept Obstetrics and Gynecology, Univ Iowa, Iowa City, IA; 3) Dept Internal Medicine, Univ Iowa, Iowa City, IA; 4) UMDNJ-Robert Wood Johnson Medical School, Dept Pharmacology, Piscataway, NJ; 5) Centre de Recherche en Nutrition Humaine d'Auvergne, INRA, Theix, St. Genes Champanelle, France; 6) Howard Hughes Medical Institute, Iowa City, IA.

Recent evidence suggests that the channel kinases, TRPM6 and TRPM7, play a key role in the regulation of magnesium homeostasis in vertebrates. Mutations in *TRPM6* cause familial hypomagnesemia with secondary hypocalcemia (HSH) (MIM 602014). TRPM7 is a ubiquitously expressed Mg permeable ion channel and TRPM7-deficient cells fail to grow in the absence of supplemental Mg. There is evidence that TRPM6 and TRPM7 combine to form heteromeric channels and regulate Mg transport into the cell. We have constructed mice with a targeted deletion of the *Trpm6* gene and two lines of *Trpm7* mutant mice, one with a complete elimination of *Trpm7* and one in which the kinase catalytic domain was deleted, leaving the channel portion intact (kinase). Some of the homozygous *Trpm6*$^{(-/-)}$ mice die *in utero*, but some are born with a reduced survival rate. Some *Trpm6*$^{(-/-)}$ mice survive to adulthood and are fertile. Homozygous mice from both lines of *Trpm7* mutant mice are embryonic lethal: mice completely lacking *Trpm7* die at day 6.5, while *Trpm7* kinase mice die at day 7.5. Heterozygous *Trpm6* and *Trpm7* kinase mice showed abnormalities of Mg handling. On a normal diet, the *Trpm6*$^{(+/-)}$ animals excreted much less Mg in their urine and on a low Mg diet had lower plasma Mg levels. *Trpm7* kinase$^{(+/-)}$ mice on a normal diet had lower plasma Mg and lower urinary Mg excretion than controls. On a low Mg diet, the *Trpm7* kinase$^{(+/-)}$ mice developed signs of neuromuscular irritability and abnormal motility and some developed seizures characteristic of a severe hypomagnesemic state. The *Trpm7* kinase$^{(+/-)}$ mice had more severe hypomagnesemia than the *Trpm6*$^{(+/-)}$ mice. Our data indicate that Trpm6 and Trpm7 are involved in Mg homeostasis.

A t(4;11)(p12;q23) in a therapy-related acute myeloid leukemia. *S.N.J. Sait[1], M.A. Claydon[1], M. Barcos[2], M.R. Baer[3]* 1) Clinical Cytogenetics Laboratory, Roswell Park Cancer Institute, Buffalo, NY; 2) Department of Pathology, Roswell Park Cancer Institute, Buffalo, NY; 3) Department of Medicine, Roswell Park Cancer Insitute, Buffalo, NY.

We describe a t(4;11)(p12;q23) in a patient with therapy-related acute myeloid leukemia (t-AML) as the third reported case of t(4;11)(p12;q23) in patients with secondary leukemias. The patient is a 56-year-old female who was diagnosed in 1998 with a small lymphocytic lymphoma and treated with fludarabine and rituximab, following which she had persistent cytopenias. In 1999, she was diagnosed with multifocal invasive ductal carcinoma of the left breast, which was treated with mastectomy without adjuvant chemotherapy due to cytopenias, and in 2004 biopsy of a left chest wall mass revealed a poorly differentiated adenocarcinoma, which was treated with radiation therapy, again without chemotherapy. Bone marrow histology and karyotype were normal in 2004, but in 2005 the patient was diagnosed with acute myeloid leukemia (FAB M1), therapy-related. The karyotype at that time was 54,XX,+1,der(1;7)(q10;p10),+3,t(4;11)(p12;q23),+der(4)t(4;11)(p12;q23),+6,+6,+8,+8,+8,+19,del(20)(q11q13),+22 in all 20 cells analyzed. Fluorescence in situ hybridization (FISH) using the MLL probe (Abbott Molecular) showed rearrangement of the MLL locus and confirmed the t(4;11)(p12;q23). The t(4;11)(p12;q23) has been reported previously in two patients-one with conversion of childhood acute lymphocytic leukemia (ALL) with a t(12;21) to juvenile myelomonocytic leukemia with t(4;11) (Manor et al, 2003; Cancer Genet Cytogenet) and the other with therapy-related ALL following breast adenocarcinoma (Hayette et al, 2005; Cancer Res). Hayette et al identified the AF4p12 gene at 4p12, a human homologue of the furry gene of Drosophila, as the fusion partner of the MLL gene in their patient. It is not known whether the same gene is involved in our patient.

Genome-wide admixture mapping for coronary artery calcification identifies atherosclerosis locus on chromosome 6 in African-Americans. *Q. Zhang[1], C.E. Lewis[2], L.E. Wagenknecht[3], R.H. Myers[4], J.S. Pankow[5], S.C. Hunt[6], K.E. North[7], J.E. Hixson[8], I. Borecki[1], M.A. Province[1]* 1) Washington University School of Medicine, St Louis, MO; 2) University of Alabama, Birmingham, AL; 3) Wake Forest University School of Medicine, Winston-Salem, NC; 4) Boston University School of Medicine, Boston, MA; 5) University of Minnesota, Minneapolis, MN; 6) University of Utah School of Medicine, Salt Lake City, UT; 7) University of North Carolina, Chapel Hill, NC; 8) University of Texas School of Public Health, Houston, TX.

To identify the genetic loci contributing to coronary artery calcification (CAC), an important measure of subclinical coronary atherosclerosis, we conducted a genome-wide scan with 374 microsatellite markers by applying admixture mapping to 618 African American participants in the NHLBI Family Heart Study (FHS). 868 Caucasian participants in FHS and 157 Africans genotyped by the Marshfield Medical Genetics Center were used as two reference founding populations of African Americans. A Bayesian method and Markov Chain Monte Carlo (MCMC) algorithm were used to estimate Caucasian and African ancestries among African Americans. A permutation test for regression of CAC score on marker specific African ancestry found 4 markers, D10S189 at 10p14 (P=0.0012), D20S159 at 20q13 (P=0.0075), D12S1294 at 12q14 (P=0.0082) and D6S1053 at 6q11 (P=0.0098), significant at the 0.01 level. D10S189 and D6S1053 were further confirmed at the 0.05 significance level by regression of CAC on allelic copy number. Two independent linkage scans in the FHS Caucasian families and in the Framingham Offspring Study have also identified significant linkage signals at one of these locations (D6S1053 @ 80cM) with LODs=2.2 and 3.3(ODonnell et al., 2006), respectively. Four other published studies show suggestive linkages (LODs between 1.0-2.2) to this same region for various measures of CHD and/or atherosclerosis. This region seems to harbor a highly promising, replicated QTL for atherosclerosis.

A molecular defect causing impaired ganglioside synthesis in a familial form of multiple sclerosis. *E. Vitale[1,4], C. Yildirim - Toruner[1], G. Mahon[2], S. Husain[1], G. Toruner[1], M. Schwalb[1], S. Cook[3]* 1) Microbiology & Mol Genetics, UMDNJ New Jersey Medical School, Newark, NJ; 2) NJMS-UH Cancer Center UMDNJ, Newark, NJ, USA; 3) Department of Neuroscience UMDNJ-New Jersey Medical School, Newark NJ, USA,; 4) CNR Institute of Cybernetics, Naples, Italy.

Rapid communication between neurons requires energy and the insulation of axons by discontinuous segment of myelin. Voltage-gated Na+ channels produce nerve impulses and are concentrated at the nodes Ranvier. The nerve impulse rapidly jumps from node to node by a process called saltatory conduction. MS is an inflammatory demyelinating disease of the central nervous system that destroys myelin, oligodendrocytes, axons and neurons. As a result the saltatory conduction is interrupted. We previously demonstrated a linkage between the MS and markers on 12p12 conditional on the presence of HLA DR15 DQ6 alleles in a pedigree of Pennsylvania Dutch extraction. We have evidence of an existing disruption of the ganglioside pathway that could lead to a defective distribution of GD3G on PBLs cell membranes thus representing a foundation of the disease in this unique pedigree. Sequencing of the GD3 Synthase gene localized in the 12p12 locus shows a novel G29A genetic variant at +29 in the 5' splice donor site of intron 4/5. Contingency table analysis on the family showed that all MS affected individuals have both the DR15 DQ6 allele and the G29A variant whereas the unaffected individuals have either one or neither of these markers ($p = 0.00011$). Molecular analyses revealed that in the MS patients in this family there is a premature degradation of GD3S mRNA and that the transcript is about 150 times lower when compared to the normal sample. We also analyzed the cell surface expression of GD3S and GD3 Gangliosides by Flow Cytometric Analyses on controls in the general population. Our results show that GD3S is mainly expressed by the B cell subpopulation of the immune system while GD3G it is ubiquitously expressed in PBLs . The observation of differences in protein and/or ganglioside profile and distribution in the patients supports the hypothesis that the genotype HLA DR15 DQ6 and G29A of GD3S are the major components in the disease.

Reading disability: is the 6p22 locus DCDC2, KIAA0319 or both? Using integromic techniques to resolve the conflict. *G.P. Page[1], A. Patki[1], K. Zhang[1], T. Mehta[1], V. Srinivasasainagendra[1], H. Main[2], J.R. Gruen[2]* 1) Department of Biostatistics Univ Alabama Birmingham, AL; 2) Department of Pediatrics, Yale University New Haven, CT.

Last year at this meeting we reported that polymorphisms and a deletion in and near the DCDC2 gene were associated with multiple reading traits. KIAA0319, a gene near DCDC2 has also been reported to be associated with reading disability. Since then several published studies have reported positive and negative replication of both associations. To further complicate matters is the complexity of the reading disability phenotypes, each study uses multiple different measures of reading disability, making thus it is difficult to assess agreement between studies. To make matter worse, most published studies have some evidence of association to markers in multiple genes in this region. We are using integromic approaches to understand reading disability in this genomic region. For association studies we typed over 200 markers and constructed haplotypes in the region. While single marker association still maximizes to DCDC2, we have found uncommon haplotypes that span the region from at least DCDC2 to THEM2 that are associated with some of the reading disability phenotypes. Using multidimensionality reduction approaches we have found also found evidence for interactions between SNPs in and around DCDC2 and KIAA0319 for reading disability traits. To assist in assigning functions to DCDC2 and KIAA0319 we have also built a co-expression engine containing data from over 2300 human expression arrays. We find that genes co-expressed with DCDC2 are highly enriched for transcriptional activator, nucleic acid binding, synaptic transmission, ubiquitin activity, and sensory perception, and zinc ion binding while and the majority are in the nucleus while KIAA0319 is enriched for microtubule motor activity, GTP binding, calcium, and calmodulin binding genes which are mostly in the membrane. These two lines of evidence suggest that the region on 6p22 may contain interacting genes that contribute to different aspects of reading, and do so through different pathways or different steps on the same pathway.

Evidence Based Information: Lessons learned from Fragile X and Duchenne/Becker Muscular Dystrophy. *M. Weaver[1], K. Reed[2], C. Greene[1], M. Blitzer[1], P. Furlong[3], K. Clapp[4], S. Terry[5]* 1) Univ. of Maryland, Baltimore, MD; 2) NCHPEG, Lutherville, MD; 3) Parent Project Muscular Dystrophy, Middletown, OH; 4) FRAXA Research Foundation, Newburyport, MA; 5) Genetic Alliance, Washington, DC.

Current focus on evidence-based medicine creates challenges for families and health professionals dealing with diagnosis and management of single-gene disorders. Although evidence basis for rare conditions is growing, significant uncertainty often remains about natural history and management options. The quality of information available is highly variable, and families and health professionals must assess and synthesize information from many sources to make informed decisions. The Access to Credible Genetics Resources Network is a collaborative effort led by the Genetic Alliance and funded by the CDC. One goal of this project is to enable access to accurate and scientifically valid information on single gene disorders for families and health professionals.

A critical step to accomplish this goal is describing evidence-based information. By analyzing methods used by other organizations to assess information quality, this project created a draft metric. The purpose of the metric is to assess quality of information in educational materials. Examples of criteria included in the metric are: currency and accuracy of information, and source of expertise. We will use the metric to inform the construction of a module designed to help families independently evaluate the quality of information. Further, we will develop an outline of content topics that families and experts believe are important to include in educational materials for the purpose of informed decision making. The development of both of these tools will be an iterative process to engage both families and health professionals. The recommendations on content and the quality metric will provide a comprehensive standard to assess existing materials and to guide the creation of new materials. The goal of presentation of the draft metric at ASHG is to invite critique by the professional community. After incorporation of comments from this meeting, a revised draft will be formally disseminated for further comment.

Evaluation and introduction of new techniques in the genetic testing service by EuroGentest. *N. van der Stoep*[1, 2], *G. Matthijs*[2], *M. Macek*[2], *E. Bakker*[1, 2] 1) Clinical Genetics, LUMC, Leiden, Netherlands; 2) EuroGentest, http://www.eurogentest.org/.

At present, the field of genetics is witnessing a fast expansion of new technologies that could have a strong potential for application in genetic testing. Unfortunately, implementation of these novel technologies is often hampered by the lack of complete evaluation of their technical performance in a diagnostic setting. EuroGenTest(EGT)is a European Network of Excellence aiming at harmonizing genetic testing services throughout Europe. One key objective is to bridge the gap observed in the chain of new technology transfer from research into implementation and ultimate accreditation in genetic diagnostics. EGT Unit 5 is specifically involved with the coordination and guidance of activities required for complete technical evaluation, validation and subsequent implementation of emerging technologies into diagnostic application. Recently several interesting new techniques were recruited by a EGT directed call for new techniques and introduced at the first EGT Unit 5 open meeting on 'New Technologies in Genome Diagnostics' at the European Society of Human Genetics Conference (ESHG) conference in Amsterdam (see our webpage for more details. As a follow up two of these new approaches in genetic testing are currently under evaluation in the EGT-setting in collaboration with their manufacturers and inventors. In this respect we are setting up a new test for BRCA1 mutation scanning using high-resolution melting curve analysis (hrMCA), a method to scan DNA fragments for the presence of mutations/variants, in collaboration with Idaho Technologies. In addition, we are evaluating Pyrophosphorolysis Activated Polymerization (PAP), a very sensitive detection method for the presence of low frequency mutations in the presence of excess wt allele, in collaboration with the City of Hope National Medical Center and Beckman Research Institute and ServiceXS. PAP technology will be primarily evaluated as a non-invasive prenatal diagnostic tool for the detection of paternal sequences in the presence of excess the maternal allele on fetal DNA isolated from serum of pregnant women.

Effects of *INSIG2* gene and substance dependence on body mass index. *B.Z. Yang[1], H.R. Kranzler[2], R. Weiss[3], K. Brady[4], L. Farrer[5], J. Gelernter[1]* 1) Dept Psychiatry, Yale Univ Sch Medicine, New Haven, CT. & VA CT, West Haven, CT; 2) Univ CT Health Center, Farmington, CT; 3) Dept Psychiatry, Harvard Med School, Boston, MA; 4) Med Univ South Carolina, Dept Psychiatry, Charleston, SC; 5) Boston Univ School Med, Boston, MA.

We investigated the effects of *INSIG2* gene and substance dependence (SD), including dependence on alcohol, cocaine, marijuana, opiates, and tobacco (AD, CD, MjD, OD, TD), on body mass index (BMI). We genotyped a single nucleotide polymorphism (rs7566605, designated as SNP-*INSIG2*) in insulin induced gene 2, a susceptibility gene variant for obesity, in a linkage sample collected for mapping genes increasing risk of CD and OD. The linkage sample contains 1485 subjects from 319 and 313 African American and European American pedigrees, respectively; 47.7% are male, and the average age is 39. 37.8% have BMI >30 (i.e., are considered obese), 43.2% AD, 81.8% CD, 26.7% MjD, 43.9% OD, and 65.1% TD. The family-based association test (FBAT) for the binary trait of obesity for the recessive genetic model of SNP-*INSIG2* showed borderline significance (p=0.057); alternatively, the FBAT for the quantitative trait of BMI after controlling for the effects of age and sex was not significant (p=0.093), but had only 5% power because of low number of informative families used in FBAT. We then sought to use all of the available data by implementing the generalized linear model via generalized estimating equations (GEE) for incorporating the familial correlation structure, which accounts for genetic and environmental heterogeneity across families. The results, after controlling for sex (p=0.0008) and age (p=0.007), showed significantly greater BMI for AD (p=0.014), CD (p=0.010), and MjD (p<0.0001), and a decreased BMI for OD (p=0.033). There was no genetic effect from SNP-*INSIG2* although homozygous CC individuals showed a trend for increased BMI, consistent with a prior report. TD was not included in the model due to colinearity with the other SD diagnoses. We conclude that the effect of SD on BMI is far greater than that of the genetic variant from SNP-*INSIG2*, which carries low relative risk in the general population.

European mitochondrial haplogroups exhibit differential risk of developing presbycusis. *S. Ramon[1], A.C. Braganza[1], F.M. Mapes[2], S.T. Frisina[2], R.D. Frisina[2], D.R. Frisina[2], D.A. Eddins[2], D.L. Newman[1]* 1) Biological Sciences, Rochester Institute of Technology, Rochester, NY; 2) International Center for Speech & Hearing Research, Rochester, NY.

The genetic basis of human presbycusis (age-related hearing loss) is unknown. This common disorder is characterized by difficulty understanding conversation, particularly in noisy backgrounds. Audiograms of presbycusics show sloping hearing loss, with greatest deficiencies at the highest frequencies, and over time an individuals hearing loss progresses into the lower frequencies that are more important for understanding speech. We investigated the hypothesis that the mitochondrial (mt) genome plays a role in presbycusis. Subjects of European ancestry, all over age 58, were tested using both classical and advanced audiometric measures and then genotyped to determine mt haplogroups. We found that subjects belonging to haplogroup H (N=93) had better hearing than other Europeans (N=80), with the greatest differences observed in the right ear at 3 kHz (p=0.017) and 10-14 kHz (p=0.016). The difference at 3 kHz correlates with the common noise notch location, and thus may indicate a difference in susceptibility to noise damage. Distortion product otoacoustic emissions also indicated better hair cell health in haplogroup H subjects, at higher frequencies and in the right ear (average DPOAE for 4-6 kHz, p= 0.010). These results support the hypothesis that a mitochondrial factor influences susceptibility to the development of presbycusis. We are currently investigating the mt genome for causative mutations linked to the haplogroups.

Mutations in LRRK2 other than G2019S are rare in familial Parkinson disease. *N. Pankratz[1], M.W. Pauciulo[2], V.E. Elsaesser[2], D.K. Marek[2], C. Halter[1], A. Rudolph[3], C.W. Shults[4], T. Foroud[1], The Parkinson Study Group - PROGENI Investigators* 1) Indiana University School of Medicine, Indianapolis, IN; 2) Cincinnati Childrens Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH; 3) University of Rochester, Rochester, NY; 4) University of California, San Diego, San Diego, CA; VA San Diego Healthcare System, San Diego, CA.

Mutations in the LRRK2 gene cause some forms of autosomal dominant Parkinson disease (PD) and have been found in 5% of familial cases and 1% of sporadic cases. We screened 956 primarily Caucasian individuals from 430 multiplex PD pedigrees for 12 previously reported, pathogenic LRRK2 mutations: R793M, L1114L, I1371V, R1441C, R1441G, R1441H, Y1699C, M1869T, I2012T, I2020T, G2385R, and IVS31 +3G>A. Previous screening identified the LRRK2 G2019S mutation in 5% of our families. Only one of the 12 newly screened mutations, R1441C, was detected in a single family in our patient cohort. Two brothers were concordant for the mutation and had an average age of onset of 53 years. The mother did not harbor the mutation, and no other family members were reported to be affected; however the father died at the age of 61. These results indicate that while the G2019S mutation remains the most common mutation identified in familial PD patients, other mutations in LRRK2 are infrequent. This will make it difficult to gather adequate penetrance data for these rarer mutations. Coupled with the presence of nonpathogenic coding variants, this will make it difficult to provide proper genetic counseling to individuals undergoing genetic testing. Until these ambiguities have been resolved, it is recommended that presymptomatic testing be delayed until more is learned about the frequency, penetrance and risk assessment of LRRK2 mutations.

The L10P polymorphism of TGF-1 shows no association with prostate cancer in a US Caucasian study population. *B. Yaspan, J. Breyer, B. Elmore, K. Bradley, K. McReynolds, J.R. Smith* Departments of Medicine and Cancer Biology, Vanderbilt University Medical Center, Nashville, TN.

Transforming growth factor (TGF-) is a tumor suppressor and a potent inhibitor of cellular proliferation. However the pathway has a dual role, as excess TGF- signaling is implicated in cancer progression. TGF- has been shown to regulate proliferation and apoptosis of prostate epithelial and stromal cells. Significantly elevated levels of TGF- expression in prostate cancer cell lines have also been observed. A well-studied polymorphism in codon 10, a T to C transition, results in a proline to leucine substitution in the hydrophobic core of the signal peptide of TGF- isoform 1 (TGF-1). Presence of the T allele has been shown to be significantly associated with increased risk of prostate cancer and of benign prostate hyperplasia in a Japanese study population. We tested the hypothesis that the presence of the T allele confers risk for prostate cancer in a Caucasian study population from Vanderbilt University. We observed no significant association of the TGF-1 L10P polymorphism with risk of prostate cancer in a study population of 597 cases, and 513 screened controls without a personal or family history of prostate cancer.

Common genetic variation in ATM does not determine susceptibility to non-Hodgkin lymphoma. *P. Sipahimalani[1], J. Spinelli[2], A. MacArthur[2], A. Lai[2], J. Connors[3], R. Gascoyne[4], R. Gallagher[2], A. Brooks-Wilson[1]* 1) Genome Sciences Centre; 2) Cancer Control Research Department; 3) Medical Oncology; 4) Pathology, British Columbia Cancer Agency, Vancouver, Canada.

The ataxia telangiectasia mutated (ATM) gene is critical for the detection and repair of DNA double-stranded breaks (dsb). Mutations in ATM cause the autosomal recessive syndrome ataxia telangiectasia, a feature of which is a high risk of lymphoma. We undertook a population-based study of 798 NHL cases and 793 controls to assess the role of genetic variation in ATM on the risk of non-Hodgkin lymphoma (NHL). Many of the subtypes encompassed by the term NHL have in common the occurrence of specific somatic translocations. We hypothesize that variants that affect ATM function could reduce dsb repair capacity, contributing to the occurrence of translocations and subsequent lymphomas.

Genetic variation in the promoter and all exons of ATM was determined by bi-directional sequencing of the germline DNA of 86 NHL patients. Sequencing revealed 79 variants, 18 of which correspond to amino acid differences. Six rare variants (0.5-1.1%) were predicted to be deleterious to protein function. Eleven variants had a minor allele frequency of 5%; these made up 10 haplotypes that could be specified by seven tagSNPs. Linkage disequilibrium across the ATM gene is high but incomplete. Six tagSNPs and the 6 putatively deleterious rare variants converted successfully to Taqman assays and were genotyped in the case/control set. Direct and haplotype-based indirect association tests were performed. Subtype specific and ethnicity specific analyses were performed when sample numbers permitted.

The results of these association tests indicated that common variants of ATM do not significantly contribute to the overall risk of NHL in the general population. The effect of multiple rare deleterious ATM variants at the population level was more difficult to assess, and may require the use of new technologies to permit extensive sequencing of hundreds or even thousands of NHL cases and controls.

Functional genetic variants of both cysteinyl leukotriene 1 and 2 receptors are independently associated with atopic asthma in the population isolated on Tristan da Cunha. *M.D. Thompson[1], J. Takasaki[2], V. Capra[3], W.M. Burnham[4], D.E. Cole[1], K.A. Siminovitch[5]* 1) Lab Medicine, Banting Inst, Univ Toronto, Toronto, ON, Canada; 2) Drug Discovery Research, Astellas Pharma Inc. Tsukuba, Ibaraki 305-8585, Japan; 3) Laboratory of Molecular Pharmacology, Section of Eicosanoid Pharmacology, Department of Pharmacological Sciences, University of Milan; 4) Department of Pharmacology, University of Toronto, Toronto, Ontario; 5) Departments Medical Genetics and Microbiology, University of Toronto, the Samuel Lunenfeld Research Institute, Mount Sinai Hospital.

The contribution to asthma of variability in the genes encoding the cysteinyl leukotriene (CysLT) system was quantitated in the population resident on the island of Tristan da Cunha. Environmental risk factors such as allergies and smoking history were controlled. Genetic variation in the cysteinyl leukotriene 1 and 2 receptors has been associated with atopic and/or asthmatic disease in some populations and with variable response to the cysteinyl leukotriene family of inflammatory mediators (LTC4, LTD4 and LTE4) that regulate human airway contraction. In particular, we report the association of CysLT1 G300S and the CysLT2 M201V variants with atopic asthma in the Tristan da Cunha. In vitro studies of these variants using a calcium flux assay suggest that discrete portions of the genes encoding the CysLT1 and CysLT2 transmembrane domains may be critical to maintaining the conformation necessary for leukotriene agonist and antagonist second messenger coupling. While the M201V variant is partially inactivating, the G300S variant is partially activating. Both variants are highly associated with atopic asthma in the study population. We augue that together these variants may give rise to atopic athma and may result in a significant alteration in drug efficacy.

Neonatal hypertrophic cardiomyopathy (HCM) due to mitochondrial SCO2 deficiency: An enzyme histochemical and targeted mutation approach to the diagnosis of a novel mutation. *H. Vogel[1,3], L.J. Wong[2], E.J. Prijoles[3], A.M. Dubin[3], G.M. Enns[3]* 1) Department of Pathology, Stanford University, Palo Alto, CA; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Department of Pediatrics, Stanford University, Palo Alto, CA.

In order to demonstrate the utility of enzyme histochemistry and targeted mutational analysis in the evaluation of mitochondrial (mt) disease, we present a neonate who developed HCM. She was the first child born to consanguineous parents of Indian ethnicity. Intrauterine growth retardation and decreased fetal movement were noted the week before delivery. At birth she required resuscitation and was hypotonic with lactic acidosis (9.0 to 21.0 mmol/L). Initial echocardiography was normal, but she developed a concentric non-compacted HCM and died at 30 days. MtDNA analysis for common mutations and testing for congenital disorders of glycosylation were normal. Electron transport chain (ETC) analysis on skin fibroblasts was normal. Muscle biopsy performed on DOL 29 revealed a neuropathic pattern suggestive of spinal muscular atrophy (SMA) and enzyme histochemistry revealed marked diffuse cytochrome oxidase (COX) deficiency. Interestingly, with additional incubation from 1 to 8 hours, COX activity was detectable. ETC analysis could not be done because of insufficient muscle. DNA analysis of nuclear encoded COX assembly genes SURF1, SCO1, SCO2 and COX10 identified a novel homozygous missense alteration (c.577G>A; p.G193S) in the SCO2 gene. The parents were heterozygous carriers of p.G193S. The amino acid glycine at position 193 in the SCO2 protein is highly conserved from yeast to human and not found in 100 normal control chromosomes. Since HCM is a hallmark of SCO2 deficiency, our results support the pathogenicity of p.G193S. The deficiency likely represents defective catalytic properties of COX rather than a truncated protein as has been linked to SCO2 deficiency with SMA. This case illustrates the importance of multiple modalities in identifying mt disease, including enzyme histochemistry and targeted gene sequencing. By identifying an etiology of HCM, appropriate genetic counseling may be provided to the family.

Whole genome association studies for ADHD using samples from the Quebec founder population. *B. Paquin, J. Raelson, P. van Eerdewegh, R. Little, R. Paulussen, V. Bruat, M. Lapalme, J. Hooper, A. Belouchi, T. Keith* Genizon Biosciences, St Laurent, PQ, Canada.

We previously performed whole genome association studies (WGAS) for six complex diseases including Crohns disease. Recently, we completed genotyping 469 parent-parent-child trios with the child of each trio being affected with attention deficit and hyperactivity disorder (ADHD). The phenotype was determined following a series of interviews and tests performed both with the affected child and one of the parents, according to the DSM-IV criteria. The 469 trios were collected from the Quebec Founder Population (QFP). A total of 317,503 SNPs were genotyped in house using the Infinium assay from Illumina, generating 447 million genotypes. An additional 60,000 SNPs consisting of tag SNPs and markers spaced according to variation in LD, determined specifically from the QFP, will also be genotyped. Utilizing parental non-transmitted chromosomes as controls, haplotype case-control association analyses were carried out using a proprietary software LDSTATS calculating exact P values based on permutation. Seven regions with -log10 p values 5 have been identified. These regions range in size from 33 to 207 kb, with an average size of 125 kb. These regions contain up to 4 genes, including 5 regions with single-gene resolution. Some of the identified genes consist of ion channels or receptors that represent strong candidate genes. We are currently in the process of evaluating the genome-wide significance of the signals using a permutation-based approach. The data is also being analyzed using a proprietary TDT algorithm applied to haplotypes and genome-wide conditional analyses using the risk and protective haplotypes from the strongest signals are being conducted. The confirmed associated genes will then be analyzed in silico for the construction of GeneMaps which will reveal the underlying genetic etiology of the disease. The results of each of these analyses will be presented. In addition to our ADHD study we are also conducting gene discovery programs in more than 20 common diseases.

Mining Affymetrix 10K SNP data from the Autism Genome Project for copy number variation. *S.W. Scherer for the Autism Genome Project* Dept Genetics & Genomic Biol, Hospital for Sick Children, Toronto, ON, Canada.

Autism is a neurodevelopmental disorder characterized by impairments in social interaction, communication, and a preference for repetitive activities. The major susceptibility genes are not known but twin studies suggest autism is mainly a genetic condition of complex etiology. Previously we have observed that chromosomal abnormalities detectable by karyotyping are more prevalent in autistic patients compared to controls. We have proposed that sub-microscopic copy number variation (CNV) may also be associated with autism. In addition to providing evidence for potential disease-associated loci, copy number information can also be used to reduce genetic heterogeneity in linkage and association studies. Further to these points, we have mined Affymetrix Mapping 10K 2.0 SNP data from the Autism Genome Project (AGP) linkage analysis study for copy number variation. Upon removal of failed and noisy experiments, 5823 10K scans from 1329 families were available for copy number analysis using DNA-Chip Analyzer (dChip) software. Samples were analyzed blindly in plate by plate batches. After normalization of scan intensities and model based expression, copy number was inferred using a Hidden Markov Model built into dChip. Despite the relatively low resolution of the Affymetrix Mapping 10K 2.0 SNP Array, we detected imbalances in greater than 10% of individuals for a total of 1317 putative CNVs (median size of 1.2 Mb). The majority (~85%) of variants were gains, likely owing to a greater tolerance in the genome for large gains versus large deletions. There were 384 regions (917 CNVs) exhibiting partial or full genomic overlap between more than one individual. Multiple occurrences within a family or overlap with Mendelian errors were interpreted as additional evidence for a particular CNV to be a real event. Of the 1317 putative CNVs, 87 were familial and 50 overlapped with obvious Mendelian errors from 1168 families used in the final linkage analysis. Families with large CNVs that are not seen in controls (e.g. 15q duplications) can be flagged for removal from linkage analysis to help stratify results and guide positional cloning studies.

Comparison of results for whole genome association studies of schizophrenia using pooled DNA samples and individually genotyped samples. *J. Raelson[1], P. Croteau[1], V. Perepetchai[1], P. Van Eerdewegh[2], R. Allard[1], H. Fournier[1], N. Laplante[1], M. Lapalme[1], Q. Nguyen-Huu[1], N. Paquin[1], B. Paquin[1], J. Segal[2], J-M. Vidal[1], T. Keith[2], A. Belouchi[1]* 1) Genetics Dept, Genizon Biosciences, St Laurent, QC, Canada; 2) Genizon Biosciences, Waltham, MA, USA.

Whole genome association studies for schizophrenia were performed using both pooled DNA and individually genotyped samples from the Quebec French Canadian founder population. The pooling study used 469 patients and 469 controls divided into 9 case and 9 control pools. Each pool contained between 51 and 52 individuals. Each pool was genotyped for approximately 200,000 SNPs by Perlegen Sciences Inc. Differences in mean allele frequencies between case and control pools were determined and exact P values for single marker association and for Fisher combined P values for multi-marker windows were determined using all permutations of pool status. The maximum possible significance was - log P = 4.687 which occured when no permutation gave a larger allele frequency difference than that observed. All markers or multi-marker windows with an exact - log P value greater than 3.5 were individually genotyped and retested using permutation of individual case/control status. Thirty-eight percent of peaks remained at - Log P > 3.5. The entire genome-wide scan was repeated using individual genotyping for 510 patients and 1235 controls. Individuals used in the pooling study form a subset of the individually genotyped sample. Each individual was genotyped for a panel of approximately 370,000 SNPs using the Illumina Infinium II platform. All significantly associated regions are currently being further fine mapped. Here we examine similarities and differences between the pooling and individual genotyped results. These studies are part of our program for gene discovery in multiple complex diseases including Crohn's, psoriasis, asthma, ADHD and Alzheimer's.

Characterization of Two Female Patients with Pelizaeus-Merzbacher disease. *R. Tenconi[1], L. Salviati[1], E. Trevisson[1], A. Friso[1], M. Clementi[1], O. Zuffardi[2], A.M. Laverda[3]* 1) Dept Pediatrics, Clinical Genetics, Univ. of Padova, Italy; 2) Biologia Generale e Genetica Medica, Univ. of Pavia, Italy; 3) Dept Pediatrics, Univ. of Padova, Italy.

Pelizaeus-Merzbacher disease (PMD) is a disorder of the central nervous system characterized by severe hypomyelination and neurological involvement with nystagmus, spasticity, ataxia,and developmental delay.It is an X-linked recessive disorder caused by mutation in the PLP1 gene. Both point mutations, deletions and duplications of the entire PLP1 gene have been described.A PMD-like disease,inherited as an autosomal recessive trait,is caused by mutations in the GJA12 gene. A 5 year-old Pakistani girl was diagnosed with nystagmus since age 3 months.MRI at 1 year of age showed diffuse hypomyelination, confirmed by a second MRI at 4 years.Neurologic examination revealed nystagmus, truncal hypotonia, lower limb spasticity, and dysarthric speech.EMG and nerve conduction studies were normal. Standard karyotype and analysis of the PLP1 gene were normal.Sequencing of the GJA12 gene showed a homozygous 34 bp deletion within the coding region of the gene. Both parents were heterozygous for the mutation. A 4 year-old Italian girl was diagnosed prenatally with a duplication of chromosome Xq.Mild dysmorphic features were noted at birth. She developed nystagmus, and marked psychomotor retardation, with ataxia, hyopotonia and spasticity. MRI at 4 years showed cerebral atrophy and marked hypomyelination. She had signs of peripheral neuropathy at EMG. FISH analysis revealed that the duplicated segment contained the PLP1 gene. High resolution CGH-array mapped the duplication to cromosome Xq22-Xq28. Female carriers of Xq duplications are usually asymptomatic,however a number of patients are reported with a severe clinical picture.It is thought that some of the genes within the duplicated region escape X inactivation in tissues.We believe that our patient is functionally disomic for part of Xq, including the PLP1 gene.In fact she displayed clinical and radiological features of PMD associated with other abnormalities probably related to the effect of other dosage-sensitive genes in the duplicated region.

Binding prediction and probe design for non-continuous probes used for molecular haplotying, genotyping and multiplex-genotyping. *G. Pont-Kingdon[1], R. L. Margraf[1], A. Phansalkar[1], A. Millson[1], K. Damjanovich[1], E. Lyon[1,2]* 1) ARUP Institute for Clinical & Experimental Pathology, ARUP Laboratories, Salt Lake City, UT; 2) Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, Utah.

Many approaches for genotyping mutations use sequence specific probes. Usually the probes are complementary to a continuous stretch of DNA that contains the variant(s). We have previously demonstrated that probes can span template sequences and analyze distant loci, causing the template to bulge. We have used probes that span several loci (loci-spanning probes) that create template bulges of up to 55 nucleotides for molecular haplotyping and for multiplex-genotyping. Selected sequence variation(s) can be masked by using probes with 1-3 base deletions to simplify melting interpretation of other mutations under the probe. Thermodynamic stability (Tm-melting temperature) of a probe hybridized to the template varies with the presence or absence of mismatches and is determined by monitoring fluorescence as a function of temperature. Prediction of probe stability allows efficient design of molecular assays. Many software programs are able to predict the Tms of continuous probes but do not allow for formation of bulges in either the template or the probe. We have been evaluating the VisualOMP folding software for the stability prediction of loci-spanning and masking-deletion probes. Comparison of experimental and predicted Tms was performed with several systems: haplotyping of 3 SNPs of the beta 2 adrenergic receptor gene, and multiplex genotyping of 3 loci of the beta globin gene using loci-spanning probes, and unambiguous genotyping of mutations in the RET protooncogene and the fibroblast growth factor-3 gene in presence of other sequence variations using masking-deletion probes. Data show that predictions can be efficiently used for the design of non-continuous probes for molecular assays.

A QTL influencing blood pressure maps to the region of the essential hypertension susceptibility locus 3 (HYT3) on chromosome 2p in Alaskan Eskimos: the Genetics of Coronary Artery Disease in Alaska Natives (GOCADAN) study. *S. Rutherford[1], S.L. Laston[1], R.B. Devereux[2], H.E. Resnick[3], J.G. Umans[3], S.O.E. Ebbesson[3], R.R. Fabsitz[4], B.V. Howard[3], J.W. MacCluer[1], A.G. Comuzzie[1], S.A. Cole[1]* 1) Southwest Foundation for Biomedical Research, San Antonio, TX; 2) Weill Medical College of Cornell University, New York, NY; 3) MedStar Research Institute, Washington, DC; 4) National Heart, Lung and Blood Institute, Rockville, MD.

Hypertension is a leading cause of death and morbidity in our society because it leads to myocardial infarction, stroke, renal failure and death due to cardiovascular disease (CVD). Despite much effort, limited progress has been made in identifying specific genes responsible for the well-documented heritability of hypertension. We performed a genome-wide scan using markers distributed at approximately 10cM density to identify loci contributing to blood pressure in the Genetics of Coronary Artery Disease in Alaska Natives (GOCADAN) study. Blood pressures were measured in 674 adult Eskimos from families living in coastal villages in the Norton Sound region. In multipoint linkage analysis using 386 microsatellite markers, evidence for linkage was observed with mean arterial blood pressure (LOD = 3.0; P= 0.0001) and less strongly with systolic (LOD = 2.6; P = 0.0003) and diastolic (LOD = 2.4; P = 0.0004) blood pressures in the region of chromosome 2p24.3-p22.1. Peak evidence for linkage occurred at 28.5Mb for mean arterial blood pressure with a 1-LOD score support interval spanning 19 Mb. Our signal on chromosome 2p replicates the findings of 13 previous linkage studies in 9 different populations that also report modest evidence for linkage to blood pressures and/or hypertension in this region. The populations in which this has been observed include Sardinians, Japanese, Chinese, Nigerians, Mexican Americans, African Americans and Caucasians. Because of this, a susceptibility locus for hypertension (hypertension susceptibility locus 3: HYT3) has been mapped to this same region, suggesting that one or more of the 164 genes positioned within our 1-LOD score support interval also influences variation in systolic and diastolic blood pressures in Alaskan Eskimos.

Complete X-linkage map in the domestic cat (*Felis catus*). *A. Schmidt-Küntzel[1,2], E. Eizirik[3], A.A. Schäffer[4], S.S. Hannah[5], B. Neelam[6], V. David[7], S.J. O'Brien[7], M. Menotti-Raymond[7]* 1) Laboratory of Genomic Diversity, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD; 2) Genetics Department, George Washington University, Washington, DC; 3) Centro de Biologia Genomica e Molecular PUCRS, Porto Alegre, RS, Brasil; 4) NLM/NCBI/CBB, National Institutes of Health, Bethesda, MD; 5) Nestlé Purina PetCare Company, Saint Louis, MO; 6) ABCC, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD; 7) Laboratory of Genomic Diversity, NCI-Frederick, Frederick, MD.

A genetic recombination map of the X-chromosome was generated in the domestic cat as a tool for the discovery of X-linked loci. Primers amplifying over thirty microsatellite markers were designed from the sequences of the cat 2x whole genome sequence (WGS) database. The microsatellites were chosen in order to obtain complete coverage of the domestic cat X chromosome. Their location was determined based on sequence alignment with the dog genome assembly. It has been previously demonstrated that the marker order on the X chromosome is completely conserved between the dog and the cat. The marker density is one or more microsatellites every 10 megabases (Mb) and includes the pseudoautosomal region 1 (PAR1). This map provides one of the first integrations of PAR1 in a linkage map for a mammalian species. Recombination frequencies were determined between markers in a multi-generational pedigree of 256 outbred cats. The recombination frequencies show extreme variation across the X chromosome in the studied pedigree, ranging from an absence of recombination events in a region estimated to be greater than 20 Mb, to recombination frequencies of 26% in a region estimated to be less than 3 Mb. Although various factors have been proposed to account for variation in recombination frequency (i.e. GC content, gene density), the phenomena is not well understood. The map is currently being used in the analysis of X-linked phenotypes in the cat. Funded by NCI Contract NO1-CO-12400.

Combined effect of several common SNPs on plasma level of HDL-cholesterol. *S. Sunyaev[1], V. Spirin[1], S. Schmidt[2], J. Cohen[3]* 1) Division of Genetics, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; 2) Max-Planck-Institut für Entwicklungsbiologie Spemannstr. 35 / I D-72076 Tübingen Federal Republic of Germany; 3) University of Texas Southwestern Medical Center, Dallas, TX.

Complex phenotypes are expected to be caused by multiple genetic and environmental factors. Identifying genetic variation underlying complex phenotypes is complicated because many allelic variants may act in concert and because frequent variants may have individually small effects. Plasma level of HDL-Cholesterol is an ideal model for studies of the relationship between genetic and phenotypic variation because genes involved in HDL metabolism are very well characterized biochemically. The impact of multiple rare alleles in genes involved in the reverse cholesterol transport pathway on HDL-C was shown previously. Here, we report the analysis of higher frequency variants in both coding and non-coding regions of genes involved in the HDL metabolism and their effect on plasma level of HDL-C. We used statistical approach based on stepwise regression to relate genetic variation in candidate genes to variation in HDL-C levels in a large panel of individuals. We incorporated a simultaneous analysis of the multiple allelic variants in several candidate genes. First, we used an ANCOVA model which included only race and gender as a seed model. Next, SNPs were subsequently added to the model based on the significance of the increase in the model likelihood as estimated by permutation test. The analysis involved 839 common SNPs from 7 candidate genes from RCT pathway genotyped in the Dallas Heart Study population. The population included 3552 males and females of African-American, European-American and Hispanic origin. An independent population was used for the final validation of the model. The final model consists of four SNPs from three genes. Epistatic interactions were not found to be significant, so our model is purely additive, and can be easily understood in terms of number of HDL-lowering alleles per individual. The model was developed using a half of the dataset and tested using the second half. The model was finally validated on a completely independent panel of ~700 individuals.

No association with risk of prostate cancer for *LDOC1* and *SPANX-C* candidate genes within the HPC-X locus in a US Caucasian study population. *J.R. Smith, B. Elmore, J. Breyer, K. Bradley, K. McReynolds, B. Yaspan*
Departments of Medicine and Cancer Biology, Vanderbilt University Medical Center, Nashville, TN.

We and others have previously undertaken linkage analysis in hereditary prostate cancer (HPC), typically in families with three or more cases, yielding significant loci difficult to confirm across study populations. Described HPC loci include Xq27-28 (HPC-X). Recent haplotype analysis of a Finnish founder population within the HPC-X locus has implicated *LDOC1* and *SPANX-C* as candidate prostate cancer genes, with a 150 kb region from markers D3S2390 to bG82i1.0 containing the critical region. *LDOC1* encodes a protein containing a leucine zipper-like motif and has been shown to be downregulated in some cancers. *SPANX-C* encodes a member of the sperm protein associated with the nucleus family, expressed solely in the testis and cancerous tissue. We sought to investigate *LDOC1* and *SPANX-C* as potential candidate genes in a study population of 597 US Caucasian prostate cancer cases and 513 controls ascertained at Vanderbilt University. We screened for common polymorphisms, and selected and genotyped 19 haplotype tagging SNPs (htSNPs) within and flanking *SPANX-C* and 1 htSNP within *LDOC1*. Analyses showed no evidence of prostate cancer risk in the study population.

Radial ray and skeletal anomalies, but not aneuploidy, are associated with RECQL4 mutations in Rothmund-Thomson syndrome. *L.L. Wang, C.A. Kozinetz, R. Naeem, M. Folsom, R. Krishnamurthy, S.E. Plon* Dept Pediatrics, Baylor Col Medicine, Houston, TX.

Background: Patients with Rothmund-Thomson syndrome (RTS) have poikiloderma, skeletal anomalies, small stature, and osteosarcoma (OS). We previously showed that patients with RECQL4 mutations (Type II) are at higher risk of developing OS compared to those without mutations (Type I). Some patients with RAPADILINO and Baller-Gerold syndromes also have RECQL4 mutations and skeletal defects. These data suggest that cancer predisposition and skeletal defects in RTS may be specific to RECQL4 mutations. Therefore, we assessed the association between RECQL4 mutations and presence of skeletal defects and chromosomal instability in RTS. Methods: 24 RTS subjects enrolled in an IRB-approved study underwent physical examination and skeletal survey at our center. Blood and skin samples were sent for karyotype and FISH analyses of chromosomes 7 and 8. Genomic DNA was analyzed for RECQL4 mutations. Genotype-phenotype analysis was performed by Fishers exact test. An additional 54 subjects enrolled in a second study were evaluated for association between RECQL4 mutation status and radial ray defect or OS by chi-square analysis. Results: Cytogenetic analysis revealed abnormalities in 50 percent of subjects. There was no significant correlation between RECQL4 mutation status and cytogenetic abnormalities ($p=0.16$). Clinical and imaging evaluations revealed bone defects in 67%. Genotype-phenotype analysis demonstrated significant association between presence of RECQL4 mutation and skeletal abnormalities ($p=0.0007$). In addition, of RTS subjects ($n=78$) from both studies, 43 (55%) had RECQL4 mutations, 14 had OS, and 16 had radial ray anomalies. Genotype/phenotype analysis demonstrated significant correlation between RECQL4 mutation status and presence of OS ($p=0.0017$) and radial ray defect ($p<0.0001$). Conclusion: These data suggest a strong correlation between RECQL4 mutations and skeletal defects and OS in RTS. In contrast, RECQL4 mutation status did not correlate with cytogenetic defects. Future studies will focus on characterizing the role of RECQL4 in bone development and transformation.

A Novel Method for Integrating SNP and Microarray Data with an Application to Chronic Fatigue Syndrome. *A. Presson[1], E. Sobel[3], J. Papp[3], A. Lusis[3], S. Horvath[2,3]* 1) Dept Statistics; 2) Dept Biostatistics; 3) Dept Human Genetics, Univ California, Los Angeles, Los Angeles, CA.

Because complex diseases involve many small-effect genes, it is easier to identify them when aided by both genetic marker and expression data. We present a novel systems biology approach that integrates SNP, microarray, and trait data to identify complex disease genes. This method relies on a weighted gene co-expression network which is constructed from Pearson correlations in mRNA levels. Using methods from network analysis, we define gene connectivity and gene modules, i.e. groups of highly interconnected genes. We use gene ontology software to characterize the biological pathway(s) composing these modules. Relating the modules to clinical data narrows the disease gene search to the most relevant module. We then correlate selected SNPs with the module to identify the SNPs that have the most influence on our module. We show that the gene co-expression model is robust to data preprocessing, and we identify several associative genes according to the following selection criteria. (1) We attempt to choose genes that belong to a disease related pathway by requiring membership to the module that is related to the clinical trait. (2) To select for genes most likely to play an essential role in the pathway, we require them to have high gene network connectivity. (3) To identify genes that are possibly under genetic control and are therefore potentially causal for the trait, we require that they have significant association with the influential SNPs. (4) Finally, we check that these genes have some direct correlation with the trait. As an illustration, we apply our methods to a real data set collected by the CDC for studying chronic fatigue syndrome (CFS) and report results from simulation studies.

Evidence that PAM is a candidate gene for autism. *S.L. Santangelo[1, 2], S. Haddad[2], T.M. Santos[2], J. Fagerness[2], I. Moilanen[3,5], M.L. Mattila[3,5], K. Jussila[3,5], S. Kuusikko[3,5], H. Ebeling[3,5], J. Ignatius[4,5], L.H. Yamaki[2], P. Moorjani[2], D.L. Pauls[1,2], V. Ramesh[1,2]* 1) Harvard Medical School, Boston, MA, USA; 2) Psychiatric & Neurodevelopmental Genetics Unit, Center for Human Genetic Research, Mass General Hosp, Boston, MA, USA; 3) University of Oulu, Department of Child Psychiatry, Oulu, Finland; 4) University of Oulu, Laboratory of Genetics, Oulu, Finland; 5) University Hospital of Oulu, Oulu, Finland.

The well known co-occurrence of autism spectrum disorders (ASD) and Tuberous Sclerosis Complex (TSC) may yield clues to the etiology of autism. We recently identified *Pam* (a Protein Associated with the Myc oncogene) as an interactor of the tuberin-hamartin protein complex in mammalian CNS. Tuberin and hamartin function together to inhibit mammalian target of rapamycin (mTOR) signaling and cause TSC. The multiple functional domains of *Pam*, its highest expression in the CNS, and the role of *Pam* homologs in synapse development make it a strong functional candidate gene for autism. The gene for *Pam* (a.k.a. MYCBP2) maps to chromosome 13q22, spans ~ 280kb, and contains 84 exons. In a sample of 156 ASD parent-offspring trios, we genotyped 70 htSNPs (MAF > 5%, $r^2 > 0.8$), 58 of which remained after excluding those with errors. Genotype success rate was > 90%. All affecteds were diagnosed via the Autism Diagnostic Interview and/or Autism Diagnostic Observation Schedule.

Using Haploview, (http://www.broad.mit.edu/mpg/haploview/), we identified a nominally significant (p=0.006) 5-SNP haplotype in block 1. We then used the sliding window approach in *whap* (http://pngu.mgh.harvard.edu/~purcell/whap/) to examine all non-overlapping 5-SNP haplotype blocks, which yielded a primary omnibus permutation test p-value of 0.02 for the first block. Individual analyses of the first 5 SNPs returned a global permutation p-value of 0.02 (corrected for multiple testing) and revealed the significance of the fourth SNP (likelihood ratio test = 8.09, primary omnibus permutation p-value = 0.007, on 10,000 permutations). We are currently carrying out a replication study in several hundred families from the AGRE repository.

Genetic mutations on acid -glucosidase gene of Brazilian Pompe patients according to the Pompe Registry. *A.A.P. Santa Rosa[1], S.K.N. Marie[2], J.C. Llerena[3], C.R. Berditchevsky[4], H. Pimentel[5], C.D. Marrone[6]* 1) Ministério da Saúde, Hospital Geral de Bonsucesso, Rio de Janeiro, Brazil; 2) Depto. Neurologia, Universidade de São Paulo, Brazil; 3) Ctro. Genética Médica, IFF/FIOCRUZ, Brazil; 4) Hospital dos Servidores do Estado, Rio de Janeiro, Brazil; 5) APAE Salvador, Brazil; 6) Hospital São Lucas, PUCRS, Brazil.

INTRODUCTION: Pompe disease or glycogen storage disease type II (OMIM#232300) is a progressive, autosomal recessive and often fatal muscle disorder caused by deficiency of lysosomal acid -glucosidase. As a rare and highly heterogeneous condition, knowledge of its natural history requires a broad effort of many physicians having affected patients under their care and willing to share their data with one another, which is why the International Pompe Registry has been developed. Since Brazil is an important contributor (the fifth in number of patients), knowledge of mutations affecting Brazilian Pompe population and increase our understanding about this rare disease.

METHODS: As Pompe Registry is strictly observational and participation is voluntary, physician enrollment and patient authorization are mandatory for entering data. below.

RESULTS: from September, 2004 to March, 2006 The total amount of Pompe patients is 150 and Brazil accounts for 15 (10%), being 7 (47%) infantile-onset and 8 (53%) late-onset. It is after Netherlands with 29 patients (19%), Germany and Italy with 27 each (18%), and USA with 18 (12%). Regarding genetic data however, 12 out of 15 (80%) Brazilian patients have undergone genetic analysis compared to 31 out of 135 (23%) non-Brazilians. The common IVS1-13G>T was found in 6 Brazilian late-onset patients. The other detected mutations are IVS17-18del, 1905C>A, 1655T>C, 1905C>A, 377G>A, and IVS7-2AG>GG, present in infantile-onset ones.

CONCLUSION: Despite accounting for only 10% of world Pompe population, Brazil is playing a pivotal role in writing the natural history of Pompe disease as Brazilian contribution to Pompe Registry is increasing our knowledge of the genetic basis of this condition.

Potential of a Sequential Replication Filter to Detect Disease Associated SNPs. *M.D. Ritchie, A.A. Motsinger, X. Liang, S.M. Dudek, J.L. Haines* Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

Whole genome association (WGA) using dense SNP sets is rapidly becoming the norm rather than the exception for studies of common, complex disease. The current challenge is not in the generation of these data, but in the analysis and interpretation of the results. The most common analysis of WGA case/control data is a chi-square test of association. One major criticism of such an approach is the large number of expected false positive results. We propose a solution that allows for identifying associated SNPs while minimizing the number of false positive results: the sequential replication filter. The SEquential Replication Filter (SERF) is based on the premise that a result that replicates in one or more independent datasets is more believable than a result that is generated in only one dataset. SERF merges the goals of cross-validation and sequential multiple decision procedures by allowing the number and size of the replication datasets to be determined dynamically from the overall dataset. Each replication begins with a randomly selected subset of the data and samples are added until the analysis reaches significance or the samples are exhausted. This allows the SNPs to be filtered based on the strength of the association signal. Intuitively, SNPs with strong associations will replicate more often than those with weaker associations. To test this approach, we simulated 1000 datasets with 500 cases and 500 controls and ten different single gene models with an odds ratio between 1.6 and 2.5. We applied SERF to chi-square tests of association, although it can be applied to any number of analytical methods. SERF replicates the functional SNP three or more times for all models simulated ($p<0.05$ based on permutation testing). More importantly, SNPs with nominal significance in the overall dataset (false positives) replicate less than two times for all simulations. Thus using a replication criterion can reduce the overall false positive rate below the nominal false positive rate of 5% (for an alpha of 0.05). Thus, SERF reduces the number of SNPs that must be examined in additional association, biological, or functional experiments.

Analysis of 144 of patients with schizophrenia reveals a variant at a highly conserved amino acid in the Disrupted in schizophrenia 1(DISC1) gene. *W. Song, J. Feng, S. Sommer* Dept Molecular Genetics, Beckman Research Inst, Duarte, CA.

Schizophrenia may be the most catastrophic of all psychological disorders. In a large Scottish pedigree, a balanced translocation t(1;11)(q42.1;q14.3) segregates with major mental illness, including schizophrenia and bipolar disorder. The translocation is predicted to result in the loss of the C-terminal region of the protein product of DISC1. In addition, a four nucleotide C-terminal deletion was reported in another Scottish family. To explore the frequency of DISC1 mutation in patients of schizophrenia, a total of 1,081 kilobases in cases and 601 kilobases in controls of genomic sequence of the DISC1 gene were scanned with DOVAM-S (Detection of Virtually All Mutations-SSCP), a method that detects virtually all mutations. Four missense variants were found in cases and controls without a significant excess in cases. An additional two missense variants were found only in cases. One of the missense variants occurs at a highly conserved amino acid, hinting that the sequence change will be of functional significance. This missense variant was found in one patient with schizophrenia, and not in 455 control samples. The presence at most one good candidate for a schizophrenia mutation in 144 patients suggests that DISC1 mutations are uncommon in schizophrenia. A larger case-control analysis may be warranted to better define the frequency of mutation in patients with schizophrenia.

Large Scale Association Study Genotype Data at the National Center for Biotechnology Information. *S.T. Sherry, M. Feolo, L. Phan, M. Mailman, M. Ward, A. Vinokurov, M. Kholodov, D. Hoffman, R. Dunivin, A. Kitts, A. Graef, J. Ostell* National Center for Biotechnologg Information, National Library of Medicine, National Institutes of Health, Bethesda, MD.

NCBIs database of genetic variation, dbSNP, currently contains over 12 million non-redundant sequence variations (single nucleotide polymorphisms, insertion/deletions, and short tandem repeats) and over 1 billion individual genotypes from HapMap and other large-scale genotyping activities. In addition, NIH has established a new genome-wide association study database at NCBI to assemble and redistribute comprehensive data sets from large scale association studies. These resources offer researchers rich datasets for researching problems in population structure, haplotype analysis, genetic epidemiology, and case-control or family based association studies. The database serves the community in dual roles; both as an author-driven archive and curated resource for whole genome annotation. Whole genome association data and metadata are integrated with other existing records such as genotype probe array configurations, genotype-specific intensity data, patterns of linkage disequilibrium, genes, OMIM, and the references curated by OMIM, RefSeq staff, and others. The expanding scale and complexity of dbSNP content will be discussed in terms of new features, tools for use, and mechanisms for Authorized Access.

Quantitative Detection of EGFR 18-bp deletion by proofreading genotyping real time PCR. *C.L. Zhou, L. Xiao, L.D. Liu, Y.F. Yin, L.L. Chen, K. Li* SNP Institute, Nanhua University, Hengyang, Hunan, China.

Except for single nucleotide substitutions, small deletions are leading mutation type. According to our recently recognized pattern of small insertion/deletion, small in-frame deletions are biomedical important in causing gain of function of the mutated gene, such as the cases of EGFR in lung cancer and PDGFR (KIT) in gastrointestinal stromal tumors. Detection of somatic mutation has been a technical challenge in molecular diagnostics. We have developed a proofreading genotyping mediated on/off molecular switch (Trends Biotechnol. 2005, 23:92-6) that is advantageous over conventional mutation detection methods using Taq DNA polymerase. This study used the 18 nucleotide in-frame deletion of EGFR gene as a target in method development. With a 3 phosphorothioated mutation specific primer of tcccgtcgctatcaaggaatcg and its downstream pairing primer, proofreading DNA polymerase Pfu only yielded specific product in correct size from mutated template at the annealing temperature of 50 to 60 C but no amplified product observed with wild type DNA template. A serial dilutions of mutated EGFR template in plasmid showed that the sensitivity of detecting the specific EGFR mutant is nearly to single molecule. As controls, false positives from wild template were obtained when mutation specific primer was not phosphorothioate modified or amplified by Taq polymerase. Interestingly, the false positive products by Taq polymerase was annealing temperature dependent: lower temperature amplified relatively longer products and higher annealing temperature yielded relatively shorter products. This 18 nucleotide specific mutation assay was further used in real time PCR platform to quantitatively determine the EGFR mutant. The combination of proofreading genotyping mediated on/off molecular switch and real time PCR technology has immediate application in molecular diagnostics with its reliability, sensitivity, and time and financial cost-effective. Similar assay targeting the 15 nucleotide in-frame deletion of EGFR is in testing. *This project is supported by Hunan Education Ministry #04A048 and Chinese Ministry of Education 2005, No3 #205106.

Statistical Considerations in the Analyses of Pharmacogenomic Studies. *S.L. Senneke[1], C.M. Bromley[1], D. Goldstaub[2], S. Close Kirkwood[3]* 1) BioStat Solutions, Inc., Mount Airy, MD 21771; 2) TEVA Pharmaceutical Industries Ltd., Kiryat Nordau, Netanya, Israel; 3) Eli Lilly and Company, Indianapolis, Indiana 46285.

Pharmacogenomic studies are different from traditional genetic studies in that the primary phenotype of interest is drug response, compounded by a disease background. Traditional approaches used to analyze genetic effects are not always directly applicable for pharmacogenomic studies. Multiple factors influence the statistical and clinical interpretation of pharmacogenomic studies. Careful specification of the variables both influenced by the genetic effects and confounding the interpretation is critical. One of the most important is the definition of response. How response is defined is dependent on the disease and drug under investigation In psychiatric disease the definition of response is often determined by a measurement instrument or scale that has not been linked to the underlying biological mechanism. Further complicating this is the fact that the definition of response varies with the scale. The characteristics of these measures such as the distribution of the data should inform the statistical methodology chosen. For example, typical statistical methods assume the data be normally distributed; however, if data are non-normal, methods such as repeated measures and the standard ANOVA may not apply. The increasingly widespread use of large marker data sets, such as genome wide SNP association studies, makes statistical methodology for multiple comparison adjustments, missing data handling including imputation, LD structure and haplotypes and validation (within study, across studies), critical to the success of the projects. Further confounding this is the quality of the genotype data, LD structure and imputation of missing observations. The methodology, results and interpretation need to be clearly conveyed to statisticians and non-statisticians alike. In analyses of pharmacogenomic studies, although several statistical methodologies may be applied, it is important to choose the method that makes the most sense with respect to the drug being investigated and relates to the hypothesis in question.

Polymorphisms in the SEC8L1 (EXOC4) gene encoding a component of the exocyst complex influence BMI, leptin, and insulin levels in the NHLBI Family Heart Study. *J.B. Wilk[1], J.M. Laramie[1], S. Williamson[1], C. Leiendecker-Foster[2], J.H. Eckfeldt[2], I.B. Borecki[3], M.A. Province[3], R.H. Myers[1]* 1) Department of Neurology, Boston University School of Medicine, Boston, MA; 2) Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN; 3) Division of Statistical Genomics, Washington University School of Medicine, St. Louis, MO.

Linkage to body mass index (BMI) was identified on chromosome 7q in the NHLBI Family Heart Study. Fine-mapping of the region with SNPs was performed in a subset of 730 people from 171 families exhibiting linkage. SEC8L1 is an 800kb gene residing within 660kb of the microsatellite with the peak evidence for linkage. The SEC8L1 protein translocates to the plasma membrane as a component of the exocyst complex (Exo70) where it is thought to influence the docking of GLUT4, an insulin-responsive transporter protein in adipocytes. BMI, glucose, insulin and plasma leptin measurements were studied. All traits were defined separately by sex adjusting for age and study center locations in MA, MN, NC, and UT. Thirty SNPs in the SEC8L1 gene were evaluated using family based association tests (FBAT) and generalized estimating equations (GEE). The best FBAT result was found for insulin (p=0.005), whereas GEE results were strongest for glucose (p=0.004) and BMI traits (p=0.009). Haplotype studies in FBAT identified a 3-SNP haplotype predicting BMI (p=0.002) and 5 and 7-SNP haplotypes predicting insulin levels (p=0.001). In a subset of 237 obese/overweight participants, a dichotomous outcome variable was defined based on the gender specific median of plasma leptin measurements (12.9 ng/mL for men; 36.7 ng/mL for women). This cutoff on leptin levels splits the sample into two groups with a mean BMI difference of 5 units (mean BMI 37 vs. 32 in men and 40 vs. 35 in women). Insulin levels were also different between the two groups. SNPs in SEC8L1 produced odds ratios of 3.2 (p-value=0.004) for a 16% frequent recessive genotype predicting high leptin/high BMI in the obese/overweight subset. These results suggest that polymorphisms in SEC8L1 may influence risk for obesity.

Case-control association testing with related individuals: A more powerful quasi-likelihood score test. *T. Thornton[1], MS. McPeek[2]* 1) Department of Statistics, University of California, Berkeley, Berkeley, CA; 2) Department of Statistics, University of Chicago, Chicago, IL.

Case-control studies have been extremely valuable in evaluating associations between candidate genes and complex diseases. Traditional case-control studies use unrelated subjects and compare allele or genotype frequencies of the cases and the controls at genetic markers. When affected related individuals are used in association studies, the power to detect an association is increased since affecteds with affected relatives have a higher expected frequency of the alleles that increase susceptibility for a genetic trait than do affected individuals that do not have affected relatives. When related individuals are used in a study, the correlations among the relatives must be taken into account to ensure validity of the test, and consideration of these correlations can also improve power. To test for allelic association with a binary trait, we propose a new test, the MQLS test, that is an extension of the quasi-likelihood score test of Bourgain et al. (2003) and which takes advantage of the fact that affected individuals that have affected relatives are more likely to have the predisposing variants than individuals that do not have affected relatives. One of the motivations for using the MQLS test is that for any arbitrary set of outbred individuals, the MQLS statistic has maximal non-centrality parameter in a general class of linear statistics, for all 2 allele disease models, as the effect size tends to zero. Simulations are performed to compare the type I error and power of the new test with those of competitors. We apply the allelic methods to analyze data on an alcoholism related phenotype in a sample of moderate-size outbred Caucasian pedigrees from GAW 14 data provided by the Collaborative Study on the Genetics of Alcoholism (U10AA008401). This research was supported by National Institutes of Health grant HG001645.

DISCOVERY OF HIGHER-ORDER FUNCTIONAL DOMAINS WITHIN THE HUMAN GENOME. *R. Thurman[1], W.S. Noble[2], J.A. Stamatoyannopoulos[2]* 1) Medical Genetics, University of Washington, Seattle, WA; 2) Genome Sciences, University of Washington, Seattle, WA.

It has long been thought that the human and other large genomes are organized into higher-order (i.e., greater than gene-sized) functional domains. Under the NHGRI ENCODE Project, a number of functional experimental data types including both activating and repressive histone modifications, RNA output, and DNA replication timing have been measured in a nearly continuous fashion across 1% of the human genome. We hypothesized that these data, viewed collectively, could reveal coherent higher-order features that may in turn illuminate the underlying functional architecture of the genome. To address this, we developed a novel approach combining wavelet analysis and hidden Markov models for unbiased discovery of 'domain-level' behavior in high-resolution functional genomic data. We find that higher-order patterns in histone modifications, transcription, and DNA replication timing are generally concordant and can be used to define discrete active and inactive functional domains ranging from 15-500kb in size. Active and inactive domains differ markedly from one another with respect to annotated genomic features including gene content, CpG islands, the spectrum of repetitive elements, the frequency of polymorphisms, and the density of known human disease-causing mutations. One striking feature of this domain map is the degree to which different genomic territories highlighted by integrating multiple functional data types reflects an organization that cannot be readily predicted from the distribution of non-coding evolutionary conservation. Our results collectively provide powerful new insights into the functional organization of the human genome.

Beyond the TDT: Application of new analytical methods to high density SNP data reveals association, inherited deletions, and regions of IBD on Chromosome 17 in autistic trios. *J. Stone[1], B. Merriman[1], Z. Chen[1], R.M. Cantor-Chiu[1], D.H. Geschwind[1,2], S.F. Nelson[1]* 1) Dept Human Gen, Gonda, #5554, Univ California, Los Angeles, Los Angeles, CA; 2) Dept Neurology, Univ California, Los Angeles, Los Angeles, CA.

Recently, male-specific linkage to chromosome 17 has been established (Stone et al., 2004; Cantor et al., 2005) in a large sample of ASPs with autism. To follow-up on these linkage findings, 2053 SNPs were genotyped in 219 trios to test for association of autism to these common variants within the region. Standard application of the TDT to the families contributing to the male-specific linkage peak originally highlighted 43 genes (112 SNPs, P0.05) for further study. To go beyond this, and leverage the strong sex-specific linkage, we established case and control groups consisting of affected male children from the most linked families (male-only) and parents from the least linked (female containing) families, respectively. Requiring further significance in this subdivision highlighted 11 genes, most notably MYO1D and ACCN1. These data were also analyzed for inherited deletions. Three different deletions were discovered and confirmed in three different families. Consequently, genes within these regions become prime candidate genes for contributing to autism susceptibility, including KCNJ12, MYO1D and four function-unknown genes. Affected children of unknown relationship to each other were further scanned for extended regions inherited IBD from a hypothetical unknown ancestor from whom they have inherited a susceptibility allele. Ancestral IBD sharing of long contiguous stretches of 14cM and 17cM were identified overlapping the KCNJ12 gene. Another region of apparent IBD was identified spanning 11cM, and smaller subcomponents of this very long inferred haplotype were enriched in autistic individuals relative to a control population, further highlighting the MYO1D locus. These additional observations provide supporting evidence that MYO1D and KCNJ12 may have both common and rare autism susceptibility alleles.

Premature Graying of hair: study of two large families with autosomal dominant inheritance. *P.Y. Hasmukhlal*
Nuclear Medicine Department, Dubai Hospital, Dubai, United Arab Emirates.

One of the most recognizable early signs of aging is gradual development of gray hair. It is caused by the gradual decrease of pigmentation that occurs when melanin ceases to be produced in the hair root, and new hairs grow in without pigment. The change naturally occurs as people age, usually turning hair from its natural color to gray, then to white. Usually, majority of persons have some gray hair through their 40 years of age. Premature Graying of hair (MIM 139100) or whiteness of the hair is called as a condition that if grey hairs appear as early as the teens and twenties for some, even in childhood. There are families with autosomal dominant (J. Hered. 20: 31-32, 1929) premature whitening of hair and association between known disorders, such as Book syndrome, Waardenburg syndrome; Lison syndrome and pernicious anemia have been reported. Conditions such as tobacco smoking may cause premature graying (BMJ ; 313:1616, 1996). We have studied two large autosomal dominant families of Asian origin with premature graying of hair. The pedigrees consist of 115 individuals with 20 affected males and 25 affected females. The age of onset ranged from 8-14 years and the degree of graying was variable ranged from 20-50% and in some it was over 50% at 25 years of their age? Male to male transmission was seen in both the families and skipping of a generation was observed in one pedigree. Eyebrows of all affected were normal and no other anomalies were present in these pedigrees. The present study indicates a monogenic mode of inheritance with complete expression. Systematic genome-wide linkage analyses, using larger families that show autosomal dominant may reveal chromosomal loci for the phenotype. E-mail: yogen4@yahoo.com.

Respecting patient confidentiality and professional disclosure while avoiding potential patient harm. *J.S. Wilbur, J.L. Kent, J.S. Gass, E.K. Brown, R.D. Legare* Cancer Risk Assessment and Prevention Program, Program in Womens Oncology, Women & Infants Hospital, Brown Medical School, Providence, RI.

One benefit of genetic testing in BRCA gene positive families is the ability to greatly reduce cancer risk in non-carriers. We present a case of a woman who requested that her BRCA test result, if negative, not be disclosed to her breast surgeon in apprehension that she would not be able to continue annual breast MRI screening. This case presents a challenging ethical and medical dilemma in regards to the attempt to avoid potential patient harm while remaining cognizant of professional disclosure and patient confidentiality. The proband is a 48 year old woman who was referred to our high risk clinic for predictive BRCA gene testing as her sister was found positive for the BRCA2 deleterious gene mutation, 4075delGT. The patient was apprehensive about disclosing a negative BRCA test to her surgeon as she would no longer be considered a candidate for breast MRI screening and her insurance could deny coverage for this procedure. According to the ASHG consensus statement, in order to override patient confidentiality, the harm from failing to disclose should outweigh the harm from disclosure, thus, one could argue that the patient may actually suffer more from disclosure if denied MRI because a cancer may be detected at a later stage, or the patient may suffer from heightened anxiety. However, because the surgeon felt bound to adhere to contemporary guidelines for breast cancer screening, she would not recommend MRI if a negative BRCA2 test resulted. Therefore, the patient chose not to proceed with BRCA testing through our institution. This case brings to light the issues of patient confidentiality and professional disclosure, as well as the need to assess the effectiveness of screening breast MRI for average risk women. However, only with technologic advancement will breast MRI costs decrease and therefore become a more realistic option for screening but until that time, and with the increasing uptake of cancer genetic testing, healthcare practitioners should be aware of the heightened potential for ethically and medically challenging cases within a high risk clinic setting.

Discrepancy of karyotype and phenotype in monochorionic twins. *J. Pappas[1], K. Daley[1], A. Roman[2]* 1) Human Gnetics Program, New York Univ, Sch Medicine, New York, NY; 2) Department of Obstetrics nad Gynecology, New York Univ, Sch Medicine, New York, NY.

We present two sets of unrelated monochorionic diamniotic female twins with phenotypic and karyotypic discrepancy. The first set of twins were product of in vitro fertilization. Two embryos were transferred and implanted and one spontaneously aborted. This suggested that the twins were monozygotic. Amniocentesis was performed and twin A had normal chromosomes 46,XX. Twin B was not sampled because of the high probability of monozygosity. Midtrimester prenatal sonography did not reveal fetal abnormalities. At 15 months twin B had generalized hypotonia, strabismus, downslanting eyes, elongated face, narrow high arched palate, submucosal cleft, posteriorly rotated ears with a history of bilateral myringotomies, prominent grooves of the philtrum, thick eyebrows and pectus excavatum. Twin A was developmentally normal and was not dysmorphic. Peripheral blood chromosome studies from twin B revealed mosaicism of a small ring chromosome 47,XX,+r[8]/46,XX[12]. Spectral karyotyping (SKY) and centromeric FISH probe studies revealed that the supernumerary ring chromosome originated from chromosome 1. Parental karyotypes were normal. More than 100 cells from twin A were reviewed utilizing the amniotic fluid cytogenetic slides and there was no evidence of mosaicism. The second set of twins presented prenatally with discrepant karyotypes in CVS and amniocentesis. The karyotype for twin B was 47,XX+13 with consistent midtrimester ultrasonographic abnormalities. Twin A had 46,XX chromosomes and normal midtrimester ultrasonographic phenotype. Zygosity studies utilizing DNA markers revealed that the twins were monozygotic. The pregnancy was terminated and pathology confirmed the prenatal findings. A few cases of monochorionic twins with discrepant karyotypes were reported in the medical literature (P. F. Colm et al, 2004, A. Nieuwint et al, 1999). Our cases demonstrate that monochorionic/monozygotic twins can have karyotypic and phenotypic discrepancies even when prenatal sonography is unremarkable. This report supports a recommendation for sampling both monochorionic twins for prenatal testing.

Two new novel point mutations localized upstream and downstream of the HMG box region of the SRY gene in three Indian 46,XY females with sex reversal and gonadal tumour formation. *M. Shahid, V.S. Dhillion, M. Raish, A. Ahmad, N.J. Khan, Rahimunnisha, S. A. Husain* Department of Biosciences, Jamia Millia Islamia,, New Delhi, New Delhi, India.

The Y chromosome-specific gene SRY is one of the key genes involved in human sex determination. The SRY gene encodes a testis-specific transcription factor that plays a key role in sexual differentiation and development in males and is located on the distal region of the short arm of the Y chromosome. Mutations in SRY gene result in XY sex reversal and pure gonadal dysgenesis. SRY expression initiates a network of gene activity that transforms the undifferentiated gonad, genital ridge into testis. Mutations in the SRY gene have been considered to account for only 10-15% of 46,XY gonadal dysgenesis cases, whereas the majority of the remaining cases may have mutation(s) in the SRY regulatory elements or other genes involved in the sex differentiation pathway. Patients both with gonadal dysgenesis and Y-chromosome presence are at high risk of developing gonadoblastoma. Using PCR, single strand conformational polymorphism (SSCP) and automated DNA sequencing, we analysed the mutations in the SRY gene in three 46,XY sex reversal patients. Two patients demonstrated nucleotide substitution (A G) within the open reading frame just outside and upstream of the conserved DNA-binding motif called the high-mobility group (HMG) box, replacing glutamine at codon 57 with arginine. Altered SSCP patterns were also observed in these patients. Histological examination of gonads in patient 1 revealed the formation of gonadoblastoma. Patient 3 demonstrated A T substitution which replaces serine at codon 143 with cysteine, just outside but downstream of the HMG box. Results suggest the involvement of SRY gene in sex reversal which further supports the relationship between SRY alterations, gonadal dysgenesis and/or primary infertility.

The rare nonsynonymous SCN5A-S1103Y variant in Caucasians is due to recent African Admixture as revealed by 100k SNP genotyping. *A. Pfeufer[1,2], M. Akyol[1,2], M. Sinner[1,2], S. Jalilzadeh[1,2], A. Rauch[3], A. Reis[3], T. Illig[4], H.E. Wichmann[4], M. Hinterseer[5], S. Kääb[5], T. Meitinger[1,2]* 1) Institute of Human Genetics, TU Munich and GSF Research Center, Munich, Bavaria; 2) Institute of Human Genetics, GSF National Research Center, D-85764 Neuherberg; 3) Institute of Human Genetics, University of Erlangen, D-91054 Erlangen; 4) Institute of Epidemiology, GSF National Research Center, D-85764 Neuherberg; 5) Department of Medicine I, Klinikum Grosshadern, University of Munich, D-81366 Munich.

The SCN5A-S1103Y variant is an established and confirmed risk factor conferring an odds ratio up to 8.5 for cardiac ventricular arrhythmias and sudden cardiac death (Splawski et al, Science, 2002, Burke et al., Circulation, 2005, Plant et al., J. Clin. Invest. 2006). In Africans it is a common nonsynonymous SNP (MAF=8%), but it is rarely observed in Caucasians (Chen et al, J. Med. Genet. 2002). In a Bavarian family appearing of entirely Caucasian descent and affected with long QT Syndrome we have detected this variant in heterozygote state as the only causal nonsynonymous variation upon diagnostic ion channel resequencing. To resolve the question, whether in the family the variant was (a) of ancient African descent, (b) due to recent African admixture or (c) a de novo mutation, we analyzed the genetic segment it resided on. Dense SNP genotyping in admixed individuals allows to infer the ethnicity of chromosomal regions if allele frequencies are known in the original populations. Ethnicity inference for any given locus can be carried out by applying the product rule to a sliding window of neighboring SNPs or via modeling ancestry by hidden Markov Chain Monte Carlo Methods (Tang et al. Am. J. Hum. Genet, 2006). By 100k SNP genotyping of the Bavarian family, we demonstate that the S1103 variant is due to recent African admixture (b) and could rule out possibilities (a) and (c). This application demonstrates that inferring ethnicity of chromosomal regions by high density SNP genotyping is a powerful approach with prospects also to admixture mapping of disease loci and population stratification correction of genomewide association mapping of complex disease loci.

Japanese public attitudes toward blood donation for genomic research. *K. Muto[1, 2], I. Ishiyama[3], A. Nagai[3], A. Tamakoshi[4], M. Kokado[5], K. Mimura[6], Z. Yamagata[3]* 1) Department of Health Sciences, Shinshu University, Japan; 2) Institute of Medical Science, University of Tokyo, Japan; 3) Department of Health Sciences, Interdisciplinary Graduate School of Medicine and Enginering, University of Yamanashi, Japan; 4) Department of Clinical Research Management, National Hospital for Geriatric Medicine, Japan; 5) Center for Life Science and Society, Japan; 6) School of Interdisciplinary Gender Studies, Ochanomizu University, Japan.

Objectives: While large-scale and long-term human genomic studies were launched in Japan, its not clear public attitudes toward blood donations for research. The purpose of our study is to examine the validity of the deficit model (Ziman 1991) on genome sciences in Japan on the ground that the willingness of blood donation for genome research would be one of the indicators of public understanding of genome science. Participants and methods: We used a dataset compiled from a postal questionnaire survey in 2005, which was mailed to 4,000 Japanese adults who were sampled randomly from the whole population. Results: A total of 2,171 completed the questionnaire (991 men and 1,180 women; 18-60 years old). The response rate was 54.3%. 69.6% of all respondents agree to promote healthcare research. On the other hand, those who expressed willingness blood donations are much fewer (39.1%). Educational backgrounds on genetics slightly affect their willingness to donate. By estimating the multi-nominal logit model, it's indicated if all respondents had obtained the full scientific literacy score, the want to donate group would increase up to 78%(from current 39.1%). This group would require scientists to disclose analyzed data of themselves (85%) and safe environment for donation (72.4%) in return for donation. Discussions: These data shows that the deficit model has still some power to explain in case of Japanese citizens. However, genomic research doesn't always return direct benefit to each participant though some may donate without any scientific interests. We need further discussions and research to analyze what determines the attitudes toward blood donation.

Introduction and overview: Local versus federal regulations regarding genetic studies in humans. *M.L. Marazita*
Center for Craniofacial and Dental Genetics, University of Pittsburgh, Pittsburgh, PA.

Session Descriptions:
For many human genetics researchers, the current regulatory climate is a source of increasing concern, depending on the specific policies of their local Institutional Review Boards (IRB). In this session, speakers and panelists will address several aspects of this complex topic, such as: Is genetic research inherently different from other research on human beings? What about genetic research on children? How does federal policy become implemented at the local IRB, with specific reference to genetic risk and benefit estimates? Are there consent issues specific to large-scale genomic studies with open-access databases? Do regulatory bodies have a more constricted view of risk than research participants, and is this appropriate? A final question and answer session will explore avenues by which human geneticists can work more effectively with their regulatory counterparts to enable genetic research on human subjects, while protecting them from exploitation or unnecessary risk.

Consent issues for large-scale studies where data access is increasingly open. *F.S. Collins* NHGRI/NIH, Bethesda, MD.

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For many human genetics researchers, the current regulatory climate is a source of increasing concern, depending on the specific policies of their local Institutional Review Boards (IRB). In this session, speakers and panelists will address several aspects of this complex topic, such as: Is genetic research inherently different from other research on human beings? What about genetic research on children? How does federal policy become implemented at the local IRB, with specific reference to genetic risk and benefit estimates? Are there consent issues specific to large-scale genomic studies with open-access databases? Do regulatory bodies have a more constricted view of risk than research participants, and is this appropriate? A final question and answer session will explore avenues by which human geneticists can work more effectively with their regulatory counterparts to enable genetic research on human subjects, while protecting them from exploitation or unnecessary risk.

From subjects to participants: At the crux of benefit and risk. *S.F. Terry* CEO, The Genetic Alliance, Washington, DC.

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Policies and Practices of Genetics and Genomics Research. *S.B. Haga* Institute for Genome Sciences and Policy, Duke University, Durham, NC.

Session Descriptions:
For many human genetics researchers, the current regulatory climate is a source of increasing concern, depending on the specific policies of their local Institutional Review Boards (IRB). In this session, speakers and panelists will address several aspects of this complex topic, such as: Is genetic research inherently different from other research on human beings? What about genetic research on children? How does federal policy become implemented at the local IRB, with specific reference to genetic risk and benefit estimates? Are there consent issues specific to large-scale genomic studies with open-access databases? Do regulatory bodies have a more constricted view of risk than research participants, and is this appropriate? A final question and answer session will explore avenues by which human geneticists can work more effectively with their regulatory counterparts to enable genetic research on human subjects, while protecting them from exploitation or unnecessary risk.

Pharmacogenetics: Current progress and future prospects. *D.B. Goldstein* Institute for Genome Sciences and Policy, Duke University, Durham, NC.

Session Descriptions:
Pharmacogenetics, the study of the genetic basis for differences in drug response, promises the tailoring of therapeutic regimes to specific patients genomic profiles, an advance widely anticipated to revolutionize both drug evaluation and clinical practice. While the advantages of using genetic information to increase drug efficiency and safety are clear, many important questions remain about the ways in which genetic variation relevant to drug response will be characterized, how such characterizations will direct the stratification of clinical trial participants in an effort to expedite drug development, and whether these choices will promote or impede the broad availability of desired therapeutic interventions. This session will examine the complex nexus of basic science, translational advance, and clinical application relevant to these challenging questions with the aim of identifying the specific social and ethical implications of current practice in this rapidly developing area.

The role of pharmacogenomics in drug development: Economic and regulatory considerations. *A.M. Issa* Univ of Houston and The Methodist Hospital, Houston, TX.

Session Descriptions:
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Ethical and social implications of genetically targeted therapeutics. *S.S.-J. Lee* Center for Biomedical Ethics, Stanford University Medical School, Palo Alto, CA.

Session Descriptions:
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Going beyond nature versus nurture: Advances and challenges in studying genetic and environmental factors together in association studies. *M.J. Khoury* Centers for Disease Control and Prevention, Atlanta, GA.

Session Descriptions:
Many birth defects appear to be complex, resulting from a combination of risk factors. Even for cases where defects are largely determined by exposures, the genetic background of the infant, mother or both may confer increased susceptibility to environmental effects. Therefore, examining exposures without considering genetic background might obscure the causative role of an exposure. Likewise, studying genetic factors alone might not show associations. Thus, genetic and environmental factors must be considered simultaneously. This session will present techniques, advances and challenges in the identification of gene-environment interactions contributing to birth defects. The session will review the study of gene-environment interactions, discussing accomplishments and limitations using traditional epidemiological study designs and how future studies and alternative designs may address these issues. Specific examples of birth defects studies combining environmental and genetic factors will be presented, as well as methods for identifying novel gene-environment interactions involved in birth defects using animal models.

Maternal smoking, genetic variation of Glutathione S-Transferases, and risk for orofacial clefts. *E.J. Lammer*
Children's Hospital Research Institute, Oakland, CA.

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Many birth defects appear to be complex, resulting from a combination of risk factors. Even for cases where defects are largely determined by exposures, the genetic background of the infant, mother or both may confer increased susceptibility to environmental effects. Therefore, examining exposures without considering genetic background might obscure the causative role of an exposure. Likewise, studying genetic factors alone might not show associations. Thus, genetic and environmental factors must be considered simultaneously. This session will present techniques, advances and challenges in the identification of gene-environment interactions contributing to birth defects. The session will review the study of gene-environment interactions, discussing accomplishments and limitations using traditional epidemiological study designs and how future studies and alternative designs may address these issues. Specific examples of birth defects studies combining environmental and genetic factors will be presented, as well as methods for identifying novel gene-environment interactions involved in birth defects using animal models.

You are what you eat: The importance of gene-nutrient interactions in the etiology of birth defects. *R.H. Finnell*
Institute of Biosciences & Technology, Houston, TX.

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Many birth defects appear to be complex, resulting from a combination of risk factors. Even for cases where defects are largely determined by exposures, the genetic background of the infant, mother or both may confer increased susceptibility to environmental effects. Therefore, examining exposures without considering genetic background might obscure the causative role of an exposure. Likewise, studying genetic factors alone might not show associations. Thus, genetic and environmental factors must be considered simultaneously. This session will present techniques, advances and challenges in the identification of gene-environment interactions contributing to birth defects. The session will review the study of gene-environment interactions, discussing accomplishments and limitations using traditional epidemiological study designs and how future studies and alternative designs may address these issues. Specific examples of birth defects studies combining environmental and genetic factors will be presented, as well as methods for identifying novel gene-environment interactions involved in birth defects using animal models.

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Of Mice and Men: Using mouse models to study gene-environment interactions in birth defects. *K. Sulik* Bowles Center for Alcohol Studies, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Session Descriptions:
Many birth defects appear to be complex, resulting from a combination of risk factors. Even for cases where defects are largely determined by exposures, the genetic background of the infant, mother or both may confer increased susceptibility to environmental effects. Therefore, examining exposures without considering genetic background might obscure the causative role of an exposure. Likewise, studying genetic factors alone might not show associations. Thus, genetic and environmental factors must be considered simultaneously. This session will present techniques, advances and challenges in the identification of gene-environment interactions contributing to birth defects. The session will review the study of gene-environment interactions, discussing accomplishments and limitations using traditional epidemiological study designs and how future studies and alternative designs may address these issues. Specific examples of birth defects studies combining environmental and genetic factors will be presented, as well as methods for identifying novel gene-environment interactions involved in birth defects using animal models.

Population genetics in common diseases. *K. Stefansson* Decode Genetics, Reykjavik, Iceland.

Session Descriptions:
Special populations had discrete founders whose descendants experienced genetic isolation as the result of geography, religion, or cultural practice. In turn, these populations accumulated prevalent founder mutations for Mendelian conditions with extended intervals of linkage disequilibrium (LD). The LD has been used for identifying founder mutations for diseases and coalescence times for these mutations. Once identified, these founder mutations have been used for studies of prevalence and penetrance and, in turn, for the creation of genetic programs for prevention or early identification of disease. Among the special populations that have been studied are Icelandics, Sardinians, Amish and Mennonites, and Jews. Over the last 5 to 10 years, several common diseases have been extensively studied in these relatively isolated populations. This approach has come of age and has yielded several genes for common diseases such as schizophrenia, stroke, hyperlipidemia, nephrolithiasis, and myocardial infarction.

The Sardinian genetic park of Ogliastra: An ideal isolated population for complex traits. *M. Pirastu* National Research Council of Italy and Shardna Lifesciences, Località Piscinamanna, 09010 Pula (CA), Italy.

Session Descriptions:
Special populations had discrete founders whose descendants experienced genetic isolation as the result of geography, religion, or cultural practice. In turn, these populations accumulated prevalent founder mutations for Mendelian conditions with extended intervals of linkage disequilibrium (LD). The LD has been used for identifying founder mutations for diseases and coalescence times for these mutations. Once identified, these founder mutations have been used for studies of prevalence and penetrance and, in turn, for the creation of genetic programs for prevention or early identification of disease. Among the special populations that have been studied are Icelandics, Sardinians, Amish and Mennonites, and Jews. Over the last 5 to 10 years, several common diseases have been extensively studied in these relatively isolated populations. This approach has come of age and has yielded several genes for common diseases such as schizophrenia, stroke, hyperlipidemia, nephrolithiasis, and myocardial infarction.

Strategies for gene identification, characterization of molecular lesions, and delivery of efficient testing among Amish and Mennonite populations. *E. Puffenberger* Clinic for Special Children, Strasburg, PA.

Session Descriptions:
Special populations had discrete founders whose descendants experienced genetic isolation as the result of geography, religion, or cultural practice. In turn, these populations accumulated prevalent founder mutations for Mendelian conditions with extended intervals of linkage disequilibrium (LD). The LD has been used for identifying founder mutations for diseases and coalescence times for these mutations. Once identified, these founder mutations have been used for studies of prevalence and penetrance and, in turn, for the creation of genetic programs for prevention or early identification of disease. Among the special populations that have been studied are Icelandics, Sardinians, Amish and Mennonites, and Jews. Over the last 5 to 10 years, several common diseases have been extensively studied in these relatively isolated populations. This approach has come of age and has yielded several genes for common diseases such as schizophrenia, stroke, hyperlipidemia, nephrolithiasis, and myocardial infarction.

The 4000-year genetic history of Jewish populations. *H. Ostrer* Human Genetics Program, NYU School of Medicine, New York, NY.

Session Descriptions:
Special populations had discrete founders whose descendants experienced genetic isolation as the result of geography, religion, or cultural practice. In turn, these populations accumulated prevalent founder mutations for Mendelian conditions with extended intervals of linkage disequilibrium (LD). The LD has been used for identifying founder mutations for diseases and coalescence times for these mutations. Once identified, these founder mutations have been used for studies of prevalence and penetrance and, in turn, for the creation of genetic programs for prevention or early identification of disease. Among the special populations that have been studied are Icelandics, Sardinians, Amish and Mennonites, and Jews. Over the last 5 to 10 years, several common diseases have been extensively studied in these relatively isolated populations. This approach has come of age and has yielded several genes for common diseases such as schizophrenia, stroke, hyperlipidemia, nephrolithiasis, and myocardial infarction.

The ENCODE project: functional annotation of one percent of the human genome. *A. Reymond* Center for Integrative Genomics, Lausanne, Switzerland.

Session Descriptions:
One of the biggest problems in the post-genome era is the interpretation of the nature of variation, nucleotide or large scale, in the human genome. Although we have some idea of how to interpret variation within coding sequences, the majority of functional DNA is non-coding and variation within noncoding sequences is difficult to interpret. In addition, many aspects of coding variation are still unknown (e.g., positive selection). In this session the speakers will present their research efforts in interpreting the function and phenotypic impact of such variation in both coding and noncoding regions and how this relates to the identification of disease variants. These efforts will have a high impact on the understanding of phenotype-genotype associations and the pathophysiology of genetic disorders.

Functional and regulatory variation in the ENCODE regions. *E.T. Dermitzakis* Population & Comparative Genomics, Wellcome Trust Genome Campus, Hinxton Cambridge, United Kingdom.

Session Descriptions:
One of the biggest problems in the post-genome era is the interpretation of the nature of variation, nucleotide or large scale, in the human genome. Although we have some idea of how to interpret variation within coding sequences, the majority of functional DNA is non-coding and variation within noncoding sequences is difficult to interpret. In addition, many aspects of coding variation are still unknown (e.g., positive selection). In this session the speakers will present their research efforts in interpreting the function and phenotypic impact of such variation in both coding and noncoding regions and how this relates to the identification of disease variants. These efforts will have a high impact on the understanding of phenotype-genotype associations and the pathophysiology of genetic disorders.

Inference of functional variation in coding and noncoding DNA. *A.G. Clark* Dept. of Molecular Biology & Genetics, Cornell University, Ithaca, NY.

Session Descriptions:
One of the biggest problems in the post-genome era is the interpretation of the nature of variation, nucleotide or large scale, in the human genome. Although we have some idea of how to interpret variation within coding sequences, the majority of functional DNA is non-coding and variation within noncoding sequences is difficult to interpret. In addition, many aspects of coding variation are still unknown (e.g., positive selection). In this session the speakers will present their research efforts in interpreting the function and phenotypic impact of such variation in both coding and noncoding regions and how this relates to the identification of disease variants. These efforts will have a high impact on the understanding of phenotype-genotype associations and the pathophysiology of genetic disorders.

Structural variation and disease in the human genome. *E. Eichler* Genome Sci, HSB K336B, University of Washington, Seattle, WA.

Session Descriptions:
One of the biggest problems in the post-genome era is the interpretation of the nature of variation, nucleotide or large scale, in the human genome. Although we have some idea of how to interpret variation within coding sequences, the majority of functional DNA is non-coding and variation within noncoding sequences is difficult to interpret. In addition, many aspects of coding variation are still unknown (e.g., positive selection). In this session the speakers will present their research efforts in interpreting the function and phenotypic impact of such variation in both coding and noncoding regions and how this relates to the identification of disease variants. These efforts will have a high impact on the understanding of phenotype-genotype associations and the pathophysiology of genetic disorders.

What can genetics say (or not say) about complex traits? *D. Goldstein* Duke University, 2006 Durham, NC.

Session Descriptions:
Behavioral genetics raises a number of issues due to the complexity of human behavior. Understanding what genetic tools can say about such complex and socially-charged traits as intelligence, sexuality, or aggression is an important consideration. Researchers may see the scientific value in and validity of such studies. How these findings are interpreted and used by the public and societal institutions, however, are other important considerations. This session will identify some of the most significant ethical, legal, social, and policy issues raised by anticipated research on behavioral genetics. The role of geneticists in incorporating consideration of these issues into the conduct of research will be discussed. What is the responsibility of researchers who study the genetics of behavior to deal with the potential positive and negative consequences of their work? How, if at all, should ethical, legal, social, and policy considerations affect study design, data analysis, and reporting results?

How should ethical, legal, and social considerations affect study design, analysis, and reporting? *L. Baker*
University of Southern California, Los Angeles, CA.

Session Descriptions:
Behavioral genetics raises a number of issues due to the complexity of human behavior. Understanding what genetic tools can say about such complex and socially-charged traits as intelligence, sexuality, or aggression is an important consideration. Researchers may see the scientific value in and validity of such studies. How these findings are interpreted and used by the public and societal institutions, however, are other important considerations. This session will identify some of the most significant ethical, legal, social, and policy issues raised by anticipated research on behavioral genetics. The role of geneticists in incorporating consideration of these issues into the conduct of research will be discussed. What is the responsibility of researchers who study the genetics of behavior to deal with the potential positive and negative consequences of their work? How, if at all, should ethical, legal, social, and policy considerations affect study design, data analysis, and reporting results?

What are the ethical, legal, social, and policy issues to consider? *T. Simoncelli* ACLU, NY, NY.

Session Descriptions:
Behavioral genetics raises a number of issues due to the complexity of human behavior. Understanding what genetic tools can say about such complex and socially-charged traits as intelligence, sexuality, or aggression is an important consideration. Researchers may see the scientific value in and validity of such studies. How these findings are interpreted and used by the public and societal institutions, however, are other important considerations. This session will identify some of the most significant ethical, legal, social, and policy issues raised by anticipated research on behavioral genetics. The role of geneticists in incorporating consideration of these issues into the conduct of research will be discussed. What is the responsibility of researchers who study the genetics of behavior to deal with the potential positive and negative consequences of their work? How, if at all, should ethical, legal, social, and policy considerations affect study design, data analysis, and reporting results?

Direct-to-consumer DNA Testing: The way to the future. *H. Coleman* Chairman and CEO, Genelex Corporation, Seattle, Washington.

Session Descriptions:
Some genetic testing companies are beginning to sell tests directly to consumers, eliminating the need to go to a doctors office. Test results usually are made available online, in the privacy of ones own home. Will at home genetic tests provide useful genetic information in a private, nonthreatening way? Or will these tests pose real risks to health and take advantage of people desperately seeking answers? Panelists will share their diverse perspectives and explore the commercial, legal, medical and ethical issues raised by direct-to-consumer (DTC) marketing of genetic tests. In addition, the panel and audience will consider what position, if any, the ASHG should take regarding DTC genetic testing.

At-home fetal gender DNA testing: Caveat emptor. *D. Bianchi* Professor of Pediatrics, Obstetrics, and Gynecology; Tufts New England Medical Center, Boston, MA.

Session Descriptions:
Some genetic testing companies are beginning to sell tests directly to consumers, eliminating the need to go to a doctors office. Test results usually are made available online, in the privacy of ones own home. Will at home genetic tests provide useful genetic information in a private, nonthreatening way? Or will these tests pose real risks to health and take advantage of people desperately seeking answers? Panelists will share their diverse perspectives and explore the commercial, legal, medical and ethical issues raised by direct-to-consumer (DTC) marketing of genetic tests. In addition, the panel and audience will consider what position, if any, the ASHG should take regarding DTC genetic testing.

Regulatory landscape for DTC genetic testing. *G. Javitt* Genetics and Public Policy Center, Johns Hopkins University, Washington, DC.

Session Descriptions:
Some genetic testing companies are beginning to sell tests directly to consumers, eliminating the need to go to a doctors office. Test results usually are made available online, in the privacy of ones own home. Will at home genetic tests provide useful genetic information in a private, nonthreatening way? Or will these tests pose real risks to health and take advantage of people desperately seeking answers? Panelists will share their diverse perspectives and explore the commercial, legal, medical and ethical issues raised by direct-to-consumer (DTC) marketing of genetic tests. In addition, the panel and audience will consider what position, if any, the ASHG should take regarding DTC genetic testing.

Evidence-based medicine in rare genetic conditions. *R.D. Steiner* Pediatrics, OHSU, Portland, OR.

Session Descriptions:
The practice of evidence-based medicine continues to be a challenge in the field of medical genetics. In this session, the speakers will introduce the topic of evidence-based medicine in general, and will also discuss whether or not it currently applies to both rare, pediatric disorders as well as common, chronic diseases of adulthood that have a genetic component. The evaluation of genetic tests and their entry into the clinical arena will also be discussed. Approaches for the systematic collection of evidence to guide the treatment and management of genetic conditions will be presented, including the development of national and international collaborative genetics research networks for testing and evaluating new genetic tests, treatments, and management recommendations for genetic diseases.

Evidence-based medicine in common disease genetics. *M.T. Scheuner* Rand Corporation, Health Unit, Santa Monica, CA.

Session Descriptions:
The practice of evidence-based medicine continues to be a challenge in the field of medical genetics. In this session, the speakers will introduce the topic of evidence-based medicine in general, and will also discuss whether or not it currently applies to both rare, pediatric disorders as well as common, chronic diseases of adulthood that have a genetic component. The evaluation of genetic tests and their entry into the clinical arena will also be discussed. Approaches for the systematic collection of evidence to guide the treatment and management of genetic conditions will be presented, including the development of national and international collaborative genetics research networks for testing and evaluating new genetic tests, treatments, and management recommendations for genetic diseases.

Evidence-based evaluation of genetic tests. *W. Burke* Univ Washington Sch Med, Seattle, WA.

Session Descriptions:
The practice of evidence-based medicine continues to be a challenge in the field of medical genetics. In this session, the speakers will introduce the topic of evidence-based medicine in general, and will also discuss whether or not it currently applies to both rare, pediatric disorders as well as common, chronic diseases of adulthood that have a genetic component. The evaluation of genetic tests and their entry into the clinical arena will also be discussed. Approaches for the systematic collection of evidence to guide the treatment and management of genetic conditions will be presented, including the development of national and international collaborative genetics research networks for testing and evaluating new genetic tests, treatments, and management recommendations for genetic diseases.

Efforts for collecting the missing evidence base in medical genetics. *M.S. Watson* ACMG, Bethesda, MD.

Session Descriptions:
The practice of evidence-based medicine continues to be a challenge in the field of medical genetics. In this session, the speakers will introduce the topic of evidence-based medicine in general, and will also discuss whether or not it currently applies to both rare, pediatric disorders as well as common, chronic diseases of adulthood that have a genetic component. The evaluation of genetic tests and their entry into the clinical arena will also be discussed. Approaches for the systematic collection of evidence to guide the treatment and management of genetic conditions will be presented, including the development of national and international collaborative genetics research networks for testing and evaluating new genetic tests, treatments, and management recommendations for genetic diseases.

Whole genome association studies: The why and how. *A. Chakravarti* McKusick-Nathans Inst Gen Med, Baltimore, MD.

Session Descriptions:
Identifying genetic factors that influence health, disease, and response to treatment is essential to reduce the burden of disease. With the sequencing of the human genome, more efficient genotyping and sequencing technologies, and the International Haplotype Map project, we now have powerful research tools for identifying genetic variants that contribute to common diseases. Researchers can now employ whole genome association studies to uncover regions of the genome and even specific genes contributing to disease susceptibility. We have an historic opportunity to understand key aspects of the complex contributions of genetic variation to health, with major consequences for prevention, diagnosis, and treatment. This session will draw upon recent large, coordinated whole genome association studies to consider the scientific opportunities presented by such studies and the challenges they face, how to mine and analyze the data from such projects, and the opportunities they offer for devising new diagnostics and therapeutics.

Whole genome association studies: Mining and analyzing data. *D. Altshuler* Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, MA.

Session Descriptions:
Identifying genetic factors that influence health, disease, and response to treatment is essential to reduce the burden of disease. With the sequencing of the human genome, more efficient genotyping and sequencing technologies, and the International Haplotype Map project, we now have powerful research tools for identifying genetic variants that contribute to common diseases. Researchers can now employ whole genome association studies to uncover regions of the genome and even specific genes contributing to disease susceptibility. We have an historic opportunity to understand key aspects of the complex contributions of genetic variation to health, with major consequences for prevention, diagnosis, and treatment. This session will draw upon recent large, coordinated whole genome association studies to consider the scientific opportunities presented by such studies and the challenges they face, how to mine and analyze the data from such projects, and the opportunities they offer for devising new diagnostics and therapeutics.

Whole genome association studies: Therapeutic opportunities. *P. Milos* Executive Director, Molecular Profiling, Pfizer Global Research and Development, Pfizer, Inc., Groton, CT.

Session Descriptions:
Identifying genetic factors that influence health, disease, and response to treatment is essential to reduce the burden of disease. With the sequencing of the human genome, more efficient genotyping and sequencing technologies, and the International Haplotype Map project, we now have powerful research tools for identifying genetic variants that contribute to common diseases. Researchers can now employ whole genome association studies to uncover regions of the genome and even specific genes contributing to disease susceptibility. We have an historic opportunity to understand key aspects of the complex contributions of genetic variation to health, with major consequences for prevention, diagnosis, and treatment. This session will draw upon recent large, coordinated whole genome association studies to consider the scientific opportunities presented by such studies and the challenges they face, how to mine and analyze the data from such projects, and the opportunities they offer for devising new diagnostics and therapeutics.

The Type 1 Diabetes Genetics Consortium: Linkage, fine mapping of the human MHC and beyond. *S.S. Rich*
Public Health Services, Wake Forest Univ Sch of Medicine, Winston-Salem, NC.

Session Descriptions:
This session will cover the current progress being made in understanding the genetic basis of type 1 diabetes. Four presentations will provide updates on genomewide linkage studies, including an in-depth examination of the MHC, in humans (Type 1 Diabetes Genetics Consortium); results from the first major genomewide association study of type 1 diabetes in humans (Wellcome Trust Case Control Consortium); progress in resolving the genetic basis of type 1 diabetes in mouse; and the search for genes that influence risk of micro- and macrovascular complications of type 1 diabetes. Studies in type 1 diabetes genetics may serve as a model for other complex human diseases, including the coordination of international efforts to collect, characterize, and analyze samples. The use of human (linkage and association) and model system approaches and resources for performing these genetic studies may provide insights for addressing the genetic basis of other "classically complex human traits."

Whole genome association approaches to the genetic basis of type 1 diabetes: the Wellcome Trust Case Control Consortium. *J. Todd* Medical Genetics, Wellcome Trust/JDRF Diabetes Inflammation Laboratory, CB2 2XY Cambridge, United Kingdom.

Session Descriptions:
This session will cover the current progress being made in understanding the genetic basis of type 1 diabetes. Four presentations will provide updates on genomewide linkage studies, including an in-depth examination of the MHC, in humans (Type 1 Diabetes Genetics Consortium); results from the first major genomewide association study of type 1 diabetes in humans (Wellcome Trust Case Control Consortium); progress in resolving the genetic basis of type 1 diabetes in mouse; and the search for genes that influence risk of micro- and macrovascular complications of type 1 diabetes. Studies in type 1 diabetes genetics may serve as a model for other complex human diseases, including the coordination of international efforts to collect, characterize, and analyze samples. The use of human (linkage and association) and model system approaches and resources for performing these genetic studies may provide insights for addressing the genetic basis of other "classically complex human traits."

Identification and modulation of molecular genetic mechanisms leading to type 1 diabetes in the NOD mouse. *L. Wicker* Medical Genetics, Addenbrooke Hospital, Cambridge, United Kingdom.

Session Descriptions:
This session will cover the current progress being made in understanding the genetic basis of type 1 diabetes. Four presentations will provide updates on genomewide linkage studies, including an in-depth examination of the MHC, in humans (Type 1 Diabetes Genetics Consortium); results from the first major genomewide association study of type 1 diabetes in humans (Wellcome Trust Case Control Consortium); progress in resolving the genetic basis of type 1 diabetes in mouse; and the search for genes that influence risk of micro- and macrovascular complications of type 1 diabetes. Studies in type 1 diabetes genetics may serve as a model for other complex human diseases, including the coordination of international efforts to collect, characterize, and analyze samples. The use of human (linkage and association) and model system approaches and resources for performing these genetic studies may provide insights for addressing the genetic basis of other "classically complex human traits."

Genetic basis of complications of type 1 diabetes. *A. Krolewski* Genetics and Epidemiology, Joslin Diabetes Center, Boston, MA.

Session Descriptions:
This session will cover the current progress being made in understanding the genetic basis of type 1 diabetes. Four presentations will provide updates on genomewide linkage studies, including an in-depth examination of the MHC, in humans (Type 1 Diabetes Genetics Consortium); results from the first major genomewide association study of type 1 diabetes in humans (Wellcome Trust Case Control Consortium); progress in resolving the genetic basis of type 1 diabetes in mouse; and the search for genes that influence risk of micro- and macrovascular complications of type 1 diabetes. Studies in type 1 diabetes genetics may serve as a model for other complex human diseases, including the coordination of international efforts to collect, characterize, and analyze samples. The use of human (linkage and association) and model system approaches and resources for performing these genetic studies may provide insights for addressing the genetic basis of other "classically complex human traits."

Introduction. *N. Blau* Department of Pediatrics, University of Zurich, 8032 Zurich, ZH, Switzerland.

Session Descriptions:
Phenylketonuria (PKU) is a disease of temperate-zone human populations and certain mutations are surprisingly prevalent. How might one explain these features? PKU is the flagship for treatable human genetic disease. Restriction of phenylalanine intake prevents brain damage but disagreement persists on how long the diet should be continued. Adolescents and young adults tend not to comply with dietary treatment and 2/3 of pregnant women (in the USA) do not follow the diet before becoming pregnant. New approaches to treatment are of interest: 1. use of the r- AAV2/8 vector to correct hyperphenylalaninemia in a murine model; 2. treatment of murine PKU with recombinant phenylalanine ammonia lyase; 3. use of tetrahydrobiopterin (6R- BH4) to treat a relatively high proportion of patients with PAH-mutant hyperphenylalaninemia who benefit from the cofactor acting either as a chaperone-like molecule or by overcoming adverse binding kinetics.

Advances in treatment of PKU: an overview. *C.R. Scriver* Montreal Children's Hospital Research Institute, McGill University, Montreal Quebec H3H 1P3, Canada.

Session Descriptions:
Phenylketonuria (PKU) is a disease of temperate-zone human populations and certain mutations are surprisingly prevalent. How might one explain these features? PKU is the flagship for treatable human genetic disease. Restriction of phenylalanine intake prevents brain damage but disagreement persists on how long the diet should be continued. Adolescents and young adults tend not to comply with dietary treatment and 2/3 of pregnant women (in the USA) do not follow the diet before becoming pregnant. New approaches to treatment are of interest: 1. use of the r- AAV2/8 vector to correct hyperphenylalaninemia in a murine model; 2. treatment of murine PKU with recombinant phenylalanine ammonia lyase; 3. use of tetrahydrobiopterin (6R- BH4) to treat a relatively high proportion of patients with PAH-mutant hyperphenylalaninemia who benefit from the cofactor acting either as a chaperone-like molecule or by overcoming adverse binding kinetics.

Efficiency of tetrahydrobiopterin in cofactor- responsive PKU patients. *H. Levy* Department of Medicine, Children's Hospital Boston, Boston, MA.

Session Descriptions:
Phenylketonuria (PKU) is a disease of temperate-zone human populations and certain mutations are surprisingly prevalent. How might one explain these features? PKU is the flagship for treatable human genetic disease. Restriction of phenylalanine intake prevents brain damage but disagreement persists on how long the diet should be continued. Adolescents and young adults tend not to comply with dietary treatment and 2/3 of pregnant women (in the USA) do not follow the diet before becoming pregnant. New approaches to treatment are of interest: 1. use of the r- AAV2/8 vector to correct hyperphenylalaninemia in a murine model; 2. treatment of murine PKU with recombinant phenylalanine ammonia lyase; 3. use of tetrahydrobiopterin (6R- BH4) to treat a relatively high proportion of patients with PAH-mutant hyperphenylalaninemia who benefit from the cofactor acting either as a chaperone-like molecule or by overcoming adverse binding kinetics.

Mechanisms underlying responsiveness to tetrahydrobiopterin in PKU mutations. *A. Martinez* Department of Biomedicine, University of Bergen, 5009 Bergen, Norway.

Session Descriptions:

Phenylketonuria (PKU) is a disease of temperate-zone human populations and certain mutations are surprisingly prevalent. How might one explain these features? PKU is the flagship for treatable human genetic disease. Restriction of phenylalanine intake prevents brain damage but disagreement persists on how long the diet should be continued. Adolescents and young adults tend not to comply with dietary treatment and 2/3 of pregnant women (in the USA) do not follow the diet before becoming pregnant. New approaches to treatment are of interest: 1. use of the r- AAV2/8 vector to correct hyperphenylalaninemia in a murine model; 2. treatment of murine PKU with recombinant phenylalanine ammonia lyase; 3. use of tetrahydrobiopterin (6R- BH4) to treat a relatively high proportion of patients with PAH-mutant hyperphenylalaninemia who benefit from the cofactor acting either as a chaperone-like molecule or by overcoming adverse binding kinetics.

Gene and stem cell therapies for PKU. *C.O. Harding* Molecular and Medical Genetics, Oregon Health & Science University, Portland, OR.

Session Descriptions:
Phenylketonuria (PKU) is a disease of temperate-zone human populations and certain mutations are surprisingly prevalent. How might one explain these features? PKU is the flagship for treatable human genetic disease. Restriction of phenylalanine intake prevents brain damage but disagreement persists on how long the diet should be continued. Adolescents and young adults tend not to comply with dietary treatment and 2/3 of pregnant women (in the USA) do not follow the diet before becoming pregnant. New approaches to treatment are of interest: 1. use of the r- AAV2/8 vector to correct hyperphenylalaninemia in a murine model; 2. treatment of murine PKU with recombinant phenylalanine ammonia lyase; 3. use of tetrahydrobiopterin (6R- BH4) to treat a relatively high proportion of patients with PAH-mutant hyperphenylalaninemia who benefit from the cofactor acting either as a chaperone-like molecule or by overcoming adverse binding kinetics.

Large-scale mutation discovery in ion channels. *R.A. Gibbs* Department of Molecular and Human Genetics, Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX.

Session Descriptions:

The triumph of the Human Genome Project requires little embellishment. The availability of the entire euchromatic genomic sequence serves as a routine starting point for a huge multidisciplinary investigator base and has accelerated our understanding of human genome content, organization and evolution. Yet, the greatest fruits of having such an initial blueprint are still to come, as we unravel how genomic changes lead to a plethora of human diseases and to our inter-individual phenotypic diversity. Continued advances in DNA sequencing methodologies and their further cost reductions will significantly increase clinical access to such technology and exponentially increase the availability of medical sequence- based datasets. These resources will undeniably further our understanding of the genetic bases of human disease susceptibility but will also present significant computational challenges to properly interpret their biological relevance.

Genetic architecture of plasma lipoprotein levels. *M. Meyerson* Department of Pathology, Dana-Farber Cancer Institute, Dept of Medical Oncology, Boston, MA.

Session Descriptions:
The triumph of the Human Genome Project requires little embellishment. The availability of the entire euchromatic genomic sequence serves as a routine starting point for a huge multidisciplinary investigator base and has accelerated our understanding of human genome content, organization and evolution. Yet, the greatest fruits of having such an initial blueprint are still to come, as we unravel how genomic changes lead to a plethora of human diseases and to our inter-individual phenotypic diversity. Continued advances in DNA sequencing methodologies and their further cost reductions will significantly increase clinical access to such technology and exponentially increase the availability of medical sequence- based datasets. These resources will undeniably further our understanding of the genetic bases of human disease susceptibility but will also present significant computational challenges to properly interpret their biological relevance.

Gene copy number variation in cancer and other human diseases. *M. Wigler* , Cold Spring Harbor Laboratories, Cold Spring Harbor, NY.

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The triumph of the Human Genome Project requires little embellishment. The availability of the entire euchromatic genomic sequence serves as a routine starting point for a huge multidisciplinary investigator base and has accelerated our understanding of human genome content, organization and evolution. Yet, the greatest fruits of having such an initial blueprint are still to come, as we unravel how genomic changes lead to a plethora of human diseases and to our inter-individual phenotypic diversity. Continued advances in DNA sequencing methodologies and their further cost reductions will significantly increase clinical access to such technology and exponentially increase the availability of medical sequence- based datasets. These resources will undeniably further our understanding of the genetic bases of human disease susceptibility but will also present significant computational challenges to properly interpret their biological relevance.

Genomic characterization of human cancers. *R.K. Wilson* Genome Sequencing Center, Washington University School of Medicine, St Louis, MO.

Session Descriptions:

The triumph of the Human Genome Project requires little embellishment. The availability of the entire euchromatic genomic sequence serves as a routine starting point for a huge multidisciplinary investigator base and has accelerated our understanding of human genome content, organization and evolution. Yet, the greatest fruits of having such an initial blueprint are still to come, as we unravel how genomic changes lead to a plethora of human diseases and to our inter-individual phenotypic diversity. Continued advances in DNA sequencing methodologies and their further cost reductions will significantly increase clinical access to such technology and exponentially increase the availability of medical sequence- based datasets. These resources will undeniably further our understanding of the genetic bases of human disease susceptibility but will also present significant computational challenges to properly interpret their biological relevance.

Lessons from re-sequencing candidate genes. *D. Nickerson* Department of Genome Sciences, University of Washington, School of Medicine, Seattle, WA.

Session Descriptions:
The triumph of the Human Genome Project requires little embellishment. The availability of the entire euchromatic genomic sequence serves as a routine starting point for a huge multidisciplinary investigator base and has accelerated our understanding of human genome content, organization and evolution. Yet, the greatest fruits of having such an initial blueprint are still to come, as we unravel how genomic changes lead to a plethora of human diseases and to our inter-individual phenotypic diversity. Continued advances in DNA sequencing methodologies and their further cost reductions will significantly increase clinical access to such technology and exponentially increase the availability of medical sequence- based datasets. These resources will undeniably further our understanding of the genetic bases of human disease susceptibility but will also present significant computational challenges to properly interpret their biological relevance.

Animal models of developmental diaphragmatic defects: Gateway to candidate gene discovery in humans. *K.G. Ackerman* Genetics, Brigham and Women's Genetics, Boston, MA.

Session Descriptions:

Congenital diaphragmatic hernia (CDH) is an extremely common birth defect affecting ~1/2500 births and is estimated to constitute up to eight percent of all congenital malformations. The condition remains associated with a high mortality and considerable morbidity among long-term survivors. The etiology is currently unknown in most cases, but new developments from human and model organism investigations indicate a major role for genetic causation of CDH. Four talks will highlight the following: 1) diaphragm and lung embryology; basis of pulmonary hypoplasia; genetic and teratogenic model organisms; 2) prevalence of common cytogenetic abnormalities including microdeletions detected by array based CGH; suggested testing guidelines for both isolated CDH and CDH with anomalies; 3) Fryns syndrome; single gene disorders associated with CDH; differential diagnoses; 4) mutations associated with human CDH; candidate gene sequence analysis and explanation for major pathways currently being sequenced; multiplex families with CDH and linkage analysis.

Fryns syndrome and single gene disorders associated with CDH. *A.M. Slavotinek* Department of Pediatrics, U585P, Univ California, San Francisco, San Francisco, CA.

Session Descriptions:
Congenital diaphragmatic hernia (CDH) is an extremely common birth defect affecting ~1/2500 births and is estimated to constitute up to eight percent of all congenital malformations. The condition remains associated with a high mortality and considerable morbidity among long-term survivors. The etiology is currently unknown in most cases, but new developments from human and model organism investigations indicate a major role for genetic causation of CDH. Four talks will highlight the following: 1) diaphragm and lung embryology; basis of pulmonary hypoplasia; genetic and teratogenic model organisms; 2) prevalence of common cytogenetic abnormalities including microdeletions detected by array based CGH; suggested testing guidelines for both isolated CDH and CDH with anomalies; 3) Fryns syndrome; single gene disorders associated with CDH; differential diagnoses; 4) mutations associated with human CDH; candidate gene sequence analysis and explanation for major pathways currently being sequenced; multiplex families with CDH and linkage analysis.

Findings from cytogenetics and array-based comparative genomic hybridization: Methods to identify loci associated with CDH. *K. Klein* Department of clinical Genetics, Erasmus MC, Rotterdam, The Netherlands.

Session Descriptions:
Congenital diaphragmatic hernia (CDH) is an extremely common birth defect affecting ~1/2500 births and is estimated to constitute up to eight percent of all congenital malformations. The condition remains associated with a high mortality and considerable morbidity among long-term survivors. The etiology is currently unknown in most cases, but new developments from human and model organism investigations indicate a major role for genetic causation of CDH. Four talks will highlight the following: 1) diaphragm and lung embryology; basis of pulmonary hypoplasia; genetic and teratogenic model organisms; 2) prevalence of common cytogenetic abnormalities including microdeletions detected by array based CGH; suggested testing guidelines for both isolated CDH and CDH with anomalies; 3) Fryns syndrome; single gene disorders associated with CDH; differential diagnoses; 4) mutations associated with human CDH; candidate gene sequence analysis and explanation for major pathways currently being sequenced; multiplex families with CDH and linkage analysis.

Genetic causation of CDH in human populations: Evidence from family studies and candidate gene analyses. *B.R. Pober* Pediatrics, Mass General Hospital for Children, Boston, MA.

Session Descriptions:
Congenital diaphragmatic hernia (CDH) is an extremely common birth defect affecting ~1/2500 births and is estimated to constitute up to eight percent of all congenital malformations. The condition remains associated with a high mortality and considerable morbidity among long-term survivors. The etiology is currently unknown in most cases, but new developments from human and model organism investigations indicate a major role for genetic causation of CDH. Four talks will highlight the following: 1) diaphragm and lung embryology; basis of pulmonary hypoplasia; genetic and teratogenic model organisms; 2) prevalence of common cytogenetic abnormalities including microdeletions detected by array based CGH; suggested testing guidelines for both isolated CDH and CDH with anomalies; 3) Fryns syndrome; single gene disorders associated with CDH; differential diagnoses; 4) mutations associated with human CDH; candidate gene sequence analysis and explanation for major pathways currently being sequenced; multiplex families with CDH and linkage analysis.

Dynamic mutations and RNA-mediated disease. *M. Swanson* Molec Gen & Microbiology, Univ Florida Col Medicine, Gainesville, FL.

Session Descriptions:
The most widely accepted view on mechanisms leading to neuronal cell death in neurodegenerative disease is that the main culprits are proteins abnormally modified as the result of mutations, aberrant processing or post-translational modifications. However, over the past few years, new evidence has emerged for the involvement of RNA-mediated mechanisms in a number of degenerative pathologies of the central nervous system. The main objective of this session is to review the most recent advances on the groundbreaking concept of RNA neurotoxicity, and to provide a thought-provoking forum from which working models with a wide implication for human diseases will emerge. This session will particularly focus on DM1, DM2, SCA8, and FXTAS, in which the toxicity of noncoding RNAs has been demonstrated in different animal and cell models.

Insights into molecular pathogenesis through comparative studies of DM1, DM2 and SCA8. *L. Ranum* Institute of Human Genetics, University of Minnesota, Minneapolis, MN.

Session Descriptions:
The most widely accepted view on mechanisms leading to neuronal cell death in neurodegenerative disease is that the main culprits are proteins abnormally modified as the result of mutations, aberrant processing or post-translational modifications. However, over the past few years, new evidence has emerged for the involvement of RNA-mediated mechanisms in a number of degenerative pathologies of the central nervous system. The main objective of this session is to review the most recent advances on the groundbreaking concept of RNA neurotoxicity, and to provide a thought-provoking forum from which working models with a wide implication for human diseases will emerge. This session will particularly focus on DM1, DM2, SCA8, and FXTAS, in which the toxicity of noncoding RNAs has been demonstrated in different animal and cell models.

Molecular pathogenesis of FXTAS. *P.J. Hagerman* Biochemistry & Molecular Medicine, University of California Sch Med, Davis, Davis, CA.

Session Descriptions:
The most widely accepted view on mechanisms leading to neuronal cell death in neurodegenerative disease is that the main culprits are proteins abnormally modified as the result of mutations, aberrant processing or post-translational modifications. However, over the past few years, new evidence has emerged for the involvement of RNA-mediated mechanisms in a number of degenerative pathologies of the central nervous system. The main objective of this session is to review the most recent advances on the groundbreaking concept of RNA neurotoxicity, and to provide a thought-provoking forum from which working models with a wide implication for human diseases will emerge. This session will particularly focus on DM1, DM2, SCA8, and FXTAS, in which the toxicity of noncoding RNAs has been demonstrated in different animal and cell models.

From fruit fly to bedside: translating lessons from a Drosophila model of FXTAS. *P. Jin* Human Genetics, Emory University SOM, Atlanta, GA.

Session Descriptions:
The most widely accepted view on mechanisms leading to neuronal cell death in neurodegenerative disease is that the main culprits are proteins abnormally modified as the result of mutations, aberrant processing or post-translational modifications. However, over the past few years, new evidence has emerged for the involvement of RNA-mediated mechanisms in a number of degenerative pathologies of the central nervous system. The main objective of this session is to review the most recent advances on the groundbreaking concept of RNA neurotoxicity, and to provide a thought-provoking forum from which working models with a wide implication for human diseases will emerge. This session will particularly focus on DM1, DM2, SCA8, and FXTAS, in which the toxicity of noncoding RNAs has been demonstrated in different animal and cell models.

Introduction to DNA damage response genes and their role in preventing human cancer. *M. Meyn* Dept Genetics, Hospital for Sick Children, Toronto, Ontario.

Session Descriptions:
The DNA damage response (DDR) protects us against the harmful effects of DNA and chromosomal breakage. The impact of loss of these defenses extends beyond the rare situations in which loss or altered function of the ataxia telangiectasia gene, ATM and its downstream targets in the DDR (e.g., BRCA, FANC genes) cause familial cancer syndromes and in some cases, chromosomal breakage syndromes. This session will review the DDR pathways and the chromosomal instability syndromes, the impact of DDR gene mutations and polymorphisms on cancer susceptibility in carriers, mouse models for various genes in the DDR and their value in understanding the DDR pathways and cancer susceptibility, interindividual variation in DNA repair capacity and cancer susceptibility, recent research findings on the cellular response to DNA breaks, and pertinent clinical and genetic counseling issues. Genome instability and cancer susceptibility is an important emerging area of cancer genetics and public health genetics.

Mouse models of genome instability syndromes. *L. Niedernhofer* University of Pittsburgh Cancer Institute, Hillman Cancer Center, 2.6, Pittsburgh, PA.

Session Descriptions:
The DNA damage response (DDR) protects us against the harmful effects of DNA and chromosomal breakage. The impact of loss of these defenses extends beyond the rare situations in which loss or altered function of the ataxia telangiectasia gene, ATM and its downstream targets in the DDR (e.g., BRCA, FANC genes) cause familial cancer syndromes and in some cases, chromosomal breakage syndromes. This session will review the DDR pathways and the chromosomal instability syndromes, the impact of DDR gene mutations and polymorphisms on cancer susceptibility in carriers, mouse models for various genes in the DDR and their value in understanding the DDR pathways and cancer susceptibility, interindividual variation in DNA repair capacity and cancer susceptibility, recent research findings on the cellular response to DNA breaks, and pertinent clinical and genetic counseling issues. Genome instability and cancer susceptibility is an important emerging area of cancer genetics and public health genetics.

The role of DNA repair capacity in genetic susceptibility to cancer. *X. Wu* Dept of Epidemiology, UT MD Anderson Cancer Center, Houston, TX.

Session Descriptions:

The DNA damage response (DDR) protects us against the harmful effects of DNA and chromosomal breakage. The impact of loss of these defenses extends beyond the rare situations in which loss or altered function of the ataxia telangiectasia gene, ATM and its downstream targets in the DDR (e.g., BRCA, FANC genes) cause familial cancer syndromes and in some cases, chromosomal breakage syndromes. This session will review the DDR pathways and the chromosomal instability syndromes, the impact of DDR gene mutations and polymorphisms on cancer susceptibility in carriers, mouse models for various genes in the DDR and their value in understanding the DDR pathways and cancer susceptibility, interindividual variation in DNA repair capacity and cancer susceptibility, recent research findings on the cellular response to DNA breaks, and pertinent clinical and genetic counseling issues. Genome instability and cancer susceptibility is an important emerging area of cancer genetics and public health genetics.

Visualizing the DNA damage response (DDR): How DDR and telomeric proteins protect against genetic damage.
M. Meyn Dept. Genetics, Hospital for Sick Children, Toronto, Ontario, Canada.

Session Descriptions:
The DNA damage response (DDR) protects us against the harmful effects of DNA and chromosomal breakage. The impact of loss of these defenses extends beyond the rare situations in which loss or altered function of the ataxia telangiectasia gene, ATM and its downstream targets in the DDR (e.g., BRCA, FANC genes) cause familial cancer syndromes and in some cases, chromosomal breakage syndromes. This session will review the DDR pathways and the chromosomal instability syndromes, the impact of DDR gene mutations and polymorphisms on cancer susceptibility in carriers, mouse models for various genes in the DDR and their value in understanding the DDR pathways and cancer susceptibility, interindividual variation in DNA repair capacity and cancer susceptibility, recent research findings on the cellular response to DNA breaks, and pertinent clinical and genetic counseling issues. Genome instability and cancer susceptibility is an important emerging area of cancer genetics and public health genetics.

Genetic counseling and public health implications of defects in the DNA damage response. *J. Peters* NCI/NIH, Clinical Genetics Br, DCEG, Rockville, MD.

Session Descriptions:
The DNA damage response (DDR) protects us against the harmful effects of DNA and chromosomal breakage. The impact of loss of these defenses extends beyond the rare situations in which loss or altered function of the ataxia telangiectasia gene, ATM and its downstream targets in the DDR (e.g., BRCA, FANC genes) cause familial cancer syndromes and in some cases, chromosomal breakage syndromes. This session will review the DDR pathways and the chromosomal instability syndromes, the impact of DDR gene mutations and polymorphisms on cancer susceptibility in carriers, mouse models for various genes in the DDR and their value in understanding the DDR pathways and cancer susceptibility, interindividual variation in DNA repair capacity and cancer susceptibility, recent research findings on the cellular response to DNA breaks, and pertinent clinical and genetic counseling issues. Genome instability and cancer susceptibility is an important emerging area of cancer genetics and public health genetics.

Human meiotic aneuploidy: Where weve been, where were going. *T. Hassold* School of Molecular Bioscience, Washington State University, Pullman, WA.

Session Descriptions:
In the human genetics community, we frequently take a narrow view of aneuploidy, thinking of it as due to meiotic nondisjunction and leading to either miscarriage or birth defects. Clearly, this is an important component of aneuploidy and, as part of this session, we will update recent studies of nondisjunction in humans and mammalian models. However, a variety of studies suggest that we need to expand our thinking of both the causes and consequences of aneuploidy. Accordingly, we will discuss emerging evidence that aneuploidy is an important driver for cancer and will also focus on aneuploid cells in neurons, providing data that we are all mosaics for chromosome abnormalities and that aneuploidy may be necessary for functioning neurons. Finally, we will summarize recent data indicating that exposure to endrocrine disruptors affects meiotic synapsis, recombination and chromosome segregation in mice, suggesting that the environment is an important contributor to aneuploidy.

Aneuploidy and cancer. *D. Cleveland* Neuroscience & Molecular Medicine, University of California at San Diego, La Jolla, CA.

Session Descriptions:
In the human genetics community, we frequently take a narrow view of aneuploidy, thinking of it as due to meiotic nondisjunction and leading to either miscarriage or birth defects. Clearly, this is an important component of aneuploidy and, as part of this session, we will update recent studies of nondisjunction in humans and mammalian models. However, a variety of studies suggest that we need to expand our thinking of both the causes and consequences of aneuploidy. Accordingly, we will discuss emerging evidence that aneuploidy is an important driver for cancer and will also focus on aneuploid cells in neurons, providing data that we are all mosaics for chromosome abnormalities and that aneuploidy may be necessary for functioning neurons. Finally, we will summarize recent data indicating that exposure to endrocrine disruptors affects meiotic synapsis, recombination and chromosome segregation in mice, suggesting that the environment is an important contributor to aneuploidy.

Naturally occurring aneuploidy in the brain. *J. Chun* Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA.

Session Descriptions:
In the human genetics community, we frequently take a narrow view of aneuploidy, thinking of it as due to meiotic nondisjunction and leading to either miscarriage or birth defects. Clearly, this is an important component of aneuploidy and, as part of this session, we will update recent studies of nondisjunction in humans and mammalian models. However, a variety of studies suggest that we need to expand our thinking of both the causes and consequences of aneuploidy. Accordingly, we will discuss emerging evidence that aneuploidy is an important driver for cancer and will also focus on aneuploid cells in neurons, providing data that we are all mosaics for chromosome abnormalities and that aneuploidy may be necessary for functioning neurons. Finally, we will summarize recent data indicating that exposure to endrocrine disruptors affects meiotic synapsis, recombination and chromosome segregation in mice, suggesting that the environment is an important contributor to aneuploidy.

Aneuploidy and the environment: are we making things worse? *P. Hunt* School of Molecular Bioscience, Washington State University, Pullman, WA.

Session Descriptions:

In the human genetics community, we frequently take a narrow view of aneuploidy, thinking of it as due to meiotic nondisjunction and leading to either miscarriage or birth defects. Clearly, this is an important component of aneuploidy and, as part of this session, we will update recent studies of nondisjunction in humans and mammalian models. However, a variety of studies suggest that we need to expand our thinking of both the causes and consequences of aneuploidy. Accordingly, we will discuss emerging evidence that aneuploidy is an important driver for cancer and will also focus on aneuploid cells in neurons, providing data that we are all mosaics for chromosome abnormalities and that aneuploidy may be necessary for functioning neurons. Finally, we will summarize recent data indicating that exposure to endrocrine disruptors affects meiotic synapsis, recombination and chromosome segregation in mice, suggesting that the environment is an important contributor to aneuploidy.

Cancer as a model for understanding complex trait epigenetics. *A.P. Feinberg* Molecular Biology/Genetics, Johns Hopkins Univ School of Medicine, Baltimore, MD.

Session Descriptions:
While it is well established that DNA variation contributes to complex disease, epigenetic variations, i.e., nonsequence changes associated with DNA and heritable during somatic cell division, are emerging as an additional source of diversity contributing to disease risk. In addition to cancer, in which the role of epigenetics is well established, epigenetic variation is associated with aging and environmental perturbation including diet, and it may both mediate and promote genetic alterations in somatic cells. The purpose of this session is to provide an introduction to this new frontier, focusing on examples of cancer as a model of the epigenetics of complex traits, strategies for high throughput epigenome analysis, the challenges of the resulting large quantitative data sets and their incorporation into disease risk modeling, and the epigenome as a target for environmental perturbation and aging.

Incorporating epigenetic hypotheses into genetic epidemiology. *M. Fallin* Dept of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.

Session Descriptions:
While it is well established that DNA variation contributes to complex disease, epigenetic variations, i.e., nonsequence changes associated with DNA and heritable during somatic cell division, are emerging as an additional source of diversity contributing to disease risk. In addition to cancer, in which the role of epigenetics is well established, epigenetic variation is associated with aging and environmental perturbation including diet, and it may both mediate and promote genetic alterations in somatic cells. The purpose of this session is to provide an introduction to this new frontier, focusing on examples of cancer as a model of the epigenetics of complex traits, strategies for high throughput epigenome analysis, the challenges of the resulting large quantitative data sets and their incorporation into disease risk modeling, and the epigenome as a target for environmental perturbation and aging.

Advances in epigenome technology and implications for the study of aging. *M. Esteller* Cancer Epigenetics Branch, Spanish National Cancer Center, 28029 Madrid, Spain.

Session Descriptions:
While it is well established that DNA variation contributes to complex disease, epigenetic variations, i.e., nonsequence changes associated with DNA and heritable during somatic cell division, are emerging as an additional source of diversity contributing to disease risk. In addition to cancer, in which the role of epigenetics is well established, epigenetic variation is associated with aging and environmental perturbation including diet, and it may both mediate and promote genetic alterations in somatic cells. The purpose of this session is to provide an introduction to this new frontier, focusing on examples of cancer as a model of the epigenetics of complex traits, strategies for high throughput epigenome analysis, the challenges of the resulting large quantitative data sets and their incorporation into disease risk modeling, and the epigenome as a target for environmental perturbation and aging.

Genomewide approaches to analysis of cytosine methylation. *J. Greally* Department of Molecular Genetics, Albert Einstein College of Medicine, Bronx, NY.

Session Descriptions:
While it is well established that DNA variation contributes to complex disease, epigenetic variations, i.e., nonsequence changes associated with DNA and heritable during somatic cell division, are emerging as an additional source of diversity contributing to disease risk. In addition to cancer, in which the role of epigenetics is well established, epigenetic variation is associated with aging and environmental perturbation including diet, and it may both mediate and promote genetic alterations in somatic cells. The purpose of this session is to provide an introduction to this new frontier, focusing on examples of cancer as a model of the epigenetics of complex traits, strategies for high throughput epigenome analysis, the challenges of the resulting large quantitative data sets and their incorporation into disease risk modeling, and the epigenome as a target for environmental perturbation and aging.

Environmental modification of the epigenome. *R. Jirtle* Dept. of Radiation Oncology, Duke University School of Medicine, Durham, NC.

Session Descriptions:
While it is well established that DNA variation contributes to complex disease, epigenetic variations, i.e., nonsequence changes associated with DNA and heritable during somatic cell division, are emerging as an additional source of diversity contributing to disease risk. In addition to cancer, in which the role of epigenetics is well established, epigenetic variation is associated with aging and environmental perturbation including diet, and it may both mediate and promote genetic alterations in somatic cells. The purpose of this session is to provide an introduction to this new frontier, focusing on examples of cancer as a model of the epigenetics of complex traits, strategies for high throughput epigenome analysis, the challenges of the resulting large quantitative data sets and their incorporation into disease risk modeling, and the epigenome as a target for environmental perturbation and aging.

Parathyroid tumor genetics. *A.J. Arnold* Murray-Heilig Chair in Molecular Medicine, University of Connecticut Health Center, Farmington, CT.

Session Descriptions:

New genetic and molecular information clarifies thyroid and parathyroid tumors, chromaffin tumors, multiple endocrine neoplasia types 1 and 2, and Carney Complex. There are important implications in counseling, in normal signal transduction pathways, and in tumorigenesis. An endocrine neoplasia syndrome can express striking metabolic features from hormone excess. Traditionally, these features have defined carriers and have given tools to monitor tumor burden. Other morbid features are from benign and malignant neoplasia of endocrine and sometimes non-endocrine tissues. Focus on the phenotypes from hormone excess in these syndromes has sped discoveries about genetics and tumorigenesis. Not only does mutation testing in the germline benefit rare families, but mutation testing in somatic tissues benefits management of cases with similar common variety tumors. Some of the disturbed molecular pathways are already promising for interventions against neoplasia in vitro, and some are already under exploration in clinical trials.

Genomic medicine practice: Lessons learned from pheochromocytoma. *C. Eng* Chairman and Director, Genomic Medicine Institute, Cleveland Clinic Lerner Research Institute, Cleveland, OH.

Session Descriptions:
New genetic and molecular information clarifies thyroid and parathyroid tumors, chromaffin tumors, multiple endocrine neoplasia types 1 and 2, and Carney Complex. There are important implications in counseling, in normal signal transduction pathways, and in tumorigenesis. An endocrine neoplasia syndrome can express striking metabolic features from hormone excess. Traditionally, these features have defined carriers and have given tools to monitor tumor burden. Other morbid features are from benign and malignant neoplasia of endocrine and sometimes non-endocrine tissues. Focus on the phenotypes from hormone excess in these syndromes has sped discoveries about genetics and tumorigenesis. Not only does mutation testing in the germline benefit rare families, but mutation testing in somatic tissues benefits management of cases with similar common variety tumors. Some of the disturbed molecular pathways are already promising for interventions against neoplasia in vitro, and some are already under exploration in clinical trials.

MEN1 Gene function and molecular diagnosis. *A.E. Bale* Yale University, School of Medicine, New Haven, CT.

Session Descriptions:
New genetic and molecular information clarifies thyroid and parathyroid tumors, chromaffin tumors, multiple endocrine neoplasia types 1 and 2, and Carney Complex. There are important implications in counseling, in normal signal transduction pathways, and in tumorigenesis. An endocrine neoplasia syndrome can express striking metabolic features from hormone excess. Traditionally, these features have defined carriers and have given tools to monitor tumor burden. Other morbid features are from benign and malignant neoplasia of endocrine and sometimes non-endocrine tissues. Focus on the phenotypes from hormone excess in these syndromes has sped discoveries about genetics and tumorigenesis. Not only does mutation testing in the germline benefit rare families, but mutation testing in somatic tissues benefits management of cases with similar common variety tumors. Some of the disturbed molecular pathways are already promising for interventions against neoplasia in vitro, and some are already under exploration in clinical trials.

c-AMP and protein kinase A signaling in endocrine and other tumors. *C.A. Stratakis* Chief, Heritable Disorders Branch, 10 Center Drive, Bethesda, MD.

Session Descriptions:

New genetic and molecular information clarifies thyroid and parathyroid tumors, chromaffin tumors, multiple endocrine neoplasia types 1 and 2, and Carney Complex. There are important implications in counseling, in normal signal transduction pathways, and in tumorigenesis. An endocrine neoplasia syndrome can express striking metabolic features from hormone excess. Traditionally, these features have defined carriers and have given tools to monitor tumor burden. Other morbid features are from benign and malignant neoplasia of endocrine and sometimes non-endocrine tissues. Focus on the phenotypes from hormone excess in these syndromes has sped discoveries about genetics and tumorigenesis. Not only does mutation testing in the germline benefit rare families, but mutation testing in somatic tissues benefits management of cases with similar common variety tumors. Some of the disturbed molecular pathways are already promising for interventions against neoplasia in vitro, and some are already under exploration in clinical trials.

How thyroid cancers start: Non-overlapping mutations of multiple MAPK genes. *J.A. Fagin* Vontz Center for Molecular Sciences, University of Cincinatti, Cincinatti, OH, USA.

Session Descriptions:
New genetic and molecular information clarifies thyroid and parathyroid tumors, chromaffin tumors, multiple endocrine neoplasia types 1 and 2, and Carney Complex. There are important implications in counseling, in normal signal transduction pathways, and in tumorigenesis. An endocrine neoplasia syndrome can express striking metabolic features from hormone excess. Traditionally, these features have defined carriers and have given tools to monitor tumor burden. Other morbid features are from benign and malignant neoplasia of endocrine and sometimes non-endocrine tissues. Focus on the phenotypes from hormone excess in these syndromes has sped discoveries about genetics and tumorigenesis. Not only does mutation testing in the germline benefit rare families, but mutation testing in somatic tissues benefits management of cases with similar common variety tumors. Some of the disturbed molecular pathways are already promising for interventions against neoplasia in vitro, and some are already under exploration in clinical trials.

Molecular principles of human telomerase function and dysfunction in disease. *K. Collins* Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA.

Session Descriptions:

In order to distinguish a normal telomere from a double strand break, a minimum number of telomere repeats must cap each chromosome end. The length of each repeat array will reflect a unique history of addition and losses. Telomere losses are known to occur sporadically as well as with every replication cycle. Losses of telomeric DNA are countered by the telomerase enzyme containing telomerase RNA (encoded by the TERC gene) and a reverse transcriptase protein (encoded by TERT gene) as minimal components. Telomerase activity is limiting in most somatic (stem) cells and the average length of telomere repeats in most somatic cells shows a highly significant decline with age. In this session basic aspects of telomere and telomerase biology will be reviewed together with recent observations indicating that haplo-insufficiency for either the TERC or TERT gene can give rise to disease including autosomal dominant dyskeratosis congenita (DKC) and aplastic anemia.

Dyskeratosis congenita: a disease of defective telomere maintenance. *I. Dokal* Department of Haematology, Imperial College London, London, UK.

Session Descriptions:

In order to distinguish a normal telomere from a double strand break, a minimum number of telomere repeats must cap each chromosome end. The length of each repeat array will reflect a unique history of addition and losses. Telomere losses are known to occur sporadically as well as with every replication cycle. Losses of telomeric DNA are countered by the telomerase enzyme containing telomerase RNA (encoded by the TERC gene) and a reverse transcriptase protein (encoded by TERT gene) as minimal components. Telomerase activity is limiting in most somatic (stem) cells and the average length of telomere repeats in most somatic cells shows a highly significant decline with age. In this session basic aspects of telomere and telomerase biology will be reviewed together with recent observations indicating that haplo-insufficiency for either the TERC or TERT gene can give rise to disease including autosomal dominant dyskeratosis congenita (DKC) and aplastic anemia.

Mechanisms of telomere loss. *P.M. Lansdorp* Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada.

Session Descriptions:

In order to distinguish a normal telomere from a double strand break, a minimum number of telomere repeats must cap each chromosome end. The length of each repeat array will reflect a unique history of addition and losses. Telomere losses are known to occur sporadically as well as with every replication cycle. Losses of telomeric DNA are countered by the telomerase enzyme containing telomerase RNA (encoded by the TERC gene) and a reverse transcriptase protein (encoded by TERT gene) as minimal components. Telomerase activity is limiting in most somatic (stem) cells and the average length of telomere repeats in most somatic cells shows a highly significant decline with age. In this session basic aspects of telomere and telomerase biology will be reviewed together with recent observations indicating that haplo-insufficiency for either the TERC or TERT gene can give rise to disease including autosomal dominant dyskeratosis congenita (DKC) and aplastic anemia.

Pathophysiology and treatment of bone marrow failure resulting from mutations in telomerase genes. *N. Young*
NHLBI, National Institutes of Health, Bethesda, MD.

Session Descriptions:
In order to distinguish a normal telomere from a double strand break, a minimum number of telomere repeats must cap each chromosome end. The length of each repeat array will reflect a unique history of addition and losses. Telomere losses are known to occur sporadically as well as with every replication cycle. Losses of telomeric DNA are countered by the telomerase enzyme containing telomerase RNA (encoded by the TERC gene) and a reverse transcriptase protein (encoded by TERT gene) as minimal components. Telomerase activity is limiting in most somatic (stem) cells and the average length of telomere repeats in most somatic cells shows a highly significant decline with age. In this session basic aspects of telomere and telomerase biology will be reviewed together with recent observations indicating that haplo-insufficiency for either the TERC or TERT gene can give rise to disease including autosomal dominant dyskeratosis congenita (DKC) and aplastic anemia.

Pharmacogenetics: Lessons learned and the "Blockbuster" model. *D. Goldstein* Institute for Genome Sciences & Policy (IGSP), Duke University, CIEMAS DUMC Box 3382, Durham, NC.

Session Descriptions:
Traditional models of drug discovery, particularly the "blockbuster" model, are widely believed to be in crisis. For some diseases, few new medicines are in the pipeline and there are persistent safety concerns. The genetic personalization of treatment may help, but the current research base allows few firm conclusions. Recent developments suggest that this situation may be changing. In the wake of advances from large-scale genomic initiatives, the capacity for the pharmacogenetic prediction of treatment response and adverse side-effects may be improving due to a new generation of studies. Indeed, the genetics of drug response/adverse drug reactions may be more tractable phenotypes than the genetics of disease predisposition. The purpose of this invited session is to describe these developments. We provide examples of where genotype-clinical phenotype correlations are compelling and appear to be ready for clinical translation (warfarin and gefitinib) and examples that have not worked out as consistently along with the regulatory perspective of the United States FDA.

A comprehensive pharmacogenetic analysis of antipsychotic use in the CATIE schizophrenia trial. *P.F. Sullivan*
Genetics, UNC-Chapel Hill, CB#7264, Chapel Hill, NC.

Session Descriptions:
Traditional models of drug discovery, particularly the "blockbuster" model, are widely believed to be in crisis. For some diseases, few new medicines are in the pipeline and there are persistent safety concerns. The genetic personalization of treatment may help, but the current research base allows few firm conclusions. Recent developments suggest that this situation may be changing. In the wake of advances from large-scale genomic initiatives, the capacity for the pharmacogenetic prediction of treatment response and adverse side-effects may be improving due to a new generation of studies. Indeed, the genetics of drug response/adverse drug reactions may be more tractable phenotypes than the genetics of disease predisposition. The purpose of this invited session is to describe these developments. We provide examples of where genotype-clinical phenotype correlations are compelling and appear to be ready for clinical translation (warfarin and gefitinib) and examples that have not worked out as consistently along with the regulatory perspective of the United States FDA.

The pharmacogenetics of warfarin response. *M. Rieder* Dept of Genome Sciences, University of Washington, Seattle, WA.

Session Descriptions:
Traditional models of drug discovery, particularly the "blockbuster" model, are widely believed to be in crisis. For some diseases, few new medicines are in the pipeline and there are persistent safety concerns. The genetic personalization of treatment may help, but the current research base allows few firm conclusions. Recent developments suggest that this situation may be changing. In the wake of advances from large-scale genomic initiatives, the capacity for the pharmacogenetic prediction of treatment response and adverse side-effects may be improving due to a new generation of studies. Indeed, the genetics of drug response/adverse drug reactions may be more tractable phenotypes than the genetics of disease predisposition. The purpose of this invited session is to describe these developments. We provide examples of where genotype-clinical phenotype correlations are compelling and appear to be ready for clinical translation (warfarin and gefitinib) and examples that have not worked out as consistently along with the regulatory perspective of the United States FDA.

Gefitinib: The genetics of treatment Response and resistance. *D. Bell* , Center for Cancer Research, Charlestown, MA.

Session Descriptions:
Traditional models of drug discovery, particularly the "blockbuster" model, are widely believed to be in crisis. For some diseases, few new medicines are in the pipeline and there are persistent safety concerns. The genetic personalization of treatment may help, but the current research base allows few firm conclusions. Recent developments suggest that this situation may be changing. In the wake of advances from large-scale genomic initiatives, the capacity for the pharmacogenetic prediction of treatment response and adverse side-effects may be improving due to a new generation of studies. Indeed, the genetics of drug response/adverse drug reactions may be more tractable phenotypes than the genetics of disease predisposition. The purpose of this invited session is to describe these developments. We provide examples of where genotype-clinical phenotype correlations are compelling and appear to be ready for clinical translation (warfarin and gefitinib) and examples that have not worked out as consistently along with the regulatory perspective of the United States FDA.

A regulatory perspective on pharmacogenetics in drug development and clinical practice. *L. Lesko* Director, Office of Clinical Pharmacology & Biopharmaceutics, Center for Drug Evaluation & Research, FDA, Silver Spring, MD.

Session Descriptions:
Traditional models of drug discovery, particularly the "blockbuster" model, are widely believed to be in crisis. For some diseases, few new medicines are in the pipeline and there are persistent safety concerns. The genetic personalization of treatment may help, but the current research base allows few firm conclusions. Recent developments suggest that this situation may be changing. In the wake of advances from large-scale genomic initiatives, the capacity for the pharmacogenetic prediction of treatment response and adverse side-effects may be improving due to a new generation of studies. Indeed, the genetics of drug response/adverse drug reactions may be more tractable phenotypes than the genetics of disease predisposition. The purpose of this invited session is to describe these developments. We provide examples of where genotype-clinical phenotype correlations are compelling and appear to be ready for clinical translation (warfarin and gefitinib) and examples that have not worked out as consistently along with the regulatory perspective of the United States FDA.

Traffic jams: a compendium of human diseases that affect intracellular transport processes. *M. Huizing* NHGRI, NIH, Bethesda, Maryland.

Session Descriptions:
Genetic mutations that result in human disease provide insights into the complex mechanisms of protein routing within the cell. In this session, distinct cellular compartments, their protein routing, and the associated genetic disorders will be addressed. Topics include protein folding and quality control in the endoplasmic reticulum, protein glycosylation and sorting in the Golgi complex, and endosomal and caveolar protein trafficking to and from distinct organelles and the plasma membrane. Each speaker will address groups of human genetic disorders associated with each compartment and the insights that specific disorders provide into the underlying cellular mechanisms. These cellular insights are ultimately essential for a rational approach to therapies.

Disorders of protein routing through the Golgi complex. *H.H. Freeze* Glycobiology and Carbohydrate Chemistry Program, Burnham Institute for Medical Research, La Jolla, CA.

Session Descriptions:
Genetic mutations that result in human disease provide insights into the complex mechanisms of protein routing within the cell. In this session, distinct cellular compartments, their protein routing, and the associated genetic disorders will be addressed. Topics include protein folding and quality control in the endoplasmic reticulum, protein glycosylation and sorting in the Golgi complex, and endosomal and caveolar protein trafficking to and from distinct organelles and the plasma membrane. Each speaker will address groups of human genetic disorders associated with each compartment and the insights that specific disorders provide into the underlying cellular mechanisms. These cellular insights are ultimately essential for a rational approach to therapies.

RAB GTPases and endocytic trafficking in health and disease. *A. Wandinger-Ness* Deptartment of Pathology, Univ of New Mexico Health Sciences Center, Albuquerque, NM.

Session Descriptions:
Genetic mutations that result in human disease provide insights into the complex mechanisms of protein routing within the cell. In this session, distinct cellular compartments, their protein routing, and the associated genetic disorders will be addressed. Topics include protein folding and quality control in the endoplasmic reticulum, protein glycosylation and sorting in the Golgi complex, and endosomal and caveolar protein trafficking to and from distinct organelles and the plasma membrane. Each speaker will address groups of human genetic disorders associated with each compartment and the insights that specific disorders provide into the underlying cellular mechanisms. These cellular insights are ultimately essential for a rational approach to therapies.

Caveolins, caveolae and lipid rafts in cellular transport: From cell biology to medicine. *M.P. Lisanti* Departments of Medicine and Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY.

Session Descriptions:
Genetic mutations that result in human disease provide insights into the complex mechanisms of protein routing within the cell. In this session, distinct cellular compartments, their protein routing, and the associated genetic disorders will be addressed. Topics include protein folding and quality control in the endoplasmic reticulum, protein glycosylation and sorting in the Golgi complex, and endosomal and caveolar protein trafficking to and from distinct organelles and the plasma membrane. Each speaker will address groups of human genetic disorders associated with each compartment and the insights that specific disorders provide into the underlying cellular mechanisms. These cellular insights are ultimately essential for a rational approach to therapies.

Heritability in prematurity: New insights into the genetics of preterm birth. *K. Ward* Dept of OB/GYN and Women's Health, Kapi'olani Hospital, Honolulu, Hawaii.

Session Descriptions:

Preterm birth is the number one obstetrical problem today, exacting a huge toll upon individuals, families, and society. In addition to being the leading cause of neonatal mortality, prematurity contributes to lifelong morbidity for many children. The incremental rise in preterm birth in the United States continued in 2004, reaching 12.5% (almost 500,000 births), the highest rate ever reported. But, despite the lifelong impact of prematurity on health, there are few effective strategies to predict preterm birth and fewer clinical interventions to prevent preterm birth. Prematurity is increasingly appreciated to result from the interaction of environmental influences on varying genetic backgrounds, and thus it can be approached as a common complex disorder, using the tools of genetic epidemiology and applied genomic research. This session will outline applied genomic research approaches to preterm birth and propose broad implications for clinical care and public health.

Genetics to genomics: A framework for approaching preterm birth as a common complex disorder. *S. Dolan*
OB/GYN & Reproductive Genetics, March of Dimes & Albert Einstein College of Medicine, White Plains, NY.

Session Descriptions:
Preterm birth is the number one obstetrical problem today, exacting a huge toll upon individuals, families, and society. In addition to being the leading cause of neonatal mortality, prematurity contributes to lifelong morbidity for many children. The incremental rise in preterm birth in the United States continued in 2004, reaching 12.5% (almost 500,000 births), the highest rate ever reported. But, despite the lifelong impact of prematurity on health, there are few effective strategies to predict preterm birth and fewer clinical interventions to prevent preterm birth. Prematurity is increasingly appreciated to result from the interaction of environmental influences on varying genetic backgrounds, and thus it can be approached as a common complex disorder, using the tools of genetic epidemiology and applied genomic research. This session will outline applied genomic research approaches to preterm birth and propose broad implications for clinical care and public health.

Meta-analysis and knowledge integration: Implications for research on complex disease. *J.P. Ioannidis*
Department of Hygiene and Epidemiology, University of Ioannina School of Medicine, Ioannina, Greece, Greece.

Session Descriptions:
Preterm birth is the number one obstetrical problem today, exacting a huge toll upon individuals, families, and society. In addition to being the leading cause of neonatal mortality, prematurity contributes to lifelong morbidity for many children. The incremental rise in preterm birth in the United States continued in 2004, reaching 12.5% (almost 500,000 births), the highest rate ever reported. But, despite the lifelong impact of prematurity on health, there are few effective strategies to predict preterm birth and fewer clinical interventions to prevent preterm birth. Prematurity is increasingly appreciated to result from the interaction of environmental influences on varying genetic backgrounds, and thus it can be approached as a common complex disorder, using the tools of genetic epidemiology and applied genomic research. This session will outline applied genomic research approaches to preterm birth and propose broad implications for clinical care and public health.

Implications of applied genomic research for public health: What will it take to bring down the rate of preterm birth? *M. Khoury* Coordinating Center for Health Promotion, CDC Office of Genomics & Disease Prevention, Atlanta, GA.

Session Descriptions:

Preterm birth is the number one obstetrical problem today, exacting a huge toll upon individuals, families, and society. In addition to being the leading cause of neonatal mortality, prematurity contributes to lifelong morbidity for many children. The incremental rise in preterm birth in the United States continued in 2004, reaching 12.5% (almost 500,000 births), the highest rate ever reported. But, despite the lifelong impact of prematurity on health, there are few effective strategies to predict preterm birth and fewer clinical interventions to prevent preterm birth. Prematurity is increasingly appreciated to result from the interaction of environmental influences on varying genetic backgrounds, and thus it can be approached as a common complex disorder, using the tools of genetic epidemiology and applied genomic research. This session will outline applied genomic research approaches to preterm birth and propose broad implications for clinical care and public health.

Malaria pressure on the human genome: A paradigm for genomic epidemiology of common disease. *D. Kwiatkowski* Oxford University, Wellcome Trust Centre for Human Genetics, Oxford, UK.

Session Descriptions:
Human genetic variation affects the severity of infectious disease progression for many pathogens. Individual polymorphisms, and epistatic interactions between them, substantially influence the host response to infection. Immune-response genes and non-immune genes are involved, but the innate immune response is emerging as a system in which these effects are most pronounced. Quantitative immune variation, arising from promoter mutations or from Copy-Number Polymorphisms (CNPs), influences the host response to pathogens, especially the early stages of infection, and has recently been demonstrated in the defensin and cytokine systems. Advances in genome analysis now enable these phenomena to be quantified, and candidate-gene approaches are completed by techniques that can detect patterns of disease-resistance polymorphism in whole-genome SNP datasets. This session will present examples of recent work that has utilized these approaches to detect many new loci affecting resistance to pathogens such as malaria, tuberculosis, and viral diseases such as HIV and hepatitis.

Natural selection on genes influencing susceptibility to infectious disease. *M.J. Bamshad* Dept Pediatrics, University of Washington, Seattle, WA.

Session Descriptions:

Human genetic variation affects the severity of infectious disease progression for many pathogens. Individual polymorphisms, and epistatic interactions between them, substantially influence the host response to infection. Immune-response genes and non-immune genes are involved, but the innate immune response is emerging as a system in which these effects are most pronounced. Quantitative immune variation, arising from promoter mutations or from Copy-Number Polymorphisms (CNPs), influences the host response to pathogens, especially the early stages of infection, and has recently been demonstrated in the defensin and cytokine systems. Advances in genome analysis now enable these phenomena to be quantified, and candidate-gene approaches are completed by techniques that can detect patterns of disease-resistance polymorphism in whole-genome SNP datasets. This session will present examples of recent work that has utilized these approaches to detect many new loci affecting resistance to pathogens such as malaria, tuberculosis, and viral diseases such as HIV and hepatitis.

Convergent evolution and population structure in Africa: Implications for disease intervention. *S. Tishkoff* Dept Biol, Biol/Psychology Bldg, University of Maryland, College Park, MD.

Session Descriptions:

Human genetic variation affects the severity of infectious disease progression for many pathogens. Individual polymorphisms, and epistatic interactions between them, substantially influence the host response to infection. Immune-response genes and non-immune genes are involved, but the innate immune response is emerging as a system in which these effects are most pronounced. Quantitative immune variation, arising from promoter mutations or from Copy-Number Polymorphisms (CNPs), influences the host response to pathogens, especially the early stages of infection, and has recently been demonstrated in the defensin and cytokine systems. Advances in genome analysis now enable these phenomena to be quantified, and candidate-gene approaches are completed by techniques that can detect patterns of disease-resistance polymorphism in whole-genome SNP datasets. This session will present examples of recent work that has utilized these approaches to detect many new loci affecting resistance to pathogens such as malaria, tuberculosis, and viral diseases such as HIV and hepatitis.

Innate immunity genes and susceptibility to pulmonary tuberculosis. *W. Scott* , Duke Univ Medical Ctr, Durham, NC.

Session Descriptions:
Human genetic variation affects the severity of infectious disease progression for many pathogens. Individual polymorphisms, and epistatic interactions between them, substantially influence the host response to infection. Immune-response genes and non-immune genes are involved, but the innate immune response is emerging as a system in which these effects are most pronounced. Quantitative immune variation, arising from promoter mutations or from Copy-Number Polymorphisms (CNPs), influences the host response to pathogens, especially the early stages of infection, and has recently been demonstrated in the defensin and cytokine systems. Advances in genome analysis now enable these phenomena to be quantified, and candidate-gene approaches are completed by techniques that can detect patterns of disease-resistance polymorphism in whole-genome SNP datasets. This session will present examples of recent work that has utilized these approaches to detect many new loci affecting resistance to pathogens such as malaria, tuberculosis, and viral diseases such as HIV and hepatitis.

Adaptive evolution of immune and inflammatory related genes: insights from genomewide scans of positive selection. *J. Akey* Genome Sciences, University of Washington, Seattle, WA.

Session Descriptions:
Human genetic variation affects the severity of infectious disease progression for many pathogens. Individual polymorphisms, and epistatic interactions between them, substantially influence the host response to infection. Immune-response genes and non-immune genes are involved, but the innate immune response is emerging as a system in which these effects are most pronounced. Quantitative immune variation, arising from promoter mutations or from Copy-Number Polymorphisms (CNPs), influences the host response to pathogens, especially the early stages of infection, and has recently been demonstrated in the defensin and cytokine systems. Advances in genome analysis now enable these phenomena to be quantified, and candidate-gene approaches are completed by techniques that can detect patterns of disease-resistance polymorphism in whole-genome SNP datasets. This session will present examples of recent work that has utilized these approaches to detect many new loci affecting resistance to pathogens such as malaria, tuberculosis, and viral diseases such as HIV and hepatitis.

Introduction. *W. Robinson* Department of Medical Genetics, University of British Columbia, 950 W. 28th Ave, Rm 3086, Vancouver, BC V5Z 4H4, Canada.

Session Descriptions:
While it is well-established that structural and numerical chromosome abnormalities arise frequently during meiosis and early embryo development, abnormalities in chromatin structure (i.e., epigenetic alterations) may also be important in human disorders, ranging from classical imprinting syndromes to intrauterine growth restriction, obesity and diabetes. But what do we really know about how epigenetic programming can be altered in gametogenesis and early placental and embryo development? And how can we test for such abnormalities in clinical samples? This workshop is meant to "set the stage" in terms of our knowledge of normal and abnormal epigenetic programming in mammalian development and lead to a discussion of the possible medical implications of epigenetic change. The emphasis of this session will be on the types of epigenetic alterations that may occur in early development and factors influencing epigenetic change.

Epigenetic marking during gametogenesis. *L. Lefbvre* Medical Genetics, University of British Columbia, UBC Vancouver V6T 1Z3, BC, Canada.

Session Descriptions:
While it is well-established that structural and numerical chromosome abnormalities arise frequently during meiosis and early embryo development, abnormalities in chromatin structure (i.e., epigenetic alterations) may also be important in human disorders, ranging from classical imprinting syndromes to intrauterine growth restriction, obesity and diabetes. But what do we really know about how epigenetic programming can be altered in gametogenesis and early placental and embryo development? And how can we test for such abnormalities in clinical samples? This workshop is meant to "set the stage" in terms of our knowledge of normal and abnormal epigenetic programming in mammalian development and lead to a discussion of the possible medical implications of epigenetic change. The emphasis of this session will be on the types of epigenetic alterations that may occur in early development and factors influencing epigenetic change.

Epigenetic reprogramming and instability in preimplantation embryos. *T. Haaf* Institute Of Human Genetic, Johannes Gutenberg Univ Mainz, Mainz, Germany.

Session Descriptions:
While it is well-established that structural and numerical chromosome abnormalities arise frequently during meiosis and early embryo development, abnormalities in chromatin structure (i.e., epigenetic alterations) may also be important in human disorders, ranging from classical imprinting syndromes to intrauterine growth restriction, obesity and diabetes. But what do we really know about how epigenetic programming can be altered in gametogenesis and early placental and embryo development? And how can we test for such abnormalities in clinical samples? This workshop is meant to "set the stage" in terms of our knowledge of normal and abnormal epigenetic programming in mammalian development and lead to a discussion of the possible medical implications of epigenetic change. The emphasis of this session will be on the types of epigenetic alterations that may occur in early development and factors influencing epigenetic change.

Clinical implications of epigenetic change. *R. Weksberg* Division of Clin & Metabolic Gen, Hosp Sick Children, Toronto, Canada.

Session Descriptions:
While it is well-established that structural and numerical chromosome abnormalities arise frequently during meiosis and early embryo development, abnormalities in chromatin structure (i.e., epigenetic alterations) may also be important in human disorders, ranging from classical imprinting syndromes to intrauterine growth restriction, obesity and diabetes. But what do we really know about how epigenetic programming can be altered in gametogenesis and early placental and embryo development? And how can we test for such abnormalities in clinical samples? This workshop is meant to "set the stage" in terms of our knowledge of normal and abnormal epigenetic programming in mammalian development and lead to a discussion of the possible medical implications of epigenetic change. The emphasis of this session will be on the types of epigenetic alterations that may occur in early development and factors influencing epigenetic change.

Chromosome and genomewide epigenetic change: lessons from the X. *C. Brown* Department of Medical Genetics, University of British Columbia, Vancouver V6T 1Z3, BC, Canada.

Session Descriptions:
While it is well-established that structural and numerical chromosome abnormalities arise frequently during meiosis and early embryo development, abnormalities in chromatin structure (i.e., epigenetic alterations) may also be important in human disorders, ranging from classical imprinting syndromes to intrauterine growth restriction, obesity and diabetes. But what do we really know about how epigenetic programming can be altered in gametogenesis and early placental and embryo development? And how can we test for such abnormalities in clinical samples? This workshop is meant to "set the stage" in terms of our knowledge of normal and abnormal epigenetic programming in mammalian development and lead to a discussion of the possible medical implications of epigenetic change. The emphasis of this session will be on the types of epigenetic alterations that may occur in early development and factors influencing epigenetic change.

Introduction. *E. Mardis* Lawrence Berkeley National Laboratory, Berkeley, CA.

Session Descriptions:
The triumph of the Human Genome Project requires little embellishment. The availability of the entire euchromatic genomic sequence serves as a routine starting point for a huge multidisciplinary investigator base and has accelerated our understanding of human genome content, organization and evolution. Yet, the greatest fruits of having such an initial blueprint are still to come, as we unravel how genomic changes lead to a plethora of human diseases and to our inter-individual phenotypic diversity. Continued advances in DNA sequencing methodologies and their further cost reductions will significantly increase clinical access to such technology and exponentially increase the availability of medical sequence- based datasets. These resources will undeniably further our understanding of the genetic bases of human disease susceptibility but will also present significant computational challenges to properly interpret their biological relevance.

Introduction. *S. J. Marx* NIDDK, NIH, Bethesda, MD.

Session Descriptions:
New genetic and molecular information clarifies thyroid and parathyroid tumors, chromaffin tumors, multiple endocrine neoplasia types 1 and 2, and Carney Complex. There are important implications in counseling, in normal signal transduction pathways, and in tumorigenesis. An endocrine neoplasia syndrome can express striking metabolic features from hormone excess. Traditionally, these features have defined carriers and have given tools to monitor tumor burden. Other morbid features are from benign and malignant neoplasia of endocrine and sometimes non-endocrine tissues. Focus on the phenotypes from hormone excess in these syndromes has sped discoveries about genetics and tumorigenesis. Not only does mutation testing in the germline benefit rare families, but mutation testing in somatic tissues benefits management of cases with similar common variety tumors. Some of the disturbed molecular pathways are already promising for interventions against neoplasia in vitro, and some are already under exploration in clinical trials.

Introduction. *C. A. Moore* Centers for Disease Control and Prevention, Atlanta, GA.

Session Descriptions:
Preterm birth is the number one obstetrical problem today, exacting a huge toll upon individuals, families, and society. In addition to being the leading cause of neonatal mortality, prematurity contributes to lifelong morbidity for many children. The incremental rise in preterm birth in the United States continued in 2004, reaching 12.5% (almost 500,000 births), the highest rate ever reported. But, despite the lifelong impact of prematurity on health, there are few effective strategies to predict preterm birth and fewer clinical interventions to prevent preterm birth. Prematurity is increasingly appreciated to result from the interaction of environmental influences on varying genetic backgrounds, and thus it can be approached as a common complex disorder, using the tools of genetic epidemiology and applied genomic research. This session will outline applied genomic research approaches to preterm birth and propose broad implications for clinical care and public health.

Questions and answers.

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Discussion.

Session Descriptions:
While it is well established that DNA variation contributes to complex disease, epigenetic variations, i.e., nonsequence changes associated with DNA and heritable during somatic cell division, are emerging as an additional source of diversity contributing to disease risk. In addition to cancer, in which the role of epigenetics is well established, epigenetic variation is associated with aging and environmental perturbation including diet, and it may both mediate and promote genetic alterations in somatic cells. The purpose of this session is to provide an introduction to this new frontier, focusing on examples of cancer as a model of the epigenetics of complex traits, strategies for high throughput epigenome analysis, the challenges of the resulting large quantitative data sets and their incorporation into disease risk modeling, and the epigenome as a target for environmental perturbation and aging.

Introduction. *A. Feinberg* Johns Hopkins University School of Medicine, Baltimore, MD.

Session Descriptions:
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Introduction. *P. F. Sullivan* University of North Carolina, Chapel Hill.

Session Descriptions:
Traditional models of drug discovery, particularly the "blockbuster" model, are widely believed to be in crisis. For some diseases, few new medicines are in the pipeline and there are persistent safety concerns. The genetic personalization of treatment may help, but the current research base allows few firm conclusions. Recent developments suggest that this situation may be changing. In the wake of advances from large-scale genomic initiatives, the capacity for the pharmacogenetic prediction of treatment response and adverse side-effects may be improving due to a new generation of studies. Indeed, the genetics of drug response/adverse drug reactions may be more tractable phenotypes than the genetics of disease predisposition. The purpose of this invited session is to describe these developments. We provide examples of where genotype-clinical phenotype correlations are compelling and appear to be ready for clinical translation (warfarin and gefitinib) and examples that have not worked out as consistently along with the regulatory perspective of the United States FDA.

Questions and answers.

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Questions and answers.

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New genetic and molecular information clarifies thyroid and parathyroid tumors, chromaffin tumors, multiple endocrine neoplasia types 1 and 2, and Carney Complex. There are important implications in counseling, in normal signal transduction pathways, and in tumorigenesis. An endocrine neoplasia syndrome can express striking metabolic features from hormone excess. Traditionally, these features have defined carriers and have given tools to monitor tumor burden. Other morbid features are from benign and malignant neoplasia of endocrine and sometimes non-endocrine tissues. Focus on the phenotypes from hormone excess in these syndromes has sped discoveries about genetics and tumorigenesis. Not only does mutation testing in the germline benefit rare families, but mutation testing in somatic tissues benefits management of cases with similar common variety tumors. Some of the disturbed molecular pathways are already promising for interventions against neoplasia in vitro, and some are already under exploration in clinical trials.

Discussion.

Session Descriptions:
Pharmacogenetics, the study of the genetic basis for differences in drug response, promises the tailoring of therapeutic regimes to specific patients genomic profiles, an advance widely anticipated to revolutionize both drug evaluation and clinical practice. While the advantages of using genetic information to increase drug efficiency and safety are clear, many important questions remain about the ways in which genetic variation relevant to drug response will be characterized, how such characterizations will direct the stratification of clinical trial participants in an effort to expedite drug development, and whether these choices will promote or impede the broad availability of desired therapeutic interventions. This session will examine the complex nexus of basic science, translational advance, and clinical application relevant to these challenging questions with the aim of identifying the specific social and ethical implications of current practice in this rapidly developing area.

Introduction. *W. Burke* University of Washington, Seattle.

Session Descriptions:
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Introduction. *J. M. Friedman* University of British Columbia, Children's and Women's Hospital, Vancouver, British Columbia, Canada.

Session Descriptions:
Many birth defects appear to be complex, resulting from a combination of risk factors. Even for cases where defects are largely determined by exposures, the genetic background of the infant, mother or both may confer increased susceptibility to environmental effects. Therefore, examining exposures without considering genetic background might obscure the causative role of an exposure. Likewise, studying genetic factors alone might not show associations. Thus, genetic and environmental factors must be considered simultaneously. This session will present techniques, advances and challenges in the identification of gene-environment interactions contributing to birth defects. The session will review the study of gene-environment interactions, discussing accomplishments and limitations using traditional epidemiological study designs and how future studies and alternative designs may address these issues. Specific examples of birth defects studies combining environmental and genetic factors will be presented, as well as methods for identifying novel gene-environment interactions involved in birth defects using animal models.

Introduction. *J. Gulcher* deCode Genetics, Reykjavik, Iceland.

Session Descriptions:
Special populations had discrete founders whose descendants experienced genetic isolation as the result of geography, religion, or cultural practice. In turn, these populations accumulated prevalent founder mutations for Mendelian conditions with extended intervals of linkage disequilibrium (LD). The LD has been used for identifying founder mutations for diseases and coalescence times for these mutations. Once identified, these founder mutations have been used for studies of prevalence and penetrance and, in turn, for the creation of genetic programs for prevention or early identification of disease. Among the special populations that have been studied are Icelandics, Sardinians, Amish and Mennonites, and Jews. Over the last 5 to 10 years, several common diseases have been extensively studied in these relatively isolated populations. This approach has come of age and has yielded several genes for common diseases such as schizophrenia, stroke, hyperlipidemia, nephrolithiasis, and myocardial infarction.

Introduction. *K. Hudson* Genetics & Public Policy Center, Johns Hopkins University, Washington DC.

Session Descriptions:
Some genetic testing companies are beginning to sell tests directly to consumers, eliminating the need to go to a doctors office. Test results usually are made available online, in the privacy of ones own home. Will at home genetic tests provide useful genetic information in a private, nonthreatening way? Or will these tests pose real risks to health and take advantage of people desperately seeking answers? Panelists will share their diverse perspectives and explore the commercial, legal, medical and ethical issues raised by direct-to-consumer (DTC) marketing of genetic tests. In addition, the panel and audience will consider what position, if any, the ASHG should take regarding DTC genetic testing.

Panel discussion and questions and answers.

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Introduction. *M. Feldman* Stanford University, Stanford, CA.

Session Descriptions:
Behavioral genetics raises a number of issues due to the complexity of human behavior. Understanding what genetic tools can say about such complex and socially-charged traits as intelligence, sexuality, or aggression is an important consideration. Researchers may see the scientific value in and validity of such studies. How these findings are interpreted and used by the public and societal institutions, however, are other important considerations. This session will identify some of the most significant ethical, legal, social, and policy issues raised by anticipated research on behavioral genetics. The role of geneticists in incorporating consideration of these issues into the conduct of research will be discussed. What is the responsibility of researchers who study the genetics of behavior to deal with the potential positive and negative consequences of their work? How, if at all, should ethical, legal, social, and policy considerations affect study design, data analysis, and reporting results?

Discussion.

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Questions and answers.

Session Descriptions:
The practice of evidence-based medicine continues to be a challenge in the field of medical genetics. In this session, the speakers will introduce the topic of evidence-based medicine in general, and will also discuss whether or not it currently applies to both rare, pediatric disorders as well as common, chronic diseases of adulthood that have a genetic component. The evaluation of genetic tests and their entry into the clinical arena will also be discussed. Approaches for the systematic collection of evidence to guide the treatment and management of genetic conditions will be presented, including the development of national and international collaborative genetics research networks for testing and evaluating new genetic tests, treatments, and management recommendations for genetic diseases.

Introduction. *M. Boehnke* Univ of Michigan, Dept of Biostatistics, Ann Arbor MI.

Session Descriptions:
This session will cover the current progress being made in understanding the genetic basis of type 1 diabetes. Four presentations will provide updates on genomewide linkage studies, including an in-depth examination of the MHC, in humans (Type 1 Diabetes Genetics Consortium); results from the first major genomewide association study of type 1 diabetes in humans (Wellcome Trust Case Control Consortium); progress in resolving the genetic basis of type 1 diabetes in mouse; and the search for genes that influence risk of micro- and macrovascular complications of type 1 diabetes. Studies in type 1 diabetes genetics may serve as a model for other complex human diseases, including the coordination of international efforts to collect, characterize, and analyze samples. The use of human (linkage and association) and model system approaches and resources for performing these genetic studies may provide insights for addressing the genetic basis of other "classically complex human traits."

Panel discussion and questions.

Session Descriptions:
For many human genetics researchers, the current regulatory climate is a source of increasing concern, depending on the specific policies of their local Institutional Review Boards (IRB). In this session, speakers and panelists will address several aspects of this complex topic, such as: Is genetic research inherently different from other research on human beings? What about genetic research on children? How does federal policy become implemented at the local IRB, with specific reference to genetic risk and benefit estimates? Are there consent issues specific to large-scale genomic studies with open-access databases? Do regulatory bodies have a more constricted view of risk than research participants, and is this appropriate? A final question and answer session will explore avenues by which human geneticists can work more effectively with their regulatory counterparts to enable genetic research on human subjects, while protecting them from exploitation or unnecessary risk.

Introduction. *F. S. Collins* National Human Genome Research Institute, National Institutes, of Heath, Bethesda, MD.

Session Descriptions:
Identifying genetic factors that influence health, disease, and response to treatment is essential to reduce the burden of disease. With the sequencing of the human genome, more efficient genotyping and sequencing technologies, and the International Haplotype Map project, we now have powerful research tools for identifying genetic variants that contribute to common diseases. Researchers can now employ whole genome association studies to uncover regions of the genome and even specific genes contributing to disease susceptibility. We have an historic opportunity to understand key aspects of the complex contributions of genetic variation to health, with major consequences for prevention, diagnosis, and treatment. This session will draw upon recent large, coordinated whole genome association studies to consider the scientific opportunities presented by such studies and the challenges they face, how to mine and analyze the data from such projects, and the opportunities they offer for devising new diagnostics and therapeutics.

Questions and answers.

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Our Society and the Scientist-Citizen. *S. T. Warren* Emory University School of Medicine, Atlanta, GA.

Session Descriptions:

Stephen T. Warren
ASHG President
Emory University School of Medicine, Atlanta, GA

As investigators and practitioners of our wonderful field of human genetics, it is easy to become so engrossed in our daily work that we occasionally risk losing sight of all the external influences that affect our professional lives. Social, governmental, and educational policies are evolving, and the dynamics are often driven by the most influential but not necessarily the most well informed. Governmental actions can affect how we practice our craft, indeed they can legislatively ban aspects of our work, and limit the scope of our efforts. Often the actions taken mirror social opinions that influence what should or should not be taught in our schools. As human geneticists I believe we have a special responsibility to be aware of and participate in the dialog surrounding these issues.

As a prominent biomedical society we must be more active to educate and influence our legislative bodies at the federal and state levels. Toward this end, at the spring ASHG Board of Directors meeting in Washington DC, we set aside a day to meet with members of Congress and their staff regarding genetic nondiscrimination legislation and NIH funding. I encourage you all to get involved in these advocacy efforts. Meet with your representative, either at their home office or when you visit the DC area. Invite your elected officials to your laboratory to see first hand that NIH funding goes well beyond Bethesda (a common misconception on the hill is that the NIH appropriation is for the Bethesda campus). When you receive an email from ASHG (or any other professional societies) regarding an upcoming congressional vote, please be sure to make your opinion known to your representative or senator.

In addition to active participation in science policy, education is the other cornerstone to enhanced biomedical research. The Society is expanding its efforts in public education. Consider participating by joining the ASHG mentor network; become a human genetics advocate and invest in the future of our field. People fear the unknown, and I believe some of the mistrust of genetics research by the public is due a lack of understanding and journalistic sensationalism. If you read a newspaper article that does not give a balanced view of what we do, write a letter to the editor. We do good work that benefits society and we must continually make that point clear.

Finally, not all efforts should be external to the ASHG. We need more involvement in our Society by its members. Participation is essential to the growth, vibrancy and success of the organization. Play a role in the direction that ASHG takes and let your voice be heard regarding how our Society can better serve members. For example, seek nomination to one of the ASHG committees. This year we set up a postdoctoral fellow committee to develop meeting content specifically for those in training and to let the board know the ideas and concerns of that demography of our membership. Similarly, we are developing a program to involve our early career members, for example, assistant professors and those in comparable positions, to learn more about the Society and how to play an active role in it as well as to provide networking opportunities. Please attend this years business meeting to hear updates on the exciting activities of ASHG. Only through engagement of the membership can we really be a strong and positive force influencing the many aspects of the future of human genetics.

Reponses of Cells and Organisms to Altered Telomere Maintenance. *E. H. Blackburn* University of California, San Francisco.

Session Descriptions:
A gold medal and a $250,000 prize will be presented to Dr. Elizabeth H. Blackburn, Morris Herzstein Professor of Biology and Physiology in the Department of Biochemistry and Biophysics at the University of California, San Francisco. This prize is awarded annually to a leading scientist or group of scientists working in genetics, in recognition of groundbreaking contributions and fundamental insights in the field of genetics research. These may include original discoveries in genomic organization, function, regulation variation, and transmission. The Peter Gruber Prize recognizes her achievements in research and science advocacy.

By discovering the unique structure and mechanism of replication of telomeres, the ends of chromosomes, Dr. Blackburn demonstrated that these genetic elements play a fundamental role in normal development, and in carcinogenesis.

She has characterized telomerase, the enzyme responsible for making telomeres, in normally aging cells, in cancer cells, and in stem cells, enabling the development of new drugs based on these biological roles.

Her accuracy and honesty in debates on therapeutic cloning and stem cell research have raised public awareness of the importance of this work, and she is a model for the role of the scientist as citizen.

The Peter Gruber Foundation gives international prizes in genetics, neuroscience, justice, womens rights, and cosmology. Its goal is to recognize, honor, and encourage individuals who have transformed their field, and by shining a spotlight on them, to encourage others to follow in their footsteps.

Past winners are:

Robert H. Waterston
Mary-Claire King
H. Robert Horvitz
Rudolf Jaenisch
David Botstein
Eric Knudsen
Seymour Benzer

Elizabeth Blackburn was selected to win the 2006 Prize by a distinguished advisory panel that included:

David Botstein
Uta Francke
Robert H. Waterston
Mary-Claire King
H. Robert Horvitz
Rudolf Jaenisch

For further information about the Peter Gruber Foundations prizes, please visit http://www.petergruberfoundation.org

Reponses of Cells and Organisms to Altered Telomere Maintenance
Telomeres are the structures that protect and stabilize the ends of chromosomes, ensuring genomic stability. Telomeres consist of simple DNA sequences, which bind protein factors and make a cap. Without telomeric DNA and its special way of replicating, chromosome ends dwindle away, eventually causing cells to stop dividing, a process called cellular senescence. The enzyme telomerase replenishes the DNA at telomeres, partly counteracting the progressive shortening of telomeres throughout the human life span. Although telomerase activity is normally kept in check in adult human

cells, throughout life a certain level of telomerase is still required for replenishment of tissues, such as the immune system. Recent findings have highlighted the importance of telomerase and telomere length maintenance. For example, low telomerase in white blood cells in young to middle aged humans was found to be associated with six of the known major risk factors for cardiovascular disease.

In contrast to many normal cells in human adults, late-stage cancer cells characteristically have very high telomerase levels. A major known function of telomerase in cancer is to replenish telomeric DNA and maintain cell immortality. However, knocking down the high telomerase in cancer cells also inhibited their growth surprisingly rapidly, even without telomere shortening. Rapid and distinct cellular and transcriptional responses were elicited by reducing the level of telomerase RNA component. The distinctive alterations in the gene-expression profiles were predicted to be associated with diminished cancer progression. These and other recent results indicate that telomerase likely plays roles in other aspects of cancer known to be central to cancer progression.

Having It All. *D. Warburton* Columbia University, New York, NY.

Session Descriptions:
This award, which includes $10,000 and an engraved medal, is presented for substantial scientific contributions to human and medical genetics carried out over a lifetime of scientific inquiry.

Introduced by:
Professor Patricia A. Jacobs
Co-director of Research
Wessex Regional Genetics Laboratory
Salisbury District Hospital
Salisbury, Wiltshire, United Kingdom

Recipient:
Dorothy Warburton
Professor of Clinical Genetics and Development
Columbia University, New York, NY

Having It All

Having it all is a phrase usually applied to the attempt, implicitly by a woman, to combine career and family life without short-changing either. While happily the traditional meaning is valid in my case, the phrase also describes my life in human genetics in several other ways. First, cytogenetics has allowed me to combine two seemingly disparate passions: a love of playing with numbers and a fascination with new laboratory techniques. Thus my work has involved epidemiological and population studies of spontaneous abortions, chromosome abnormalities and ovarian aging. It has also involved the development and application of cytogenetic techniques, starting with autoradioagraphy, chromosome banding, in situ hybridization and somatic cell hybridization, and moving into the molecular era of FISH, CGH and genomic microarray analysis. Secondly, I have derived great personal satisfaction from being able to combine both clinical and research activities during almost forty years of directing a clinical cytogenetics lab in an academic setting. Cytogenetic analysis has revealed a vast array of chromosome aberrations associated with congenital malformations and/or mental retardation, and defined many new syndromes. Studying these aberrations has also impacted on our basic knowledge of chromosome structure and function, epigenetics and cancer. I have seen human genetics change from a discipline where patients contemplating pregnancy could be counseled only about Mendelian or empirical risks to one that now offers prenatal diagnosis for a large number of conditions and treatment for a few.

Lastly, having it all can also be used as a metaphor for the human genome project, which I have seen come to fruition since attending the first human gene mapping conference in 1973. The new tools available have revealed that small-scale copy number variation is widespread in the genome, and may be associated with pathology or phenotypic variation. Cytogenetics has entered an exciting era of ultra high-resolution chromosome analysis via microarray, which is likely to have as big an impact on human genetics as conventional cytogenetics did in the past. At the same time, it is still true that complete trisomy, no matter how diagnosed, remains the most common cause of mortality and morbidity among human conceptions. The molecular and evolutionary explanation of why our species is so prone to meiotic error, and the extraordinary phenomenon of its association with maternal aging, remain among the most significant and intriguing unsolved problems in biology. We do not really have it all: there is much to come.

Reflections on the Value of "Sticking with" Marfan Syndrome. *H. C. Dietz* Howard Hugh Medical Institute, Johns Hopkins University School of Medicine, Baltimore, Maryland.

Session Descriptions:
The Curt Stern Award is granted yearly to a scientist or scientists for major scientific achievement in human genetics that has occurred in the last 10 years. The work could be a single discovery or a series of contributions on a similar or related topic. The Award honors the memory of Curt Stern (1902-1981) as an outstanding pioneer in human genetics and ASHG president in 1956. An engraved crystal award and $2500 will be presented to the awardee at the annual meeting.

Presenter: Victor A. McKusick, University Professor of Medical Genetics
The Johns Hopkins University School of Medicine, Baltimore, MD

Recipient: Harry C. Dietz
Victor McKusick Professor of Medicine and Genetics; Investigator, Howard Hughes Medical Institute; Professor of Pediatrics, Institute of Genetic Medicine
The Johns Hopkins University School of Medicine, Baltimore, Maryland

Reflections on the Value of Sticking with Marfan Syndrome
I am commonly asked why I stick with Marfan research given that the work after gene discovery is often more difficult and tedious than simply moving on to the next gene and disease. A growing trend in medical genetics is to emphasize common and complex traits, perhaps at the expense of dwindling excitement and resources devoted to relatively rare Mendelian disorders. Here, my thoughts are flavored by my lifes experiences as a clinician who also happens to do research. If I had been asked 10 years ago about the prospects of developing a productive medical therapy for a systemic disorder of connective tissue such as Marfan syndrome, I would have placed the chances just slightly ahead of curing a chromosomal disorder. My continuing dedication to Marfan syndrome was more a reflection of obligation to my patients, as opposed to perceived opportunity. At that time we thought that the pathogenesis of Marfan syndrome simply reflected loss of physical integrity of the tissues due to a deficiency of the structural protein fibrillin-1. It was a clinical encounter (including unbiased and astute questioning by a patient) that first challenged this paradigm. Why should weakness of the tissues lead to overgrowth of the bones? Why, for that matter, should it lead to the craniofacial features of Marfan syndrome, valve thickening, or low muscle mass and tone? We turned to mouse models of Marfan syndrome to address these issues, and learned that fibrillin-1 normally regulates the activation of and signaling by the TGFbeta family of cytokines. Furthermore, antagonism of TGFbeta using either neutralizing antibodies or the FDA-approved drug losartan could prevent the multisystem manifestation of Marfan syndrome including lung emphysema, aortic aneurysm and failed muscle regeneration in the mouse model. Having established this foundation for Marfan syndrome, we learned that excess TGFbeta signaling contributes to overlapping disease phenotypes including other forms of syndromic aortic aneurysm and emphysema and failed muscle regeneration in the mdx mouse model of Duchenne muscular dystrophy. A clinical trial of losartan for Marfan syndrome will begin soon, and this strategy has proven equally powerful in ameliorating muscle disease in mdx mice. Sticking with Marfan syndrome (or, more generally, rare Mendelian disorders) remains an obligation based upon the contributions (through personal commitment) such patients have made to the maturation of genetic technologies and human genetics as a discipline. This and other examples also underscore the privilege and opportunity that continues to be granted by these individually rare but collectively indispensable partners in research.

Genetic differences between humans and great apes: finding a bundle of needles in the haystack. *A, Varki*
University of California, San Diego.

Session Descriptions:
Despite remarkable conservation of many DNA sequences throughout evolution, cataclysmic alterations in the genes of certain pathways signal the generation of a new species. Dr. Ajit Varki illustrates this phenomenon by detailing profound differences in the glycosylation systems of apes and human. Those differences are primarily manifest by changes in the metabolic handling of sialic acid, the terminal, charged carbohydrate on N-linked and O-linked oligosaccharides that provide untold diversity to proteins and lipids alike. Nobelist Peter Agre follows with an exposition of aquaporin water channels responsible for the movement of water across membranes. The elegant discovery of these elemental systems provides new understanding of trans-cellular water fluxes and serves as a basis for therapeutic interventions. Finally, Dr. Alain Fischer explains the genetics of immune deficiency and the current state of the art regarding the application of gene therapy, complete with a rationale for the pitfalls and promise of this treatment modality in severe combined immune deficiency.

Aquaporin water channels: from atomic structure to clinical medicine. *P. Agre* The Johns Hopkins University School of Medicine, Baltimore, MD.

Session Descriptions:
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Inherited deficiencies of the immune system: from pathophysiology to therapy. *A. Fischer* Hopital Necker-Enfants Malades, Paris, France.

Session Descriptions:
Despite remarkable conservation of many DNA sequences throughout evolution, cataclysmic alterations in the genes of certain pathways signal the generation of a new species. Dr. Ajit Varki illustrates this phenomenon by detailing profound differences in the glycosylation systems of apes and human. Those differences are primarily manifest by changes in the metabolic handling of sialic acid, the terminal, charged carbohydrate on N-linked and O-linked oligosaccharides that provide untold diversity to proteins and lipids alike. Nobelist Peter Agre follows with an exposition of aquaporin water channels responsible for the movement of water across membranes. The elegant discovery of these elemental systems provides new understanding of trans-cellular water fluxes and serves as a basis for therapeutic interventions. Finally, Dr. Alain Fischer explains the genetics of immune deficiency and the current state of the art regarding the application of gene therapy, complete with a rationale for the pitfalls and promise of this treatment modality in severe combined immune deficiency.

Before submission. *C. C. Morton The American Journal of Human Genetics.*

Session Descriptions:
Publishing experimental results is a goal of most researchers, but the process is often filled with frustration and disappointment. For many authors, organizing and discussing data in a clear and concise fashion is a cumbersome task. Additionally, it is difficult to know which journal is the most appropriate for a manuscript. At the journal office, editors often receive papers that lack a few fundamental pieces that would have allowed an extra level of consideration. Also, peer-review is an essential part of the publication system, but it is only as effective as the comments that are generated and the response of the authors to the reviews. At this session, the editors from three prominent genetics journals will offer some guidelines to follow during the publication and review process. Following the speakers' presentations, there will be a 20-minute question and answer session with a panel of editors from a variety of journals.

At the Journal. *R. A. King* University of Minnesota, Minneapolis.

Session Descriptions:
Publishing experimental results is a goal of most researchers, but the process is often filled with frustration and disappointment. For many authors, organizing and discussing data in a clear and concise fashion is a cumbersome task. Additionally, it is difficult to know which journal is the most appropriate for a manuscript. At the journal office, editors often receive papers that lack a few fundamental pieces that would have allowed an extra level of consideration. Also, peer-review is an essential part of the publication system, but it is only as effective as the comments that are generated and the response of the authors to the reviews. At this session, the editors from three prominent genetics journals will offer some guidelines to follow during the publication and review process. Following the speakers' presentations, there will be a 20-minute question and answer session with a panel of editors from a variety of journals.

The peer-review process. *J. C. Carey American Journal of Human Genetics*.

Session Descriptions:
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Deep ancestry: inside the Genographic Project. *S. Wells* National Geographic Society, Washington, DC.

Session Descriptions:
The Genographic Project, directed by Explorer-in-Residence, Dr. Spencer Wells, is a five-year effort to map humanitys genetic journey through the ages. Research labs across the globe have scientists visiting remote regions in a comprehensive effort to map migration and assess the worlds remaining indigenous and ethnic population isolates. An overview of the genetic findings will help explain the diversity and source of variation in human populations.

In an effort to bring context to the variation we represent, Rick Guidotti, an energetic and talented photographer, will share his passion and vision of the importance of celebrating human differences.

This symposium will engage the audience and enlighten the scientific perspective of our daily laboratory and patient activities.

The spirit of difference. *R. Guidotti* Positive Exposure, New York, NY.

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This symposium will engage the audience and enlighten the scientific perspective of our daily laboratory and patient activities.

Forebrain neural stem cells: from basic biology to brain repair. *S. Weiss* University of Calgary, Alberta, Canada.

Session Descriptions:
The pluripotent nature of stem cells offers promise for regeneration of a variety of cell types and tissues. That promise, in turn, provides hope for victims of numerous diseases previously considered incurable. For this symposium, Dr. Weiss begins with a description of the isolation and characterization of forebrain neural stem cells and their potential use in repairing the injured brain. Dr. Daley then provides a discussion of his methodology for preparing customized stem cells, with productive results. Dr. Orkin provides details on how stem cells are controlled at the molecular level. Finally, Dr. Grompe expounds on the surprising ability of the liver to repopulate via hepatic stem cells. Each expert employs a model system to demonstrate the dynamic underpinnings of tissue homeostasis, i.e., the action of stem cells. Each presentation addresses the feasibility of using stem cells for the renewal of impaired organs and tissues, whether damaged by genetic, environmental, or traumatic causes. Stem cell therapy stands at our doorstep, about to enter the future of medicine.

Customized stem cells. *G. Daley* Children's Hospital, Boston, MA.

Session Descriptions:
The pluripotent nature of stem cells offers promise for regeneration of a variety of cell types and tissues. That promise, in turn, provides hope for victims of numerous diseases previously considered incurable. For this symposium, Dr. Weiss begins with a description of the isolation and characterization of forebrain neural stem cells and their potential use in repairing the injured brain. Dr. Daley then provides a discussion of his methodology for preparing customized stem cells, with productive results. Dr. Orkin provides details on how stem cells are controlled at the molecular level. Finally, Dr. Grompe expounds on the surprising ability of the liver to repopulate via hepatic stem cells. Each expert employs a model system to demonstrate the dynamic underpinnings of tissue homeostasis, i.e., the action of stem cells. Each presentation addresses the feasibility of using stem cells for the renewal of impaired organs and tissues, whether damaged by genetic, environmental, or traumatic causes. Stem cell therapy stands at our doorstep, about to enter the future of medicine.

Molecular control of stem cells. *S. Orkin* Dana Farber Cancer Institute, Boston, MA.

Session Descriptions:
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Liver stem cells and liver repopulation. *M. Grompe* Oregon Health and Sciences University, Portland.

Session Descriptions:
The pluripotent nature of stem cells offers promise for regeneration of a variety of cell types and tissues. That promise, in turn, provides hope for victims of numerous diseases previously considered incurable. For this symposium, Dr. Weiss begins with a description of the isolation and characterization of forebrain neural stem cells and their potential use in repairing the injured brain. Dr. Daley then provides a discussion of his methodology for preparing customized stem cells, with productive results. Dr. Orkin provides details on how stem cells are controlled at the molecular level. Finally, Dr. Grompe expounds on the surprising ability of the liver to repopulate via hepatic stem cells. Each expert employs a model system to demonstrate the dynamic underpinnings of tissue homeostasis, i.e., the action of stem cells. Each presentation addresses the feasibility of using stem cells for the renewal of impaired organs and tissues, whether damaged by genetic, environmental, or traumatic causes. Stem cell therapy stands at our doorstep, about to enter the future of medicine.

Questions and answers.

Session Descriptions:
Publishing experimental results is a goal of most researchers, but the process is often filled with frustration and disappointment. For many authors, organizing and discussing data in a clear and concise fashion is a cumbersome task. Additionally, it is difficult to know which journal is the most appropriate for a manuscript. At the journal office, editors often receive papers that lack a few fundamental pieces that would have allowed an extra level of consideration. Also, peer-review is an essential part of the publication system, but it is only as effective as the comments that are generated and the response of the authors to the reviews. At this session, the editors from three prominent genetics journals will offer some guidelines to follow during the publication and review process. Following the speakers' presentations, there will be a 20-minute question and answer session with a panel of editors from a variety of journals.

Opening Remarks. *D. Scholes* National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland; Science Policy Fellow, Washington, DC.

Session Descriptions:
This program is a special session for graduate students and post-doctoral fellows organized by the newly formed Ad Hoc Postdoctoral Committee. ASHG trainees interested in an event designed to broaden their knowledge about rewarding careers ranging from laboratory research to patent law should attend. Members of the biotechnology, patent law, clinical diagnostic, academic research, policy and journalism communities will describe what it takes for PhD recipients to be successful in different scientific careers. Finally, a network session will be open to all participants to discuss these careers and a variety of others including education, public health, and science writing. The event is limited to 250 attendees.

Keynote Address: Challenges for Young Scientists. *B. Lindstaedt* University of California, San Francisco.

Session Descriptions:
This program is a special session for graduate students and post-doctoral fellows organized by the newly formed Ad Hoc Postdoctoral Committee. ASHG trainees interested in an event designed to broaden their knowledge about rewarding careers ranging from laboratory research to patent law should attend. Members of the biotechnology, patent law, clinical diagnostic, academic research, policy and journalism communities will describe what it takes for PhD recipients to be successful in different scientific careers. Finally, a network session will be open to all participants to discuss these careers and a variety of others including education, public health, and science writing. The event is limited to 250 attendees.

Career Panel. *C. C. Morton[1], C. Gunter[2], N. Katsanis[3], P. Frosst[4], L. Sung[5]* 1) Brigham and Womens Hospital, Boston, Massachusetts; 2) Nature, Washington, DC; 3) The Johns Hopkins University School of Medicine, Baltimore, Maryland; 4) The National Human Genome Research Institute, Bethesda, Maryland; 5) University of Maryland School of Law, Baltimore, Maryland.

Session Descriptions:
This program is a special session for graduate students and post-doctoral fellows organized by the newly formed Ad Hoc Postdoctoral Committee. ASHG trainees interested in an event designed to broaden their knowledge about rewarding careers ranging from laboratory research to patent law should attend. Members of the biotechnology, patent law, clinical diagnostic, academic research, policy and journalism communities will describe what it takes for PhD recipients to be successful in different scientific careers. Finally, a network session will be open to all participants to discuss these careers and a variety of others including education, public health, and science writing. The event is limited to 250 attendees.

Networking reception with representatives from industry, academia, law, education, scientific societies, public health, policy, genetic counseling and other fields.

Session Descriptions:
This program is a special session for graduate students and post-doctoral fellows organized by the newly formed Ad Hoc Postdoctoral Committee. ASHG trainees interested in an event designed to broaden their knowledge about rewarding careers ranging from laboratory research to patent law should attend. Members of the biotechnology, patent law, clinical diagnostic, academic research, policy and journalism communities will describe what it takes for PhD recipients to be successful in different scientific careers. Finally, a network session will be open to all participants to discuss these careers and a variety of others including education, public health, and science writing. The event is limited to 250 attendees.

Leadership Award Presentation. *M. M. Kaback* Department of Pediatrics and Reproductive Medicine, University of California, San Diego.

Session Descriptions:
This award is presented to an individual whose professional achievements have fostered and enriched the development of various human genetics disciplines. Potential recipients should exemplify the enduring leadership and vision required to ensure that the field of human genetics will flourish and successfully assimilate into the broader context of science, medicine, and health. They also may have made major contributions to awareness or understanding of human genetics by policy makers or by the general public. A plaque and $2500 will be presented to the awardee at the annual meeting.

Presenter:
Michael M. Kaback, Professor
Department of Pediatrics and Reproductive Medicine,
University of California, San Diego

Recipient:
David L. Rimoin, Professor of Pediatrics, Medicine and Human Genetics
University of California, Los Angeles, School of Medicine/Cedars-Sinai Hospital, Los Angeles, CA

The Societys newest award will be presented for the first time to Dr. David Rimoin, Professor of Pediatrics, Medicine and Human Genetics, David Geffen School of Medicine at UCLA and Steven Spielberg Chair and Director of the Medical Genetics Institute, Cedars-Sinai Medical Center. In addition to his many well-recognized research and educational achievements, particularly in the area of skeletal dysplasias, he served as the founding president of the American Board of Medical Genetics (ABMG), which established the educational standards for certification of medical geneticists and the accreditation of training programs throughout the nation. He was also the founding president of the American College of Medical Genetics (ACMG) and then president of its Foundation, whose primary goal is to foster education in medical genetics to the practitioner, as well as the public. He was responsible for establishing the American College of Medical Genetics annual meetings in conjunction with the March of Dimes to provide an educational forum for clinical geneticists and other individuals providing genetic services.

ASHG Membership/Business Meeting. *S. T. Warren* Emory University School of Medicine, Atlanta, GA.

Session Descriptions:
Reports highlighting current Society business are presented to inform the members and anyone else attending the meeting. The minutes of the previous meeting are presented for approval. Committee chairpersons report on their activities for the year and discuss plans for the upcoming year. Retiring board members are thanked for their years of service.

The meeting offers an opportunity for members to discuss items of new business. All members are encouraged to attend.

Trainee Award Presentations. *D. Valle* The Johns Hopkins University, Institute of Genetic Medicine, Howard Hughes Medical Institute, Baltimore, MD.

Session Descriptions:
The Society will present $500 to each awardee for outstanding research presented by a trainee in each of the following areas: predoctoral clinical, postdoctoral clinical, predoctoral basic, postdoctoral basic, predoctoral translational, and postdoctoral translational. Finalists have been selected from nominees who submitted abstracts in the competition. Their names are listed in the section of this book entitled Trainee Awards Program..

C. W. Cotterman Award Presentation. *C. C. Morton* The American Journal of Human Genetics, Brigham & Women's Hospital, Boston, Massachusetts.

Session Descriptions:
Monetary awards of $500 each and commemorative plaques will be presented to the first authors of the best papers published in *The American Journal of Human Genetics* during the previous year on which the first author is either a pre- or postdoctoral trainee and an ASHG member. Each September, the editorial board of the Journal selects the articles that best represent outstanding contributions to the field of genetics. Two awards are presented annually.

Presenter:
Cynthia C. Morton, editor
The American Journal of Human Genetics
Brigham & Women's Hospital, Boston, Massachusetts.

Excellence in Human Genetics Education Award Presentation. *R. Pagon* University of Washington, Seattle.

Session Descriptions:
Nominees for this award have made a contribution that is recognized nationally or internationally as being of exceptional quality and great importance to human genetics education. An award of $2500 and a plaque will be presented to the awardee at the annual meeting.

Introduced by: Robert Nussbaum
Chief of the Division of Medical Genetics and Holly Smith Professor of Medicine, Department of Medicine
University of California, San Francisco.

Recipient:
Roberta Bonnie Pagon
Professor of Pediatrics
University of Washington, Seattle

This award is being presented to Dr. Pagon for her tireless work in the development of the GeneTests database that includes GeneReviews. These resources are indispensable educational tools for geneticists and counselors wishing to learn abut the practical aspects of caring for patients with genetic diseases. The information is freely available on the web and is playing a major role in the education of our genetic fellows, counseling students and clinicians.