The American Society of Human Genetics
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San Diego, CA
October 23-27, 2007

Participation from the National Human Genome Research Institute (NHGRI)

DAY II WEDNESDAY, OCTOBER 24

Plenary and Platform Presentations

08:00AM-09:30AM Room 20D
Education Session 1: Genome-Wide Association Studies in the Era of Open Data Access and Collaboration
Moderator: Francis S. Collins, National Human Genome Research Institute, Bethesda, MD

An overview of genome-wide association (GWA) studies and major initial findings from three genome-wide association resources (the Framingham SHARe Project, the Genetic Association Information Network, and the Wellcome Trust Case Control Consortium) will be presented to permit assessment of the added value of open data sharing and of conducting such programs in a collaborative mode. Early experience with open access for these databases, data-sharing and IP policies will be described, including challenges encountered and lessons learned. “Snapshots” of issues unique to collaborative GWA studies will be presented, including comparing genotyping quality and combining data across genotyping platforms, using common controls, and harmonizing datasets for cross-study use. An example of the strengths and weaknesses of combined analysis of common phenotypes (such as body mass index) across multiple studies will be provided, and the question of shared risk alleles for the four major mental illness phenotypes included in GAIN will be considered.
Session Time: Wed Oct 24, 2007
Presentation Information

Presentation 08:00AM-08:20AM
Overview of GAIN. *T. A. Manolio* Office of Population Genomics, National Human Genome Research Institute, Bethesda, MD.

Presentation 08:20AM-08:40AM
Framingham SNP Health Association Resource (SHARe). *E. G. Nabel,* Office of the Director, National Heart, Lung, and Blood institute, Bethesda, MD.

08:00AM-09:30AM Room 30
Social Issues Session 4: Genetics Policy and Educational Issues in the Response to Hurricane Katrina: Future Implications for Mass Fatalities

In August 2005, Hurricane Katrina devastated an area of the United States equaling the size of Great Britain. Over 1300 individuals lost their lives, many because of the flooding which occurred when the New Orleans’ levies broke. DNA played a major role in the identification of victims and was used exclusively in many cases where fingerprints and dental records were unavailable. The DNA identification effort was challenging because many items that could have been the source of identifying DNA, such as toothbrushes, clothing, and hairbrushes, were lost during the flooding. In addition, family members were evacuated and relocated multiple times following the storm. A total of 90 genetics professional volunteers from 20 states and Canada, representing 43 institutions/private practices, went to Baton Rouge to collect family data. We share the experiences of genetics professional volunteers and identify educational and policy needs that have implications for future mass fatality victim identification efforts.

Presentation 09:00AM-09:30AM
Lessons Learned from Hurricane Katrina: Policy Needs. *B. B. Biesecker* Social and Behavioral Research Branch, NHGRI/HIH, Bethesda, MD.

10:00AM-11:30AM Room 28
Education Session 7: The Scientist's Role in Improving Genetic Education and Awareness

Having both genetics content knowledge and the ability to utilize that knowledge in personal and civic situations is important for scientists and nonscientists alike. We, as geneticists, are in a unique position to provide the public, our trainees and our colleagues with the information and tools to achieve this genetic literacy. While each target audience requires distinct educational and training interventions, each must have the expertise of practicing geneticists to achieve success. This educational session will highlight ongoing geneticist-led educational programs for the general public, graduate students, genetic counselors and clinicians, and offer ideas and concepts attendees can incorporate in their local settings.

Presentation 11:05AM-11:25AM
The Citizen Scientist: The Role of Scientists in their Communities. *F. Collins* National Human Genome Research Institute, Bethesda, Maryland.

01:30PM-03:30PM Hall H
Plenary Session 12
Program 1 01:30PM-01:50PM
Comparative sequence analysis of primate subtelomeres.
K. Rudd[1], R. Endicott[1], C. Friedman[1], M. Walker[1], J. Young[1], K. Osoegawa[2], R. Blakesley[3], P. de Jong[2], E.D. Green[3], B. Trask[1]
1) Fred Hutchinson Cancer Res Ctr, Seattle, WA; 2) Children's Hospital of Oakland Res Inst, Oakland, CA; 3) NHGRI, NIH, Bethesda, MD.

Subtelomeric regions are among the most structurally complex, variable, and dynamic areas of the genome. Subtelomeres are

the transition zones between chromosome-specific sequences and the arrays of telomere repeats at the end of chromosomes. The identity, arrangement, and polymorphism of the blocks of subtelomeric sequence shared among multiple chromosomes suggest that subtelomeric duplications spread recently. We traced the evolutionary history of the chromosome-15 subtelomere in the genomes of human, chimpanzee, gorilla, orangutan and macaque using FISH, PCR, and sequencing of genomic clones. The ancestral locus lies internally on macaque chromosome 7; however, a chromosome fission event gave rise to two acrocentric chromosomes in the common ancestor of the great apes. Sequence originating at this fission site now resides at the terminus of 15q and the pericentromere of 14q in great apes. Subsequent exchanges have added and removed subtelomeric material on chromosome 15q, as well as transferred large subtelomeric regions to other chromosomes. At least 250 kb from the fission site region transferred to the end of chromosome 4 in the ancestor of chimpanzee and gorilla. This hybrid subtelomere contains sequences orthologous to the human 4q and 15q. Interestingly, the proximal 4q-like subtelomeric region is associated with facioscapulohumeral muscular dystrophy in humans. Eight olfactory receptor (OR) genes encompassing 125 kb have been lost from the end of the 15q subtelomere in the human and chimpanzee genomes. A terminal subtelomeric region containing a highly conserved gene has been affixed to the 15q subtelomere in the human lineage only. The orangutan chromosome 15 subtelomere is very similar to the ancestral locus, and the gorilla 15q subtelomere has lost a subset of ORs. Our detailed analysis of the chromosome 15 subtelomere has shown significant structural changes in each lineage, demonstrating that subtelomeres are one of the most rapidly evolving regions of the genome.

10:00AM-11:30AM Room 20D
Education Session 8 Designing Geneticists: Study Design Issues in Population-based Genetics and Genomics Research
Moderator: E. L. Harris Population Genomics, National Human Genome Research Institute, Bethesda, MD

Study design is a critical aspect of any research project. The study's purpose drives study design, influenced by practical issues. Genetic/genomic research increasingly uses population-based designs, such as case-control genome-wide association studies, to study genetic susceptibility to common conditions and genetic influences on quantitative traits. Epidemiologic studies of such conditions now commonly include DNA collection, and genetic information as part of the analyses. To effectively design and interpret such studies, knowledge of basic study design is critical as is an understanding of complications that genetic data introduce into such studies. Crucial design decisions include case or outcome definition, control or comparison group definition, measurement methods, and statistical analysis approach. Internal validity is imperative, and methods for assessing potential biases desirable. In this session, we will discuss: basic study design choices and rationale; designs to maximize internal validity and external validity; gene-environment interaction; and how to look for and minimize bias.

Introduction 10:00AM-10:05AM
E. L. Harris Population Genomics, National Human Genome Research Institute, Bethesda, MD.

Presentation 10:45AM-11:05AM
Evaluating potential bias in and interpreting results from epidemiologic designs. *T. A. Manolio,* Population Genomics, National Human Genome Research Institute, Bethesda, MD.

Questions and answers 11:25AM-11:30AM
E. L. Harris Population Genomics, National Human Genome Research Institute, Bethesda, MD.

Poster Presentations

Molecular Basis of Mendelian Disorders Session
Exhibit Hall E Wed 4:30PM-6:30PM, Thu 4:30PM-6:30PM, Fri 10:30AM-12:30PM

Poster 1005/W
Presentation Wed, Oct 24, 2007, 4:30PM-6:30PM
Genetic Analysis of Syndromic X-Linked Microphthalmia.
J.J. Johnston[1], E. Hilton[2,3], V. Kimonis[4], C. Schwartz[5], G.C.M. Black[2,3], L.G. Biesecker[1] 1) NHGRI, NIH, Bethesda, MD; 2) St. Mary's Hospital, Manchester, UK; 3) Manchester Royal Eye Hospital, Manchester, UK; 4) Harvard Medical School, Boston, MA; 5) Greenwood Genetics Center, Greenwood, SC.

Lenz microphthalmia is inherited in an X-linked pattern and comprises microphthalmia, mental retardation (MR), skeletal and other anomalies. This disorder has been mapped to two loci, MCOPS1 (microphthalmia with associated anomalies) at Xq27-q28 and MCOPS2 at Xp11.4. A single mutation in the BCL-6 interacting corepressor, BCOR, on chromosome Xp11.4, was identified in the family used to map the MCOPS2 locus. Mutations in BCOR have also been identified in Oculofaciocardiodental syndrome (OFCD). OFCD is inherited in an X-linked pattern with apparent male lethality and comprises microphthalmia, congenital cataracts, radiculomegaly, and cardiac and digital abnormalities. Initial studies show BCOR to be the major gene for OFCD. We have continued to screen additional patients with Lenz (2), OFCD (10), microphthalmia with or without MR (7), and X-linked MR with eye abnormalities (25) to better understand the contribution of BCOR mutations to these phenotypes, and in the case of Lenz syndrome, to identify families that map to the MCOPS1 locus. Nine out of ten patients with OFCD have had loss of function mutations in BCOR and no mutations have been identified in individuals with non-Lenz microphthalmia or in those with X-linked MR with eye anomalies. The identical substitution found in the original MCOPS2 family, p.P85L, was identified in the Lenz syndrome proband from a second family. The other proband with Lenz syndrome did not have a mutation in BCOR and the family is currently being evaluated for linkage to the MCOPS1 locus. We hypothesize that this family maps to Xq27-q28 and we will incorporate their data into our current efforts to refine the mapping of MCOPS1 in two previously reported families. In summary, loss of function mutations that affect BCOR cause OFCD as demonstrated by the mutations identified in affected individuals. Furthermore, it appears that while alterations in BCOR may contribute to Lenz syndrome, they do not appear to contribute to non-Lenz microphthalmia or X-linked MR.

Mapping, Linkage and Linkage Disequilibrium Session
Exhibit Hall E Wed 4:30PM-6:30PM, Thu 4:30PM-6:30PM, Fri 10:30AM-12:30PM

Poster 1167/W
Presentation Time: Wed, Oct 24, 2007, 4:30PM-6:30PM
Familial idiopathic scoliosis and the IRX gene family. *C. Justice[1], N.H. Miller[2], B. Marosy[3], D. Behneman[1], A.F. Wilson[1]*
1) Genometrics Section, IDRB, National Human Genome Research Institute, National Institutes of Health, Baltimore, Maryland; 2) University of Colorado, The Children's Hospital, Denver, Colorado; 3) Institue of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland.

Familial idiopathic scoliosis (FIS) is characterized by a lateral curvature of the spine present in otherwise normal individuals that affects 2 to 3% of the population. The original sample was comprised of 202 families with at least two individuals with scoliosis. Prior to analysis, three subgroups were determined including: most likely mode of inheritance (XLD vs. AD), families with at least two members with kyphoscoliosis, and families with at least two members with triple curves. Linkage analysis was performed in each subgroup with 391 STRP markers. Linkage analysis of the kyphoscoliosis subgroup (7 families, 53 individuals) identified candidate regions on chromosomes 5 and 13. The region on 5p13 (~3 Mb) contained only three genes, all belonging to the Iroquois homeobox (IRX) gene family. Three other IRX genes were located on 16q, in a region also linked to FIS in our AD subgroup.
To compare the distribution of other gene families in linked vs. non-linked regions, FIS-linked regions were defined as being ± 5 Mb from two consecutive p-values < 0.025 (26 regions ,15% of the genome). A BLAT search was performed with the IRX1 mRNA sequence, and 65% of homologous loci were in regions linked to FIS. Four other genes, three on chromosome 5 and one on chromosome 12, of similar size to IRX1, also underwent a BLAT mRNA homology search. The number or regions linked to FIS ranged from 17% to 36%, substantially less that for the IRX gene family.

Evolutionary and Population Genetics Session
Exhibit Hall E Wed 4:30PM-6:30PM, Thu 4:30PM-6:30PM, Fri 10:30AM-12:30PM

Poster 1283/W
Presentation Time: Wed, Oct 24, 2007, 4:30PM-6:30PM
Mapping complex traits in the domestic dog. *E.A. Ostrander[1], H.G. Parker[1], B. Hoopes[1], K. Bryc[3], B. vonHoldt[4], N.B. Sutter[1], K. Chase[2], K.G. Lark[2], P. Quignon[1], D.S. Mosher[1], C. Bustamante[4], R.K. Wayne[3]* 1) Cancer Genetics Branch, NHGRI/NIH, Bethesda, MD; 2) Dept. of Biology, University of Utah, SLC, UT; 3) Dept. Biological Statistics and Computational Biology, Cornell University, Ithaca, NY; 4) Dept. of Ecology and Evolution, UCLA, Los Angeles, CA.

The availability of a high quality draft sequence of the dog genome has changed the way geneticists studying companion animals are tackling the problem of finding genes that control complex traits. Of particular interest are genes controlling the morphologic differences which define different domestic dog breeds, genes regulating behavior, and those that increase disease susceptibility. Central to our ability to use the newly available resources is an understanding of dog breed structure and we herein present a detailed discussion of a new cluster analysis involving 135 U.S. breeds. Also important is an understanding of the strengths and limitations of the current molecular resources, and consideration of the traits which are likely to lend themselves to mapping using available approaches and resources. We describe our recent efforts to localize genes important in controlling body size. Our initial studies suggest a primary role for the IGF-1 gene in making small dogs small. But studies with Portuguese Water Dogs strongly suggest the existence of other loci in controlling overall body size in the dog. Building upon those findings, and using a large number of samples collected from small and large dog breeds we describe other genes and loci which potentially play a role in regulating canine morphology, particularly body size and leg length. Finally we discuss the problem of breed substructure in the context of candidate gene approaches. By way of example we discuss efforts to find genes for behavior traits in the dog, including racing speed among whippet dogs. Extending from our most recent work, we demonstrate that candidate gene analysis can work if special consideration is paid the likely occurrence of population substructure.

Poster 1296/W
Presentation Time: Wed, Oct 24, 2007, 4:30PM-6:30PM
Rate of mutation accumulation in coding and noncoding elements during mammalian evolution. *L. Parand[1], S. Nikolaev[1], J. Montoya-Burgos[2], K. Popadin[3], E.H. Margulies[4], NISC Comparative Sequencing Program[4,5], S.E. Antonarakis[1]*
1) Department of Genetics & Dev., University of Geneva Medical School; Geneva, Switzerland; 2) Department of Animal Biology, University of Geneva; Geneva, Switzerland; 3) Department of Genetics, Moscow State University; Russia; 4) Genome Technology Branch, NHGRI, NIH; Bethesda, Maryland 20892, USA; 5) NIH Intramural Sequencing Center, NHGRI, NIH; Bethesda, Maryland 20892, USA.

A comprehensive phylogenetic framework is indispensable for investigating the evolution of constrained genomic features in mammals as a whole and particularly in humans. Using the ENCODE sequence data from 1% of each of 18 mammalian genomes, we reconstructed evolutionary rates for three genomic matrices: silent (dS) substitutions, non-synonymous (dN) substitutions and Conserved Non Coding (CNC) elements. We show that synonymous substitutions (approximating neutral evolutionary rates) evolve according to the Generation Time (GT) hypothesis. Consistent with the longer generation time within mammals, primates and especially humans display a slowdown of neutral evolutionary rates. Constrained elements, however, evolve under different mechanisms. We show that dN substitutions, regarded to be slightly deleterious, are fixed as effectively neutral substitutions in species with small populations (human, chimp) and counter selected in those with large populations (mouse). We found that CNCs are more conserved than dNs in the majority of stem branches, but despite it the average rate of evolution of CNCs is 1.7 times higher than the average dN substitution evolutionary rate. This observation suggests that the selective pressure acting on a fraction of CNCs has been relaxed in a lineage specific manner not predicted by the population size or generation time hypothesis. Using the ENCODE data we detected three cases (Chimpanzee, Shrew and Eutheria) with significant relaxation among the 20 longest CNCs. Thus only a fraction of the CNCs detectable over the entire mammalian tree undergo purifying selection, while another fraction is suggested to be gradually replaced by lineage specific CNCs or those sequences become temporally unconstrained.

Poster 1307/W
Presentation Time: Wed, Oct 24, 2007, 4:30PM-6:30PM
The CanMap Project: Population Genetics and Whole Genome Association Mapping of Morphological and Behavioral Differences among Domestic Dog (Canis familiaris) Breeds. *C.D. Bustamante[1], T. Spady[2], H.G. Parker[2], B. vonHoldt[2,3], K. Bryc[1], M.H. Wright[1], N.B. Sutter[2], A. Reynolds[1], A.R. Boyko[1], M. Castelhano[1], E. Wang[4], K. Zhao[1,5], G. Johnson[6], M. Nordborg[5], R.K. Wayne[3], M. Cargill[4], E.A. Ostrander[2]* 1) Cornell University, Ithaca, NY; 2) NHGRI/NIH, Bethesda, MD; 3) UCLA, Los Angeles, CA; 4) Affymetrix, Santa Clara, CA; 5) U. Southern California, Los Angeles, CA; 6) U. of Missouri, Columbia, MO.
Domestic dog breeds exhibit great variation in behavior and morphology among breeds and low phenotypic and genetic diversity within breeds, making the dog an excellent genetic system for mapping traits of interest. Here, we present population genetic analyses and preliminary results for simultaneous whole-genome association mapping of morphological and behavioral trait differences among breeds using a panel of 1,000 dogs from 80 breeds genotyped on the Affymetrix Canine Array v.2.0 (~100,000 SNP). Population genetic analyses reveal clear genetic clustering of dogs into breeds with well defined boundaries, and shallow clustering of breeds into higher order groups. We use fine-scale recombination rate estimates across the genome to identify regions of unusually high linkage-disequilibrium within a breed, which may identify recent targets of selection during breed formation. We also estimate the domestication bottleneck size for dog as well as breed-specific bottleneck and inbreeding rates which account for dramatic differences in effective population size among popular breeds. Using a mapping strategy that accounts for expected high genetic relatedness within a breed, we aim to identify regions of the dog genome associated with skeletal conformation, hair pigmentation and texture, and behavioral trait differences including: body size, foreshortened limbs, foreshortened face, compact face and cranimum, proportional dwarfism, wire hair, curly hair, corded coat, face mask color, and prey drive. For several traits, overlying “peaks” of association with signatures of selection allows us to refine our signals to a just a few candidate genes. The approach we employ replicates previously identifies gene-trait association, including the link between IGF1 and body size.

Metabolic Disorders Session
Exhibit Hall E Wed 4:30PM-6:30PM, Thu 4:30PM-6:30PM, Fri 10:30AM-12:30PM

Poster 1442/W
Presentation Time: Wed, Oct 24, 2007, 4:30PM-6:30PM
BLOC-2 and BLOC-3 deficient melanocytes demonstrate distinct defects in TYRP1 trafficking. *A. Helip Wooley, H. Dorward, W. Westbroek, R. Hess, B. Pederson, M. Huizing, W.A. Gahl* Medical Genetics Branch, NHGRI NIH, Bethesda, MD.

Hermansky-Pudlak syndrome is an autosomal recessive disorder characterized by oculocutaneous albinism and bleeding resulting from defects in any of eight distinct genes (HPS-1 through HPS-8). With the exception of HPS-2, the human HPS genes encode proteins of unknown function. Several of these proteins interact with each other in Biogenesis of Lysosome-related Organelles Complexes or BLOCs. Specifically, HPS1 and HPS4 form BLOC-3 and HPS3, HPS5 and HPS6 comprise BLOC-2. To characterize and distinguish these BLOCs at the cellular level, we examined cultured melanocytes from individuals with HPS-1 and -4 (BLOC-3) and HPS-3, -5 and -6 (BLOC-2). BLOC-3 deficient melanocytes contained fewer dark melanosomes than BLOC-2 deficient melanocytes. Localization of melanosomal proteins by confocal immunofluorescence microscopy revealed TYRP1 staining in BLOC-3 melanocytes concentrated in the perinuclear region, with a large degree of overlap with the TGN. In BLOC-2 melanocytes, TYRP1 staining extended into the dendrites but failed to appropriately collect in the tips. Antibody uptake experiments demonstrated increased trafficking of TYRP1 via the cell membrane in BLOC-2 but not in BLOC-3 deficient melanocytes. BLOC-2 appears to sort TYRP1 from an early endosomal compartment to developing melanosomes. In the absence of BLOC-2, TYRP1 is mis-sorted to the plasma membrane. BLOC-3 likely functions earlier in the pathway such that TYRP1 does not reach the BLOC-2 endosomal compartment in BLOC-3 deficient melanocytes. BLOC-2 and BLOC-3 deficient melanocytes demonstrate distinct defects in TYRP1 trafficking, reflecting their actions in disparate steps of the pathway.

Psychiatric Genetics and Neurogenetics Session
Exhibit Hall E Wed 4:30PM-6:30PM, Thu 4:30PM-6:30PM, Fri 10:30AM-12:30PM

Poster 1875/W
Presentation Time: Wed, Oct 24, 2007, 4:30PM-6:30PM
Parkinsonian spectrum associated with glucocerebrosidase mutations. *E. Sidransky[1], G. Lopez[2], M. Hallett[2], O. Goker-Alpan[1]* 1) MGB/NHGRI/NIH, Bethesda, MD; 2) NIA/NIH, Bethesda, MD.

Alterations in the gene encoding for the lysosomal enzyme glucocerebrosidase (GBA) result in Gaucher disease (GD). Clinical, pathologic and genetic studies suggest that mutant glucocerebrosidase is associated with a phenotype characterized by parkinsonism and progressive neurologic deterioration. To define the neurologic spectrum among subjects with parkinsonism carrying GBA mutations, nine subjects (6M:3F), were followed up to 36 months in a prospective study. Cognitive function, oculomotor and motor deficits were tested by the same team. Olfactory evaluation was done using University of Pennsylvania Smell Identification Test. Genotypes were confirmed by DNA sequencing. The N370S mutation was the most common GD allele. Others included L444P, c.84insG and a recombinant allele. The mean age of onset of parkinsonian manifestations was 50 (40 -65) and disease duration was 7.4 years (1.2 -16). At presentation, four subjects had tremor, 5 had symptoms related to bradykinesia and rigidity, and one also had apraxia. Six were diagnosed with classical PD, three with the akinetic-rigid type. Three subjects were considered to have “parkinson plus” syndrome because of early cognitive changes and hallucinations. All, but one were L-Dopa responsive. Other atypical manifestation included myoclonus, EEG abnormalities and clinical seizures. Autonomic dysfunction was observed in three, and five of 6 subjects tested had olfactory loss. In half, cognitive changes were reported later in the disease course, often accompanied by depression. Glucocerebrosidase mutations are associated with a spectrum of parkinsonian phenotypes, frequently with loss of olfaction. This spectrum ranges from classic PD, mostly the akinetic type, to a less common phenotype characteristic of Lewy Body Dementia.

Statistical Genetics and Genetic Epidemiology Session
Exhibit Hall E Wed 4:30PM-6:30PM, Thu 4:30PM-6:30PM, Fri 10:30AM-12:30PM

Poster **2044/W**
Presentation Time: Wed, Oct 24, 2007, 4:30PM-6:30PM
Low correlation among association tests for quantitative traits. *J.E. Herrera-Galeano[1,2], R.A. Mathias[2], H. Sung[2], N. Faraday[1], D.M. Becker[1], L.C. Becker[1], A.F. Wilson[2]* 1) Dept of Medicine, Johns Hopkins Medical Institutions, Baltimore, MD; 2) Genometrics Section, NHGRI, NIH, Baltimore, MD.

Background: Recently, there has been a dramatic increase in the amount of genotyping data available for testing for association with quantitative traits. Several different methods of testing for associations are available; these methods use different kinds of information and have different strengths and weaknesses with respect to their statistical properties. Objective: To determine the pair-wise correlations among the following methods: ASSOC (SAGE v4.6.1), FBAT v1.7.3, GEE (SAS v8.0) and ROMP v0.2. Methods: Levels of 24 traits related to platelet aggregation were measured before and after 2 weeks of daily doses of 81 mgs of aspirin (ASA) in 541 African Americans and 955 Caucasians, in 155 and 264 families, respectively. Genotypes were determined for 2638 SNPs in 191 candidate genes using the Illumina Golden Gate platform. Tests of association were performed with each of the 4 methods in each ethnic group for each trait (506,496 total tests). Pair-wise Pearson product moment correlations were calculated, as were McNemar chi-squares categorizing p-values as significant ($p \leq 0.001$) or not significant. Results: Pair-wise correlations between the methods were evaluated only on those tests that returned a result for all four methods (57,018). The Pearson correlations were <0.14 for all pair-wise comparisons of these four methods. Furthermore, the pair-wise comparisons of the methods with the McNemar chi square tests were significant $p \leq 0.001$ for all pairs except ROMP-FBAT. Conclusions: Our results indicate that there is little correlation between the four tests of association for quantitative traits. In the absence of a consensus across association methods, the method that uses the most information should be given the greatest weight. In this case, a test of association in two and three generation family data, ASSOC, a likelihood based method that includes phenotype and genotyping information on all family members makes the fullest use of the available information.

Therapy for Genetic Disorders Session
Exhibit Hall E Wed 4:30PM-6:30PM, Thu 4:30PM-6:30PM, Fri 10:30AM-12:30PM

Poster 2246/W
Presentation Time: Wed, Oct 24, 2007, 4:30PM-6:30PM
***N*-acetylmannosamine therapy for podocytopathies and other kidney disorders due to hyposialylation.** *E. Klootwijk[1], I. Manoli[1], D. Hickey[1], C. Ciccone[1], D. Darvish[2], D. Krasnewich[1], W.A. Gahl[1], M. Huizing[1]* 1) MGB, NHGRI, NIH, Bethesda, MD; 2) HIBM Research Group, Encino, CA.

We created knock-in mice with a M712T missense mutation in *GNE*, encoding the key enzyme of sialic acid biosynthesis, UDP-GlcNAc 2-epimerase/ManNAc kinase (Gne/Mnk). Homozygous mutant ($Gne^{M712T/M712T}$) mice, deficient in sialic acid synthesis and glycoprotein sialylation, died before postnatal day 3 (P3) and exhibited severe hematuria, proteinuria and significantly abnormal glomerular structure. Ultrastructural findings included segmental splitting of the glomerular basement membrane (gbm) and effacement of the podocyte foot processes. Biochemical analysis of the mutant mice kidneys revealed decreased Gne-epimerase enzyme activity and deficient sialylation of the major podocyte sialoprotein, podocalyxin, after sialylated proteins were isolated using the sialic acid-specific lectin *Limax Flavus Agglutinin* (LFA). In contrast, overall kidney protein glycosylation, assessed by periodic acid-Schiff staining, was normal at age P2. Nor were significant differences detected in the expression of the podocyte marker podocin, the mesangial cell markers alpha smooth muscle actin and desmin, the endothelial cell marker Pecam-1, or the gmb component laminin beta-1. Oral administration of the sialic acid precursor *N*-acetylmannosamine (ManNAc) to the pregnant mothers allowed survival of 43% of the $Gne^{M712T/M712T}$ pups beyond P3. Survivors exhibited improved renal histology, increased sialylation of podocalyxin, and increased Gne/Mnk protein expression and Gne-epimerase activities. These findings establish this $Gne^{M712T/M712T}$ knock-in mouse as the first genetic model of podocyte injury due to hyposialylation. Moreover, the results support evaluation of ManNAc, a simple and well-tolerated intervention, as a treatment for renal disorders involving proteinuria and hematuria due to podocytopathy and/or segmental splitting of the gbm. Candidate disorders include Alport's syndrome, minimal change nephrosis, focal and segmental glomerulosclerosis, glomerulonephritis and other forms of idiopathic nephritic syndrome.

Poster 2261/W
Presentation Time: Wed, Oct 24, 2007, 4:30PM-6:30PM
The Evaluation of Three Novel Small Molecule Classes Identified Through Quantitative High-Throughput Screening (qHTS) as Potential Chaperones for Gaucher Disease. *D.J. Urban[1], W. Zheng[2], O. Goker-Alpan[1], E. Goldin[1], J. Inglese[2], C. Austin[2], E. Sidransky[1]* 1) Medical Genetics Branch, National Human Genome Research Institute, NIH Bld 35 Rm1A100, 35 Convent Drive, Bethesda, MD 20892-3708 USA; 2) NIH Chemical Genomics Center, National Human Genome Research Institute, NIH 9800 Medical Center Drive, MSC 3370 Bethesda, MD 20892-3370 USA.

Gaucher disease is an autosomal recessive lysosomal storage disorder caused by mutations in the glucocerebrosidase gene. Most identified mutations are missense mutations, where the reduced enzyme activity may be due to misfolding. It has been proposed that chaperone therapy with small molecule inhibitors could be used to correct the defect. Quantitative high throughput screening (qHTS) was successfully used to rapidly identify three structural series of potent, selective, non-sugar glucocerebrosidase inhibitors. These included sulfonamides, quinolines and triazines. In order to characterize the mechanism of action for these compounds and to determine their selectivity profiles, we performed enzyme kinetic assays using four different lysosomal hydrolases. We found that the glucocerebrosidase inhibitors identified in our screening were highly selective for glucocerebrosidase and not the other related hydrolyses. Structure activity relationship data was used to select compounds with high activity, which were evaluated further using both enzyme and cell-based assays. Using fibroblast cell lines from patients homozygous for N370S, we found that compounds from two identified structural series increased the activity of mutant glucocerebrosidase by 40-90%. In addition, confocal microscopy using antibodies against glucocerebrosidase demonstrated enhanced lysosomal co-localization in the treated N370S lines, indicating chaperone activity. These novel small molecules have potential as leads for chaperone therapy for Gaucher disease, and this paradigm promises to accelerate the development of leads for other rare genetic disorders.

Molecular Basis of Disorders With Complex Inheritance
Exhibit Hall E Wed 4:30PM-6:30PM, Thu 4:30PM-6:30PM, Fri 10:30AM-12:30PM

Poster 2363/W
Presentation Time: Wed, Oct 24, 2007, 4:30PM-6:30PM
Detecting Loci That Confer Susceptibility to Dust Mite-Induced Asthma Using a Combined *In Vivo* and *In Silico* Approach. *S.N.P. Kelada[1], D.M. Brass[2], S. Maruoka[2], D.A. Schwartz[2,3], F.S. Collins[1]* 1) Genome Technology Branch, National Human Genome Research Institute, Bethesda, MD; 2) Laboratory of Environmental Lung Diseases, National Heart Lung and Blood Institute, Research Triangle Park, NC; 3) National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Asthma is a disease of major public health concern. The etiology of asthma is multifactorial in nature, and involves interactions between genes and environment. Allergen exposure is a well known inciting factor for asthma among atopic individuals. In particular, house dust mite (HDM) exposure has consistently been linked to the development of asthma and exacerbations of symptoms. We aim to identify loci that confer susceptibility to HDM-induced asthma by examining the effects of HDM exposure *in vivo* across thirty inbred strains of mice whose genetic variation has previously been well characterized by a public mouse HapMap effort.

Mice are sensitized by two intra-peritoneal injections (on days 0 and 7) of purified natural dust mite allergen (nDer p 1), followed by oro-tracheal administration of the allergen on day 14. Forty-eight hours after airway challenge, cytokine levels and inflammatory cell influx into the lungs are measured, as well as pulmonary function by means of the Flexivent technique. Changes in gene expression in airway epithelial cells and T cells from lymph nodes are also examined. Strain-dependent responses (phenotypes) can then be mapped to loci *in silico* using a newly developed genome-wide association method that accounts for the population structure of the inbred strains of mice and employs a set of approximately 150,000 publicly accessible haplotype tagging SNPs. Both cis- and trans- expression determinants can be mapped using this method. Results from these experiments will be used to guide candidate gene selection in a case-control study of asthma susceptibility in humans.

Genomics Session
Exhibit Hall E Wed 4:30PM-6:30PM, Thu 4:30PM-6:30PM, Fri 10:30AM-12:30PM

Poster 2527/W
Presentation Time: Wed, Oct 24, 2007, 4:30PM-6:30PM
CGH microarray analyses in Proteus syndrome. *M.J. Lindhurst[1], J.J. Johnston[1], S.J. Vacha[2], L.G. Biesecker[1]* 1) GDRB, NHGRI/NIH, Bethesda, MD; 2) Agilent Technologies, Inc. Santa Clara, CA.

Proteus syndrome (PS) is a rare sporadic disorder that is characterized by overgrowth of multiple tissues. It is highly variable; patients have a mosaic distribution of lesions that progressively worsen with age. The hypothesis is that a genetic alteration occurs post-zygotically that results in growth dysregulation in tissues derived from the mutant cell. Because the disorder is not inherited, traditional methods for studying genetic diseases are not amenable to studying PS. We hypothesize that a subset of patients has a genomic scale duplication or deletion that causes overgrowth. We have used oligo-based CGH microarray technology to compare genomic DNA extracted from several types of patient tissue. Most comparisons were done using DNA extracted from affected and unaffected areas of the same patient using either cultured cells or DNA extracted directly from affected tissue samples. In addition, three comparisons were done between affected DNA and standard reference DNA. Initially, 14 hybridizations were performed using a CGH microarray platform containing 244K probes. Analyses of these arrays yielded no obvious aberrations, however, there were 529 regions with high LogRatio changes. To confirm these results and further characterize these regions, custom 4 x 44K oligo arrays were designed that zoomed in on each of these areas. Probes that were located within 15 Kb to either side of the probe of interest were chosen for the custom array resulting in 50-80 probes per region. Twelve hybridizations with the DNAs labeled with the opposite fluor were repeated using the custom 44K zoom-in array. Over 185 regions still remain with one or more probes that have an amplification or deletion score of 0.5 or more. Several criteria can be chosen to use for prioritizing follow up studies. However, all have caveats making the choice difficult. The sporadic, mosaic characteristics of PS provide an added challenge in interpreting high-resolution screening technologies, as any single probe outlier could be worthy of further study. We propose to share this data set collaboratively with other investigators to allow a thorough and rational approach to follow-up studies.

Poster 2690/W
Presentation Time: Wed, Oct 24, 2007, 4:30PM-6:30PM
Purine/Pyrimidine Motif Differences in Recombination Hotspots and Coldspots. *J. Cai[1], P.R. Calkins[2], J.C. Cohen[3], A.F. Wilson[1]* 1) Genometrics Section, NHGRI, NIH, Baltimore, MD; 2) Dept of Pathology, Texas Children's Hospital, Baylor College of Medicine, Houston, TX; 3) Dept of Pediatrics, Stony Brook University Health Science Center, School of Medicine, Stony Brook, NY.

During the past few years, it has become apparent that the locations of recombination events are clustered in a small proportion of the human genome. Recombination hotspots are regions of one or two thousand base pairs of DNA where the recombination rate is significantly higher than elsewhere in the genome. Hotspots are often flanked by coldspots, regions of lower than average frequency of recombination. With the identification of a large set of hotspots in the human genome, the frequency and distribution of specific sequence motifs can be compared in hotspot and coldspot regions. To date, relatively few sequence motifs have been associated with hotspots. The frequency of every 7bp motif was compared in hotspot relative to coldspot regions, and paired t-tests were calculated; however, based on previous unpublished work, sequence composition was considered at the purine/pyrimidine level (RY) rather than at the ACGT nucleotide level. Of all possible 7-mers at the purine/pyrimidine level, 0.86 fewer copies of the RYYRRYR/YRYYRRY motifs were found in the hotspots relative to the coldspots ($p < 1.37x10^{-23}$), while the RRRRRRR/YYYYYYY motifs were more frequent in the hotspots than in the coldspots (1.82 copies, $p < 1.83x10^{-30}$). When the regions flanking hotspots were compared to the regions flanking the coldspots, the differences between the frequencies of both motifs in hotspots relative to coldspots approached zero as the distance from the hotspot/coldspot boundary increased. Permutation tests, where motif frequencies in hotspot or coldspot regions were compared to those of size-matched random sequences from the genome, confirmed that the RYYRRYR/YRYYRRY motifs were less frequent in hotspots while the RRRRRRR/YYYYYYY motifs were more frequent in coldspots. Studying the composition of recombination hotspots may help elucidate the factors that

affect recombination and understand the molecular mechanism and regulation of crossover events as well as the evolutionary forces affecting recombination.

Poster 2712/W
Presentation Time: Wed, Oct 24, 2007, 4:30PM-6:30PM
Identifying Disease-causing Non-coding Mutations by Medical Sequencing. *T. Hefferon[1], S.Q. Lee-Lin[1], J. Idol[1], V. Maduro[1], S. Terry[2], A. Sharp[3], E.D. Green[1], NIH Intramural Sequencing Center* 1) NHGRI, NIH, Bethesda, MD; 2) PXE Int'l, Washington, DC; 3) Dept. Genome Sciences, U. Washington, Seattle, WA.

The comparison of genome sequences from diverse vertebrate species has enabled the identification of highly conserved regions that are under negative selection. Having resisted mutation over evolutionary time, such regions are likely to contain functional genomic elements that are important for the survival of organisms. We are using a comparative genomics approach to identify highly conserved non-coding regions in and around known human disease genes, and then screening those regions by medical sequencing for possible disease-causing mutations. In two related projects, we are studying patients with cystic fibrosis (CF) or pseudoxanthoma elasticum (PXE). The genomic regions encompassing both genes mutated in these disorders (*CFTR* and *ABCC6*, respectively) have been sequenced in multiple species, allowing the identification of multi-species conserved sequences (MCSs). We are using a medical-sequencing approach to screen DNA samples from patients where one or both mutations remain unidentified after rigorous screening of coding, splice, and promoter regions; since these patients do not appear to have two coding mutations, they may carry disease-causing changes in non-coding functional sequences. We have found multiple variants in both genes, and are following them up with further studies to define their possible functional roles. Our *CFTR* studies are being aided by the rich data sets for the corresponding genomic regions generated by the ENCODE project; these data are providing important insights about the possible function of the conserved non-coding regions being examined. Meanwhile, our *ABCC6* studies are complicated by the presence of two partial pseudogenes in the genomic region of interest, which are products of segmental duplications; this raises the possibility that copy-number changes may account for the disease in some patients. Together, these projects illustrate the complexities associated with the search for disease-causing mutations in some genetic diseases and the important interface between comparative genomics and medical sequencing in human genetics studies.

Genetics Education Session
Exhibit Hall E Wed 4:30PM-6:30PM, Thu 4:30PM-6:30PM, Fri 10:30AM-12:30PM

Poster 823/W
Presentation Time: Wed, Oct 24, 2007, 4:30PM-6:30PM
Felix the Double Helix: Teaching Elementary Students about DNA. *H.D. Edwards[1], W.J. Introne[1], A.M. Garcia[1], T.C. Markello[1], H.M. Dorward[1], M.A. Kayser[1], D.M. Krasnewich[1], G.A. Gahl[1,2], M.A. Merideth[1,2]* 1) NHGRI, NIH, Bethesda, MD; 2) Intramural ORD, NIH, Bethesda, MD.

Recent evidence supports the theory that early science education in children improves their natural scientific and math abilities (1). Given the paucity of curriculum material for genetics education of elementary-age children, we have designed an interactive educational project to teach kindergarten through second grade students about DNA through the use of a life-size costume: Felix the Double Helix. The main goals of this community outreach project are to introduce elementary students to “science in action,” and promote an interest in science The presentation, which lasts 30 minutes, incorporates the use of songs, a game and audience participation to meet 4 main teaching objectives: 1) What is DNA? 2) Where can we find DNA? 3) Why does Felix the Double Helix look the way he does? 4) How can we protect our DNA? The presentation is given in both English and Spanish to meet the needs of the primarily Spanish-speaking student population. Ultraviolet light beaded bracelets are distributed at the end of the program to reinforce the message about protecting DNA from sun damage by using sunscreen. Evaluation forms are given to the teachers and reviewed by the team to adjust the presentation based on their feedback . Continued development of curriculum to educate elementary school children will assist in meeting the goal of promoting science and math. Future plans for this project include finding optimal tools to assess the comprehension level of the children and expanding the program presentation materials to higher elementary school grade learning levels. 1) Gallenstein, NL. Engaging young children in science and mathematics. Journal of Elementary Science Education, 9/22/05.

Poster 827/W
Presentation Time: Wed, Oct 24, 2007, 4:30PM-6:30PM
Methods of Educating the Next Generation in Genetics and Genomics Science. *S.E. Harding, V.L. Bonham, C.L. Easter, D.H. Lea, J. Witherly* Educ and Community Involv, National Human Genome Research Institute/NIH, Bethesda, MD.

The overall goal of this presentation is to describe two genomic science education programs developed by the National Human Genome Research Institute (NHGRI) for students and faculty. The NHGRI Education and Community Involvement Branch (ECIB), created in 2003, serves as a liaison between NHGRI and the public to inform the public of the latest advances in genomics. One of ECIB’s main strategies is to reach out to high school and college faculty who have shown an interest in genetics and genomics but who have not yet integrated these topics into their curricula, as well as high school and college students who have shown an interest in science and genetics but have not yet determined their career path. To that end NHGRI established a Current Topics in Genomic Research Short Course in 1997 to engage students and faculty from underrepresented minority institutions to incorporate genomics into the curriculum and to expose students to genomic research careers. Over the past 10 years, 300 faculty and students from underrepresented minority and rural institutions have participated in the Short Course. ECIB also reaches out to students across the country with National DNA Day, a nationally recognized science education program aimed at high school students. NHGRI partners with ASHG, the Genetic Alliance and the National Society of Genetic Counselors to connect genetics professionals with science classrooms around the country. Through the use of educational materials, online resources and speakers, students learn about the latest advances in genetics, as well as ways they might get involved in the field. Beginning in 2005, high school students across the nation have been invited to take part in a live, on-line Chatroom staffed by NHGRI. In 2007 NHGRI staff received a 52 percent increase in questions from 2006 and responded to a total of 648 questions answered in the 10 hour period. In this presentation, the two programs will be described including the number of students and faculty reached; the number and type of institutions participating; results of evaluations indicating how information has been used by participants; and how these programs can be adapted.

DAY III THURSDAY, OCTOBER 25

Plenary and Platform Presentations

Metabolic Disorders Session Room 28
Thu Oct 25, 2007 08:00AM-10:30AM

Platform Presentation 51
Presentation Time: 09:00AM-09:15AM
In Chediak-Higashi syndrome melanocytes, giant melanosomes do not target to the dendritic tip's actin network. *W. Westbroek, A. Helip-Wooley, H. Dorward, W.A. Gahl* Medical Genetics Branch, NHGRI/MGB/NIH, Bethesda, MD.

Chediak-Higashi syndrome (CHS) is a rare autosomal recessive disorder caused by mutations in the CHS1 gene. Clinical characteristics include partial oculocutaneous albinism, recurrent infections, a bleeding diathesis, enlarged lysosomes in every cell type, and late-onset progressive neurological impairment. We report a CHS patient with two truncating CHS1 mutations, i.e., a nonsense (p.R514X)) and a frameshift mutation (p.F3298fsX3304). This patient had significant hypomelanosis of the skin, hair and eye. In normal melanocytes, melanosomes undergo microtubule and actin-dependent transport toward the dendritic periphery. Actin-mediated transport is dependent on the Rab27a/Melanophilin/Myosin Va tripartite complex. Rab27a-GTP interacts through its geranylgeranyl lipid tail with the melanosomal membrane, where it acts as a receptor for its effector, Melanophilin, and the Myosin Va motor protein. We investigated whether the melanosomes in CHS were correctly tethered to the actin filaments in the dendritic tips. Bright field microscopy revealed that CHS melanocytes harbor enlarged melanosomes that localized to the cell body and dendrites, but not to the dendritic tips, as observed in normal melanocytes. Confocal microscopy showed that Rab27a did not associate with enlarged melanosomes in cultured CHS melanocytes. Furthermore, Melanophilin and Myosin Va did not co-localize with the enlarged melanosomes; in normal melanocytes, Melanophilin and Myosin Va nearly always co-localized with peripheral melanosomes. Next, we employed a melanosome-specific transcript of Myosin Va fused to GFP for additional studies. In normal melanocytes, Myosin Va-GFP co-localized with Rab27a, Melanophilin, and melanosomes, while in CHS co-localization occurred only with Melanophilin in the dendritic tips. This investigation showed that the Rab27a/Melanophlin/Myosin Va tripartite complex did not form on enlarged CHS melanosomes. Absence of melanosome tethering to the actin in dendritic tips of melanocytes could explain the skin hypomelanosis associated with CHS.

Genomics Session Room 20D
Thu Oct 25, 2007 08:00AM-10:30AM

Platform Presentation 46
Presentation Time: 10:15AM-10:30AM
The Pattern of RET Mutations and Variants in Hirschsprung Disease: A Medical Sequencing Case Study.
L. Hao[1], S. Arnold[1], J. Albertus[1], M. Dao[1], A. Rea[1], P. Cruz[2], J. Mullikin[2], A. Young[2], E.D. Green[2], A. Chakravarti[1] 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Genome Technology Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD.

Gene-based medical sequencing is critical to our understanding of complex diseases, following the discovery of new genes by association studies. One limitation for medical sequencing studies is the difficulty of interpreting base changes when functional annotation is incomplete. We present here a case study for a model complex genetic disorder, Hirschprung disease (HSCR), in which enteric ganglion cells are absent along variable lengths of the GI tract. RET, encoding a receptor tyrosine kinase, is functionally necessary, but not sufficient, for normal enteric development. Genetic data suggest that RET mutations must exist in each affected despite the involvement of other genes and, thus, many RET mutations and polymorphisms interact to produce disease. To identify both common and rare genetic variants, we are sequencing all 20 exons and 20 additional conserved non-coding regions at RET from 680 individuals including 237 probands and their families (~20Mb). Based on our analysis of 67% of the data, we have identified very high genetic variability with a total of 239 variants including 10 indels and 37 coding alterations, most of which are novel, rare and non-synonymous. The comparison of the frequency of sequence changes associated with transmitted and non-transmitted alleles in HSCR families validated the association of a previously identified RET enhancer variant. Interestingly, we identified a new premature stop mutation in the RET kinase domain that appears to interact with the non-coding enhancer mutation and contribute to the severest forms of HSCR. In addition, sequencing in families has allowed us to identify a few potentially large indels from Mendelian inconsistencies that would have been missed without family data. Our data answers questions such as the contribution of RET to HSCR, the parental origin of mutation and the role of rare and common mutations and, thus, their genetic mechanisms of action.

Invited Session 24 Room 20D
Thu Oct 25, 2007 11:00AM-01:00PM
Complex Human Disease Genes: Help from Animal Models Session

The speakers in this session will illustrate the synergistic power of human/animal comparative genetic approaches in the identification of genes involved in complex human diseases. In addition there will examples of how new bioinformatic resources can be exploited to more rapidly identify genes in animal models and accelerate human studies. Dr. Beverly Paigen will discuss the use of mouse-human comparative QTL analysis to identify genes involved in atherosclerosis. Dr. Elaine Ostrander will describe approaches to identify genes involved in cancer susceptibility using human genetics with help from underutilized canine genetic resources. Dr. Lisa Tarantino will describe her studies using mouse genetic, genomic, and bioinformatic resources to identify genes involved in anxiety. Dr. Abraham Palmer will describe his research using both mouse genetics and human association

studies to identify genes involved in methamphetamine sensitivity.

Presentation 11:30AM-11:55AM
Mapping Complex Traits of Concern for Humans in Dogs. *E. A. Ostrander* Cancer Genetics Branch, NHGRI/NIH, Bethesda, MD.

Genetic Counseling and Clinical Session Room 29
Thu Oct 25, 2007 02:00PM-04:30PM

Platform Presentation 129
Presentation Time: 02:30PM-02:45PM
Social support, communal coping and psychological status in sisters in Hereditary Breast and Ovarian Cancer (HBOC) families. *J.A. Peters[1], L. Koehly[2], L. Hoskins[1], N. Kuhn[2], A. Letocha[2], R. Kenen[3], J. Loud[1], M.H. Greene[1]* 1) Clinical Genetics Branch, DCEG, NCI/NIH/DHHS, Rockville, MD; 2) SBRB/NHGRI/NIH/DHHS, Bethesda, MD; 3) The College of New Jersey, Ewing, NJ.

Adult sisters in HBOC families often undergo genetic counseling and testing together but the social context of their long-term adjustment to genetic information is rarely a focus of research. We conducted a quantitative, descriptive, cross-sectional study of 65 sisters from 31 HBOC families within a larger Breast Imaging Study (NCI-01-C-009) for high risk women. The aims were to consider how the size of the sisters' social networks and which communal coping measures related to psychological distress. We performed social network analyses using data from the Brief Symptom Inventory-18 to determine anxiety, somatization and depression and the Colored Eco Genetic Relationship Map (CEGRM) to identify family and non-family members of participants' social support networks. Intra-family correlation coefficients suggest that these sisters share perceptions of breast cancer risk and worry, but not ovarian cancer risk and worry. Additionally, sisters indicated shared levels of anxiety and somatization, but not depressive symptoms. Communal coping indices of shared support resources were related to anxiety and somatization, with larger numbers of shared emotional supports associated with lower levels of anxiety and lower levels of somatization. Having more shared informants regarding cancer risk was positively associated with somatization. Having a large emotional support network was negatively associated with anxiety. Participants with lower depression scores had more persons playing multiple support roles and fewer individuals providing tangible assistance. In summary, we found that quantity, function, and communal aspects of social exchanges are differentially correlated with self-reported anxiety, somatization and depression. Understanding the specific ways in which quality, quantity and types of supportive relationships impact sisters' well-being will allow us to develop appropriate management strategies to help cancer-prone families better adjust to their cancer risk.

Complex Disease Mechanisms Session Room 20A
Thu Oct 25, 2007 02:00PM-04:30PM

Platform Presentation 87
Presentation Time: 02:00PM-02:15PM
Disruption of an AP-2 binding site upstream of *IRF6* is commonly associated with nonsyndromic cleft lip and palate. *F. Rahimov[1], M.J. Hitchler[2], F.E. Domann[2], A. Jugessur[3], R.T. Lie[3], A.J. Wilcox[4], K. Christensen[5], E.D. Green[6], M. L. Marazita[7], B.C. Schutte[1], J.C. Murray[1]* 1) Dept Pediatrics, Univ Iowa; 2) Dept Rad Onc, Univ Iowa; 3) Univ Bergen, Norway; 4) NIEHS, Durham, NC; 5) Univ Southern Denmark; 6) NHGRI, NIH; 7) Center Craniof Dent Genet, Univ Pittsburgh.

Nonsyndromic cleft lip and palate (NSCLP) is a common craniofacial birth defect. We discovered that mutations in *IRF6* underlie Van der Woude syndrome (VWS), an orofacial clefting disorder where lower lip pits are the only features distinguishing VWS from NSCLP. Subsequently, we reported a strong association between SNPs in the *IRF6* locus and NSCLP. We observed a particularly strong overtransmission of the ancestral allele V of the rs2235371 (V274I) SNP in individuals of Asian and South American ancestry. However, the frequency of the risk allele is over 97% in European and African populations making it an unlikely candidate for the etiological mutation. Direct sequencing of the coding regions of *IRF6* did not detect potential causative mutations. We postulated that the causative variant(s) are in linkage disequilibrium with V274I and could reside in the regulatory element(s) of *IRF6*. Using comparative genomic sequence analysis from 14 vertebrate species, we detected a highly conserved region 9.7kb upstream of *IRF6*. Family-based association analysis in Norwegian, Danish and Filipino populations showed strong overtransmission of a conserved SNP (rs642961) in this region ($p<2x10^{-8}$). The ancestral allele G and the derived allele A of rs642961 split the V allele of V274I into two haplotypes. The V-A haplotype is significantly overtransmitted ($p<3x10^{-8}$), whereas transmission of the V-G haplotype is not distorted ($p<0.7$). Gel shift assays showed that the A allele of rs642961 disrupts binding activity of the transcription factor AP-2 alpha. *TFAP2A* is highly expressed in craniofacial structures and knockout mice have multiple facial anomalies. A ChIP assay showed that AP-2 binds to its consensus binding sites *in vivo* suggesting that it could function upstream of *IRF6*. In total, our data suggests that a common functional variant upstream of *IRF6* contributes to NSCLP and implicates AP-2 in the IRF6 developmental pathway.

Poster Presentations

Cancer Genetics Session
Exhibit Hall E Wed 4:30PM-6:30PM, Thu 4:30PM-6:30PM, Fri 10:30AM-12:30PM

Poster 389/T
Presentation Time: Thu, Oct 25, 2007, 4:30PM-6:30PM
Fine-mapping of 42 hereditary prostate cancer families narrows the interval for a susceptibility locus on chromosome 22q12.3. *B. Johanneson[1], S.K. McDonnell[2], D.M. Karyadi[1], S.J. Hebbring[3], L. Wang[3], K. Deutsch[6], L. McIntosh[4], E.M. Kwon[1], M. Suuriniemi[1], J. Stanford[4,5], D.J. Schaid[2], E.A. Ostrander[1], S.N. Thibodeau[2]* 1) Institute, National Institutes of Health, Bethesda, MD 20892; 2) Department of Health Sciences Research, Mayo Clinic, Rochester, MN 55905; 3) Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN 55905; 4) Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Box 19024, Seattle, WA 98109; 5) School of Public Health and Community Medicine, University of Washington, Seattle, WA 98115; 6) Institute for Systems Biology, Seattle, WA 98103. Genetic studies suggest that hereditary prostate cancer is a genetically heterogeneous disease with multiple contributing loci. Studies of high-risk prostate cancer families selected for aggressive disease, analysis of large multigenerational families, and a meta-analysis from the International Consortium for Prostate Cancer Genetics (ICPCG), all highlight

chromosome 22q12.3 as a susceptibility locus. Our study is the most detailed fine-mapping analysis of this region to date. Of 173 high-risk families from the Mayo Clinic and 254 from the Prostate Cancer Genetic Research Study (PROGRESS), 42 were identified as having a shared haplotype among all affected men that overlapped the 22q12.3 region. In 35 of the families, an overlapping consensus region of 8.73 Mb is defined. However, in the subset of 14 families with ≥ 5 affected men per family, a 2.53 Mb shared consensus segment is identified in 12 of the families that overlaps with previously published intervals. Combining these results with data from the other published studies, a three-recombinant consensus interval is found in 52 of 54 families which narrows down the region to 1.36 Mb between 33.72 Mb and 35.08 Mb. Overall, our results provide the most comprehensive framework achievable for candidate gene testing. Ongoing studies are aimed at evaluating genes in this region for variants associated with prostate cancer risk.

Clinical Genetics, Malformations and Dysmorphology Session
Exhibit Hall E Wed 4:30PM-6:30PM, Thu 4:30PM-6:30PM, Fri 10:30AM-12:30PM

Poster 506/T
Presentation Time: Thu, Oct 25, 2007, 4:30PM-6:30PM
Detailed analysis of the 17p11.2 region in 59 patients with Smith-Magenis syndrome. *M. Huizing[1], H. Edwards[1], C. Ciccone[1], M.P. Jones[1], S.C. Chandrasekharappa[1], C. Bendavid[2], J. Blancato[3], W.A. Gahl[1], A.C.M. Smith[1]* 1) NHGRI/NIH, Bethesda, MD; 2) Univ Rennes, France; 3) Georgetown Univ, Washington, DC.

Smith-Magenis syndrome (SMS) is characterized by distinct craniofacial and skeletal anomalies, speech/language delays, psychomotor and growth retardation, a striking neurobehavioral phenotype, and chronic sleep disorder related to an inverted circadian melatonin rhythm. Most cases are due to an interstitial deletion of 17p11.2; however, rare 'non-deletion' cases can be due to *RAI1* mutations. We performed a genotype-phenotype correlation on 59 SMS patients. Phenotype studies revealed some unique and variable clinical features, including hearing loss, low IgA levels, high cholesterol and skeletal features. We employed a dense map of 17p markers to determine the parent of origin of the deleted allele and found a slight skewing towards the maternal (63%) versus paternal (37%) allele, though this was not statistically significant. FISH analysis and quantitative real-time PCR (qPCR) were performed to determine the copy number of genes in the 17p11.2 area. qPCR assays for six genes of interest surrounding the SMS breakpoints were designed, including *RAI1* and *RASD1* (implicated in circadian rhythm), *MYO15A* (involved in hearing loss), *FLI1* (related to immune response), *PEMT* (functions in choline metabolism) and *TNFRSF13B* (implicated in IgA deficiency). The majority (56%) of patients had the common 17p11.2 deletion (3.5Mb), as expected. 11 patients (19%) had variable breakpoints, however, their clinical features could not be directly related to the copynumber of our 6 genes. 15 patients (25%) did not show a 17p11.2 deletion by FISH or qPCR, *RAI1* mutation analysis so far showed a novel mutation (P242L) in one of these patients. The non-deleted patients are being screened by whole genome CGH-array for possible novel chromosomal rearrangements. Our study emphasizes the value of a natural history study to recognize novel clinical features and outlier patients. We were unable to show a strong genotype-phenotype correlation. However, determination of exact breakpoints and the influence of genes outside the breakpoints on the resultant phenotype may shed more light on this.

Epigenetics Session
Exhibit Hall E Wed 4:30PM-6:30PM, Thu 4:30PM-6:30PM, Fri 10:30AM-12:30PM

Poster 712/T
Presentation Time: Thu, Oct 25, 2007, 4:30PM-6:30PM
Genome-Wide Analysis of Alterations in Histone Methylation and Gene Expression in Hutchinson-Gilford Progeria Syndrome. *K. Cao, D. Faddah, M.R. Erdos, B.C. Capell, F.S. Collins* National Human Genome Research Institute, National Institutes of Health, Bethesda, MD.

Hutchinson-Gilford progeria syndrome (HGPS) is a rare genetic disorder with widespread phenotypic features of premature aging. Classic HGPS is caused by a de novo point mutation in exon 11 of the LMNA gene, activating a cryptic splice donor and resulting in a mutant lamin A protein termed “progerin” that lacks 50 amino acids near the carboxyl terminus. During interphase, progerin anchors to the nuclear membrane, disrupting the nuclear scaffold and causing nuclear blebbing that has been referred to as the cellular hallmark of HGPS. Given the known interactions between the nuclear lamina and transcription factors, as well as the evidence that changes in modified histones predate the blebbed nuclear morphology in HGPS, we hypothesized that progerin causes cell damage not only by its structural effects, but in the way it alters chromatin structure and transcriptional regulation. To test our hypothesis, we have implemented a combined approach using expression array analysis and ChIP-chip (chromatin immunoprecipitation coupled with DNA microarray technology). We studied fibroblasts from normal and HGPS individuals, and generated tet-inducible cultured cells expressing progerin to assess the early events following progerin expression. Expression microarray analysis defined a set of 235 genes that show at least a two-fold, statistically significant change in HGPS. Parallel ChIP-chip analysis using ENCODE and human promoter arrays generated high-resolution maps for the distribution of H3K4, H3K27, and H3K36 trimethylation. Combining these data sets led to the identification of an initial list of differentially active and suppressed genes that may explain some of the cellular phenotypic features of HGPS. Furthermore, we have recently employed the Illumina/Solexa sequencing technology to map histone methylation patterns across the entire genome in HGPS. This study provides novel insights into the complex relationship between transcriptional regulation and chromatin organization in both HGPS and normal aging.

Poster 729/T
Presentation Time: Thu, Oct 25, 2007, 4:30PM-6:30PM
DNA methylation profiles in diffuse large B-cell lymphoma and their relationship to gene expression status. *X. Wang[1], T.C. Greiner[2], B.L. Pike[1], D.D. Weisenburger[2], Y. Hsu[1], G. Renaud[3], T.G. Wolfsberg[3], M. Kim[1], D.J. Weisenberger[1], K.D. Siegmund[1], W. Ye[1], S. Groshen[1], R. Mehrian-Shai[1], W.C. Chan[2], P.W. Laird[1], J.G. Hacia[1]* 1) Biochemistry & Molecular Biology, University of Southern California, los angeles, CA; 2) University of Nebraska Medical Center, Omaha, NE; 3) National Institutes of Health, Bethesda, Maryland.

While gene expression, genomic copy number, and mutational analyses have provided key insights into the genetic basis for the extensive pathologic and biologic heterogeneity in diffuse large B-cell lymphoma (DLBCL), considerably less is known about its epigenetic underpinnings. Here, we evaluated the DNA methylation levels of over 500 unique gene-associated

CpG islands in fourteen DLBCL tumors using McrBC-based CpG island microarray, MethyLight, and bisulfite sequencing analyses. Although we observed variation in DNA methylation across all DLBCL, we identified twelve CpG islands (*AR*, *CDKN1C*, *DLC1*, *DRD2*, *GATA4*, *GDNF*, *GRIN2B*, *MTHFR*, *MYOD1*, *NEUROD1*, *ONECUT2*, and *TFAP2A*) showing significant methylation in greater than 85% of the tumors surveyed. Interestingly, we found that the methylation levels of CpG islands proximal to *FLJ21062* and *ONECUT2* differed between activated B-cell-like (ABC-DLBCL) and germinal center B-cell-like (GCB-DLBCL) subtypes, which have distinct clinical outcomes. In addition, we compared the methylation and expression status of sixty-seven genes located within 500-bp of our methylation assays. Our observations are more consistent with the potential involvement of DNA methylation in the maintenance relative to the initiation of gene silencing. Nevertheless, the proportional reductions in *BNIP3*, *MGMT*, *RBP1*, *GATA4*, *IGSF4*, *CRABP1* and *FLJ21062* expression with increasing methylation suggests that epigenetic processes could be causally involved in the initial stages of gene silencing. Overall, the genes highlighted in our analyses warrant further investigation into their roles in the development and progression of DLBCL and potential as clinical biomarkers.

Mapping, Linkage and Linkage Disequilibrium Session
Exhibit Hall E Wed 4:30PM-6:30PM, Thu 4:30PM-6:30PM, Fri 10:30AM-12:30PM

Poster 1215/T
Presentation Time: Thu, Oct 25, 2007, 4:30PM-6:30PM
Fine-mapping of genome-wide breast cancer association study. *M.S. Udler[1], K.A. Pooley[2], A.M. Dunning[2], P.D. Pharoah[2], D.G. Ballinger[3], J.P. Struewing[4], R. Luben[1], S. Ahmed[2], C.S. Healey[2], Search Collaborators[2], C.A. Haiman[5], P. Brennan[6], C.Y. Shen[7], D. Kang[8], D.R. Cox[3], E.A. Ostrander[9], B.A.J. Ponder[10], D.F. Easton[1]* 1) Department of Public Health and Primary Care, University of Cambridge, UK; 2) Department of Oncology, University of Cambridge, UK; 3) Perlegen Sciences, Inc., Mountain View, CA; 4) Laboratory of Population Genetics, NCI, Bethesda, MD; 5) Department of Preventive Medicine, Keck School of Medicine, USC, Los Angeles, CA; 6) International Agency for Research on Cancer, Lyon, France; 7) Institute of Biomedical Sciences, Academia Sinica, Taipeii, Taiwan; 8) Seoul National University College of Medicine, Seoul, Korea; 9) Cancer Genetics Branch, NHGRI, Bethesda, MD; 10) CR-UK Cambridge Research Institute, UK.

Genome-wide association (GWA) studies utilize linkage disequilibrium (LD) between Single Nucleotide Polymorphisms (SNPs) in a population to identify genetic variants that are associated with increased risk of disease. Since SNPs located close to each other on a chromosome may be correlated, it is often difficult to determine which is the causal SNP. We have applied statistical techniques for fine-mapping to susceptibility loci identified in a breast cancer GWA study. The three strongest associations from the study were in *FGFR2*, *TNRC9*, and *MAP3K1*, located in LD blocks on chromosomes 10, 16, and 5, respectively. Here we investigate the use of single and multiple SNP analyses as well as haplotype analyses using data from 8,792 cases and 8,200 controls from five studies of European and Asian descent. For the haplotype analyses, an Ancestral Recombination Graph-based approach (Margarita[1]) and a clustering algorithm (HapCluster[2]) were utilized. Through these approaches, the number of candidate causal SNPs was considerably decreased. In *FGFR2*, of 117 SNPs in the region, all but six were excluded at odds of 100:1 after applying these methods. In this case, logistic regression following genotype imputation provided a straightforward analytical approach, and haplotype-based analysis did not provide additional precision.

[1]Minichiello MJ, Durbin R. Am J Hum Genet. 2006;79:910-22.
[2]Waldron ERB, et al. Genetic Epidemiology. 2006;30:170-9.

Cardiovascular Genetics Session
Exhibit Hall E Wed 4:30PM-6:30PM, Thu 4:30PM-6:30PM, Fri 10:30AM-12:30PM

Poster 1783/T
Presentation Time: Thu, Oct 25, 2007, 4:30PM-6:30PM
Genome-wide association scan for HDL cholesterol, LDL cholesterol and triglyceride levels in 9,000 individuals. *C.J. Willer[1], A. Scuteri[2,3], L.L. Bonnycastle[4], S. Sanna[5], A.U. Jackson[1], A. Maschio[5], W.L. Duren[1], F. Busonero[5], R. Pruim[6], Diabetes Genetics Initiative[7], R.M. Watanabe[8], S.S. Najjar[2], L.J. Scott[1], M. Uda[2], J. Tuomilehto[9], G.R. Abecasis[1], F.S. Collins[4], D. Schlessinger[2], K.L. Mohlke[10], E.G. Lakatta[2]* 1) Dept Biostatistics, Univ Michigan; 2) Gerontology Research Center, National Institute on Aging; 3) Unita Operative Geriatrica INRCA, Rome Italy; 4) Genome Technology Branch, National Human Genome Research Institute; 5) Istituto di Neurogenetica e Neurofarmacologia, CNR, Cagliari, Italy; 6) Dept Mathematics & Statistics, Calvin College; 7) Broad Institute of Harvard & MIT, Lund Univ, & Novartis Institutes of BioMedical Research; 8) Dept of Physiology and Biophysics, Keck School of Medicine, Univ Southern California; 9) Dept Epidemiology & Health Promotion, Dept Biochemistry, National Public Health Institute, Helsinki, Finland; 10) Dept Genetics, Univ North Carolina.

Cardiovascular diseases (CVD) are the leading cause of death in industrialized countries. Low density lipoprotein cholesterol (LDL) is a major risk factor for CVD whereas high density lipoprotein cholesterol (HDL) protects against CVD. Triglyceride levels (TG) may also be associated with risk of coronary artery disease. Heritability of these traits is between 30 and 60%. We have combined genome-wide association data from the ProgeNIA study of 4,301 Sardinian individuals from 450 families, the FUSION study of 2,337 Finnish individuals and 2,659 Caucasian individuals from the Diabetes Genetics Initiative (DGI). To allow for meta-analysis with genotyped SNPs from two platforms (Affymetrix 500k and Illumina 300k), we imputed genotypes for untyped SNPs in the FUSION individuals. Meta-analysis provided clear association with several previously reported loci, including *APOC1* (LDL, $p = 1 \times 10^{-18}$), *GCKR* (TG, $p = 3 \times 10^{-16}$), *CETP* (HDL, 6×10^{-16}), *LPL* (TG, $p = 7 \times 10^{-15}$), *APOB* (TG, 9×10^{-10}), and *LIPC* (HDL, 2×10^{-8}). We detected second independent association signals in 5 of these genes ($p < 5 \times 10^{-6}$). We observed 15 new loci with $p < 5 \times 10^{-6}$ that we are in the process of genotyping in 7,300 individuals. The new loci appear to be involved in pathways such as cell adhesion and lipid metabolism.

Statistical Genetics and Genetic Epidemiology Session
Exhibit Hall E Wed 4:30PM-6:30PM, Thu 4:30PM-6:30PM, Fri 10:30AM-12:30PM

Poster 2167/T
Presentation Time: Thu, Oct 25, 2007, 4:30PM-6:30PM
Genome-wide association scan for height in 6,671 individuals from Finland and Sardinia. *S. Sanna[1,2], A.U. Jackson[1], G. Usala[2], C.J. Willer[1], M. Dei[2], L.L. Bonnycastle[3], S. Lai[2], Y. Li[1], M. Uda[2], M.R. Erdos[3], H. Shen[4], A. Shuldiner[4], A. Cao[2], R.M. Bergam[5], D. Schlessinger[2,6], F.S. Collins[3], M. Boehnke[1], G.R. Abecasis[1], R. Nagaraja[5], K.L. Mohlke[7]* 1) Dept Biostatistics, Univ Michigan, Ann Arbor, MI; 2) National Human Genome Research Institute, Bethesda, MD; 3) Istituto di Neurogenetica e Neurofarmacologia (INN), CNR, Cagliari, Italy;

4) University of Maryland, School of Medicine, Baltimore, MD; 5) Keck School of Medicine of USC, Los Angeles, CA; 6) Gerentology Reasearch Center, NIA, Baltimore, MD; 7) Dept Genetics, University North Carolina, Chapel Hill, NC.

Height represents a classic example of a highly heritable quantitative trait. In our sample, heritability analysis shows that genes can explain >80% of the variation in height. Nevertheless, with the exception of a few rare Mendelian syndromes, gene-identification has proved difficult despite many parallel mapping efforts. Genetic influences on height are probably due to the contribution of several loci of small effect. We have carried out a meta-analysis of genome-wide association results from two different groups, ProgeNIA and FUSION. The first sample consist of 4,305 individuals from 570 families from Sardinia, the second includes 2,366 mostly unrelated Finnish individuals. Since the two groups worked with two different platforms (Illumina 300K and Affymetrix 500K respectively), SNPs appearing only in one platform were imputed to allow direct comparison of results across studies. To control inflation of type I error due to outliers and departure from normality, quantile normalization was applied to each trait prior the analysis. In both GWA scans, we evaluated the additive effect of each SNP, adjusting the model for familiality and covariates. In our combined results, the top associated SNP (p=4.0*10-7) maps to a region of LD containing several genes, including one previously implicated in growth. Replication is ongoing, but preliminary results on 2017 Finnish and 858 Amish samples support our initial finding (p=1.7*10-3), with the same direction of effect. Further detailed SNP analysis of the region is necessary to refine the responsible gene.

Metabolic Disorders Session
Exhibit Hall E Wed 4:30PM-6:30PM, Thu 4:30PM-6:30PM, Fri 10:30AM-12:30PM

Poster 1517/T
Presentation Time: Thu, Oct 25, 2007, 4:30PM-6:30PM
Serum levels of the KL-6 epitope of MUC1 correlate with pulmonary fibrosis in Hermansky-Pudlak syndrome. *T.C. Markello[1,2], M. Anahtar[2], I. Bernardini[2], B.B. Gochuico[2], K. O'Brien[2], G.A. Golas[2], W.A. Gahl[2]* 1) 1 Department of Genetics & Metabolism, Children's National Medical Center, Washington, DC; 2) 2Section on Human Biochemical Genetics, Medical Genetics Branch, NHGRI, NIH, Bethesda MD.

Elevated serum levels of the KL-6 epitope of MUC1 have previously been correlated with the presence of interstitial lung diseases, including Idiopathic pulmonary fibrosis (IPF). Little is known about serum levels of KL-6 prior to the onset of clinical symptoms, since patients with IPF typically present after significant fibrosis has occurred, prompting medical attention for the resulting respiratory compromise. Type 1 Hermansky-Pudlak Syndrome (HPS) has a frequency of pulmonary fibrosis that approaches 100% in the 4th through 6th decades of life. Because patients with HPS are first ascertained by their oculocutaneous albinism and their bleeding diathesis, it is possible to analyze serum samples in HPS patients prior to the onset of clinically significant pulmonary fibrosis. We tested archival serum samples from patients with Hermansky Pudlak syndrome who were seen at the NIH Clinical Center between 1998 and 2007. These samples include HPS types 1 and 3, i.e., subtypes with and without pulmonary fibrosis. Compared to normal controls, both HPS type 1 and type 3 patients have a 2.2 fold elevation in KL-6/MUC1 levels prior to onset of clinical pulmonary fibrosis (N=14, range 1.12 to 3.14 fold vs normal controls). HPS type 1 patients have a 12 fold elevation in serum KL-6/MUC1 levels after the onset of clinical pulmonary fibrosis (n=13, range 4.7 to 42.0 fold vs normal controls). These results suggest a potential relationship between a genetic disorder of intracellular vesicle trafficking (i.e., HPS) and a protein that requires intracellular trafficking for proper glycosylation and apical targeting. Further characterization of KL-6/MUC1 and other pneumoproteins in HPS may be useful for early diagnosis and prognosis of pulmonary fibrosis. Serum levels of pneumoproteins, including KL-6/MUC1, may also serve as outcome parameters for future therapeutic interventions in HPS patients with pulmonary fibrosis.

Therapy for Genetic Disorders Session
Exhibit Hall E Wed 4:30PM-6:30PM, Thu 4:30PM-6:30PM, Fri 10:30AM-12:30PM

Poster 2295/T
Presentation Time: Thu, Oct 25, 2007, 4:30PM-6:30PM
Nitisinone (OrfadinR) reduces the massive fractional excretion of homogentisic acid in alkaptonuria patients. *M. Kayser, W. Introne, K. O'Brien, I. Bernardini, R. Kleta, W. Gahl* Human Biochemical Genetics Section, Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD.

Alkaptonuria (AKU), a rare metabolic disorder of impaired tyrosine catabolism, is due to deficiency of homogentisic acid oxygenase. An organic compound, homogentisic acid (HGA), accumulates and binds to connective tissue causing darkened urine, darkened cartilage (ochronosis), joint destruction, and cardiac valve deterioration. Homogentisic acid is actively secreted through organic anion transporters in the renal tubules at levels 3-4 times the glomerular filtration rate. In AKU patients, mean plasma levels of HGA are 6.6 ug/ml and urinary HGA excretion averages 4.2 grams per day, more than 100 times normal. Nitisinone (NTBC), a potent reversible inhibitor of p-hydroxyphenylpyruvic acid dioxygenase, was shown in two separate, small studies to reduce urine homogentisic acid excretion in AKU patients up to 95%. We measured urine and plasma HGA levels in 42 AKU patients enrolled in either a natural history or a long-term treatment trial evaluating the clinical efficacy of nitisinone. Plasma HGA, measured using an HPLC/UV method, was 0.355 ug/ml (0.148-0.815) in the 6 patients receiving nitisinone and 5.65 ug/ml (2.62-11.2) in the 36 patients not receiving nitisinone. and Urine HGA, measured using a stable isotope dilution GC/MS technique, was 9.9 mg/dl (1.13-24.8) in the nitisinone-treated patients and 255.4 mg/dl (42-585.9) in those not receiving nitisinone. The average fractional excretion of HGA was 276% (90-520) in the nitisinone-treated patients vs 422% (80-1328) in those not receiving nitisinone. In conclusion, nitisinone reduces the filtered load of HGA, resulting in decreased tubular secretion through the organic anion transporter systems and, consequently, decreased urine HGA excretion.

DAY IV FRIDAY, OCTOBER 26

Plenary and Platform Presentations

Platform Session 1 Room 20D
Letting the Genie Out of the Bottle: Genotype/Phenotype Correlations
Fri Oct 26, 2007 08:00AM-10:30AM

Platform Presentation 180
Presentation Time: 08:45AM-09:00AM
Hutchinson-Gilford Progeria Syndrome(HGPS): Comprehensive characterization of 15 children. *M.A. Merideth[1,2], W.J. Introne[1], L.B. Gordon[3], M.B. Perry[4], S.B. Clauss[5], V. Sachdev[6], C.K. Zalewski[7], C.C. Brewer[7], J. Kim[7,8], J.C. Graf[4], A.C.M Smith[1,8], L.H. Gerber[9], J.A. Yanovski[10], D.L. Domingo[11], T.C. Hart[11], F.S. Collins[1], E.G. Nabel[6], R.O. Cannon[6], W.A. Gahl[1,2]* 1) NHGRI, NIH, Bethesda, MD; 2) Intramural ORD, NIH, Bethesda, MD; 3) Brown Univ, Providence, RI; 4) CC, NIH, Bethesda, MD; 5) CNMC,Washington, DC; 6) NHLBI, NIH, Bethesda, MD; 7) NIDCD, NIH, Bethesda, MD; 8) Georgetown UMC, Washington, DC; 9) GMU, Fairfax, VA; 10) NICHD, NIH, Bethesda, MD; 11) NIDCR, NIH, Bethesda, MD.

Hutchinson-Gilford Progeria Syndrome (HGPS), a sporadic autosomal dominant premature aging syndrome, has an incidence of 1/4-8 million. The cause is an abnormal lamin A protein(progerin), produced by a cryptic splice donor site activated by a GGC>GGT change in codon 608 of exon 11 of LMNA. HGPS is a multisystemic disease, uniformly fatal at an average age of 13y, with mortality primarily caused by cardiovascular disease. Progerin disrupts the nuclear scaffold and interferes with transcription; it also accumulates with age in normal cells, supporting HGPS as a model for studying the normal aging process. Fifteen children with HGPS, aged 1-17y, were investigated at the NIH between Feb 2005 and May 2006. Our studies confirmed the universal presence of sclerotic skin changes, bone abnormalities, joint contractures, alopecia, growth impairment, and decreased body fat; CV and CNS complications also occurred. New clinical findings included prolonged prothrombin times, elevated platelet counts and serum phosphorus levels, dental and oral soft tissue abnormalities, and a low-frequency conductive hearing loss. Bone density improved with age until 7y; % body fat decreased with age. Growth impairment was not due to inadequate nutrition, impaired insulin action, or growth hormone(GH) deficiency. GH treatment increased height growth by 10% and weight growth by 50%. Increased BP was common, and arterial studies identified diminished arterial distensibility, and increased carotid intima-medial thickness and augmentation indices. This comprehensive evaluation of the HGPS phenotype defines potential outcome parameters for therapeutic interventions, which may also apply to the normal aging process.

Poster Presentations

Cancer Genetics Session
Exhibit Hall E Wed 4:30PM-6:30PM, Thu 4:30PM-6:30PM, Fri 10:30AM-12:30PM

Poster 415/F
Presentation Time: Fri, Oct 26, 2007, 10:30AM-12:30PM
Association of the ARLTS1 Gly65Val and Cys148Arg variants with breast and prostate cancer risk. *J. Schleutker[1], S. Siltanen[1], K. Syrjakoski[1], R. Fagerholm[2], T. Ikonen[1], P. Lipman[3], K. Holli[4], T. Tammela[5,6], HJ. Jarvinen[7], JP. Mecklin[8], K. Aittomaki[9], C. Blomqvist[10], JE. Bailey-Wilson[3], H. Nevanlinna[2], LA. Aaltonen[11], P. Vahteristo[11]* 1) Laboratory of Cancer Genetics, Institute of Medical Technology, Univ of Tampere, Tampere, Finland; 2) Dept of Obstetrics and Gynaecology, Helsinki Univ Central Hospital (HUCH), Helsinki, Finland; 3) Inherited Disease Research Branch, National Human Genome Research Institute, NIH, Baltimore, Maryland; 4) Dept of Oncology, UTA and TAUH, Tampere, Finland; 5) Dept of Urology, TAUH, Tampere, Finland; 6) Medical School, UTA, Tampere, Finland; 7) Second Dept of Surgery, HUCH, Helsinki, Finland; 8) Dept of Surgery, Jyväskylä Central Hospital, Jyväskylä, Finland; 9) Dept of Clinical Genetics, HUCH, Helsinki, Finland; 10) Dept of Oncology, HUCH, Helsinki, Finland; 11) Dept of Medical Genetics, Univ of Helsinki, Helsinki, Finland.

ARLTS1 was recently found as a tumor susceptibility gene when a nonsense mutation Trp149Stop was found more frequently in familial cancer cases than in sporadic cancer patients and healthy controls. We screened the ARLTS1 gene for 1242 breast cancer, 541 prostate cancer, and 241 colorectal cancer cases as well as for 809 healthy population controls by direct sequencing. The Trp149Stop was found at frequencies 0.5-1.2% in all cancer patient subgroups, and with the highest frequency among controls. The recessive model of Cys148Arg variant was found to be more common among breast cancer cases (OR=1.48, 95% CI 1.16-1.87, p=0.001) and in prostate cancer patients (OR 1.50, 95% CI 1.13-1.99, p=0.005) when compared to controls. A novel variant that may have an effect on cancer risk is a Gly65Val alteration that was found at higher frequency among familial prostate cancer patients (8/164, 4.9%) when compared to the controls (13/809, 1.6% OR 3.14, 95% CI 1.28-7.70, p=0.016). No association was found with any of the variants and colorectal cancer risk. Our results suggest that Trp149Stop is not a predisposition allele in breast, prostate or colorectal cancer in the Finnish population, whereas the Gly65Val increase the familial prostate cancer risk and the Cys148Arg both prostate and breast cancer risk.

Clinical Genetics, Malformations and Dysmorphology Session
Exhibit Hall E Wed 4:30PM-6:30PM, Thu 4:30PM-6:30PM, Fri 10:30AM-12:30PM

Poster 578/F Fri, Oct 26, 2007, 10:30AM-12:30PM
Update on the NIH Study on ARPKD/CHF and other Ciliopathies. *M. Gunay-Aygun[1,2], E. Font-Montgomery[1], M. Parisi[3], D. Adams[1], H. Edwards[1], L. Lukose[1], P. Choyke[4], R. Fischer[1], I. Bernardini[1], J. Bryant[1], B. Gochuico[1], L. Guay-Woodford[5], H. Heller[6], P. Mohan[7], K. Daryanani[8], W. Gahl[1,2]* 1) MGB, NIH/ NHGRI, Bethesda, MD; 2) Intramural Office of Rare Diseases, NIH; 3) University of Washington, Seattle, WA; 4)

NCI, NIH; 5) University of Alabama, Birmingham AL; 6) NIDDK, NIH; 7) CNMC, Washington, DC; 8) NIH Clinical Center.

Human ciliopathies are a group of distinct syndromes with overlapping features caused by defects of the cilia or its basal body/centriole. These include the autosomal dominant (ADPKD) and recessive (ARPKD) polycystic kidney diseases, nephronophthisis (NP), Joubert (JS) and related cerebello-oculo-renal syndromes (CORS), and Bardet-Biedl (BBS), Meckel-Gruber (MGS), Oral-Facial-Digital (OFD), and Alstrom syndromes (AS). ARPKD, the most common pediatric ciliopathy, is characterized by progressive renal insufficiency and congenital hepatic fibrosis (CHF). Although a subset of the patients with JS/CORS, BBS, OFD, and AS are known to have kidney and liver involvement, the nature of kidney and liver disease in these syndromes is poorly defined, largely because pertinent data are limited and retrospective. We have recently expanded the ongoing NIH natural history study on ARPKD/CHF (www.clinicaltrials.gov, trial NCT00068224) to include other ciliopathies. Here we present MRI and high resolution ultrasound (HR-US) results, correlated with liver and kidney function data, on 88 patients with 95 admissions (60 ARPKD/CHF, 6 JS/CORS, 8 ADPKD/CHF and 14 unknown type of PKD/CHF). In ARPKD/CHF, kidney size and extent of cyst involvement on imaging did not correlate with creatinine clearance, except for the very mild patients. MR cholangiogram and HR-US were the most useful imaging modalities for biliary abnormalities and mild kidney involvement, respectively. Three JS/CORS patients had enlarged kidneys with diffuse cystic changes diagnosed perinatally, indistinguishable from ARPKD. We continue to enroll patients to this study to define the full phenotypic spectrum of ciliopathies and to produce comprehensive longitudinal data to provide the groundwork for more focused studies and future therapeutic interventions.

Poster 580/F
Presentation Time: Fri, Oct 26, 2007, 10:30AM-12:30PM
Intrafamilial Variability in Autosomal Recessive Polycystic Kidney Disease/Congenital Hepatic Fibrosis (ARPKD/CHF).
L. Lukose[1], E. Font-Montgomery[1], D. Adams[1], H. Edwards[1], A. Garcia[1], J. Bryant[1], P. Choyke[3], T. Heller[5], P. Mohan[6], K. Daryanani[7], L. Guay-Woodford[4], W. Gahl[1], M. Gunay-Aygun[1,2] 1) MGB, NHGRI, NIH, Bethesda, MD; 2) Intramural Office of Rare Diseases, NIH; 3) NCI, NIH; 4) University of Alabama,Birmingham, AL; 5) NIDDK, NIH; 6) CNMC, Washington DC; 7) NIH CC.

ARPKD/CHF is characterized by progressive renal insufficiency and CHF complicated by portal hypertension (PH). It is caused by mutations in PKHD1, which encodes fibrocystin. The majority of ARPKD/CHF patients present early in childhood, mostly perinatally with enlarged microcystic kidneys, oligohydramnios and hypoplastic lungs. A minority present later in childhood or in adulthood with PH. A subset of patients also have macrocysts of the bile ducts (Caroli's syndrome) predisposing to cholangitis. Chronic renal insufficiency, hypertension, recurrent cholangitis, esophageal varices and hypersplenism are the major sources of morbidity and mortality. The severity and rate of progression of the kidney and liver disease can be variable even within the same family. As part of an ongoing NIH study on ARPKD/CHF and other ciliopathies (www.clinicaltrials.gov, NCT00068224), we have evaluated 60 ARPKD/CHF patients. In this group, 5 families had 2 and 1 had 4 affected sibs. In one family, one of the sibs was diagnosed prenatally and required kidney transplantation at age 18, whereas the NIH evaluation of her 3 sibs at ages 28, 23 and 21, revealed cysts confined to the renal medulla on high resolution ultrasound; their creatinine clearances were 94, 76, and 122 ml/min/1.73 m2, respectively. In another family, the proband presented at birth with enlarged kidneys, whereas his asymptomatic 12-year old sister, who had normal abdominal ultrasound at age 2, manifested cysts confined to the renal medulla. In another sibship, the 7-year old proband presented with splenomegaly at age 3 and had a severely echogenic liver, marked splenomegaly and grade III esophageal varices. His 9-year old asymptomatic sister had a mildly echogenic liver and borderline splenomegaly. This wide intrafamilial variability suggests the presence of strong genetic modifiers.

Poster 620/F
Presentation Time: Fri, Oct 26, 2007, 10:30AM-12:30PM

A newly recognized overgrowth syndrome distinct from Proteus syndrome.
J.C. Sapp[1], R.D. Clark[2], J.T. Turner[1], J. van de Kamp[3], F. van Dijk[3], R.B. Lowry[4], L.G. Biesecker[1] 1) National Human Genome Research Institute, Bethesda, MD; 2) Loma Linda University Medical Center, Loma Linda, CA; 3) VU Medisch Centrum, Amsterdam, Netherlands; 4) Alberta Children's Hospital, Calgary, Canada.

Syndromes with overgrowth as a major manifestation are clinically and phenotypically heterogeneous and incompletely defined. Proper clinical delineation of these syndromes is important both for research and for clinical care. We present here a series of eight patients who were previously diagnosed with Proteus syndrome but who do not meet published diagnostic criteria for this disorder and whose natural history is distinct. This newly delineated phenotype comprises progressive, complex, and mixed truncal vascular malformations, dysregulated adipose tissue, varying degrees of scoliosis, and enlarged, yet not distorted, bony structures without progressive bony overgrowth. Similarities between these patients' phenotype and that of Proteus syndrome include vascular malformations (low flow blood vessels and lymphatics), linear pigmented nevi, and excess fat deposition or lipomas. Differences between this newly described entity and Proteus syndrome are that the former includes non-progressive, non-distorting overgrowth that is generally congenital and of the ballooning type, and a stereotypical distribution of lesions that includes complex truncal vascular malformations, bilateral foot overgrowth, and lack of cerebriform connective tissue nevi. We conclude that the patients presented here have a phenotype that is both recognizable and distinct from Proteus syndrome and other overgrowth conditions.

Poster 754/F
Presentation Time: Fri, Oct 26, 2007, 10:30AM-12:30PM
Autosomal Recessive Polycystic Kidney Disease/Congenital Hepatic Fibrosis (ARPKD/CHF) Associated with Congenital Anomalies.
E. Font-Montgomery[1], H. Edwards[1], D. Adams[1], P. Held[1], P. Choyke[2], L. Guay-Woodford[3], T. Heller[4], P. Mohan[5], K. Daryanani[6], W. Gahl[1,7], M. Gunay-Aygun[1,7] 1) MGB, NHGRI/NIH, Bethesda, MD; 2) NCI, NIH; 3) University of Alabama, Birmingham AL; 4) NIDDK, NIH; 5) CNMC, Washington, DC; 6) NIH CC; 7) Intramural Office of Rare Diseases, NIH.

ARPKD/CHF, the most common childhood ciliopathy, is characterized by dilated renal collecting ducts resulting in renal insufficiency and ductal plate malformation of the biliary system resulting in CHF. It is caused by mutations in PKHD1, which encodes fibrocystin, a protein located on the primary cilia-basal body/centriole. Other ciliopathies, commonly associated with

overlapping features, include Joubert Syndrome (JS) and related cerebello-oculo-renal syndromes (CORS), Bardet-Biedl (BBS), Meckel-Gruber (MGS) and Oral-Facial-Digital-1 (OFD1) syndromes and potentially other, yet-to-be-discovered disorders. Although many ciliopathy genes have been identified, for most of these disorders the processes of gene identification and phenotype delineation remain incomplete. The current consensus clinical diagnostic criteria for ARPKD/CHF require characteristic kidney and liver involvement, family history consistent with autosomal recessive inheritance, and absence of congenital anomalies. In the ongoing NIH natural history study on ARPKD/CHF and other ciliopathies (www.clinicaltrials.gov, trial NCT00068224), we have evaluated 88 patients, 72 of whom were referred with a diagnosis of ARPKD/CHF. NIH evaluations including high resolution ultrasound, MRI and sequencing of the PKHD1 gene confirmed ARPKD/CHF in 59 of the 72 patients. Here we present 5 of the 72 patients who had congenital abnormalities in addition to the typical kidney and liver disease of ARPKD/CHF. These include a patient with tetralogy of Fallot and another with unilateral cleft lip/palate, both of whom have two pathogenic mutations in PKHD1. PKHD1 sequencing was negative in the other 3 patients, one of whom had craniofacial dysmorphism associated with enlarged basilar cisterns. We continue enrolling patients to better delineate the phenotypic spectrum and improve diagnostic accuracy of these disorders.

Development Session
Exhibit Hall E Wed 4:30PM-6:30PM, Thu 4:30PM-6:30PM, Fri 10:30AM-12:30PM

Poster 941/F
Presentation Time: Fri, Oct 26, 2007, 10:30AM-12:30PM
Mouse Mutants as Models for Human Developmental Malformations: The Extra-Toes Spotting (Xs^J) Mouse. *D.E. Gildea[1,2], S.K. Loftus[1], Y. Yang[1], W.J. Pavan[1], L.G. Biesecker[1]* 1) GDRB, NHGRI, NIH, Bethesda, MD; 2) Genetics, The George Washington University, Washington, DC.

Greig cephalopolysyndactyly syndrome (GCPS) is a malformation syndrome that includes limb anomalies, specifically polydactyly and syndactyly. GCPS is caused by mutations in the Glioma-associated oncogene-3 (GLI3), which plays a role in Sonic hedgehog (SHH) signaling. The GLI3/SHH pathway regulates many developmental processes, including limb patterning. Dysregulation of this pathway due to mutations in GLI3 can result in limb malformation. The Extra-toes ($Gli3^{XtJ}$) mouse is an excellent animal model for GCPS. Like the human phenotype, $Gli3^{XtJ}$ mice exhibit preaxial polydactyly. Another mouse model for polydactyly, Extra-toes spotting (Xs^J), shares a similar phenotype with the $Gli3^{XtJ}$ mouse. Xs^J mice exhibit preaxial polydactyly and/or belly spotting. Both the $Gli3^{XtJ}$ and the Xs^J phenotypes are inherited semidominantly. Previous linkage mapping has excluded mouse *Gli3* as the Xs^J gene, and the gene and Xs^J mutation remain unknown. To identify the gene, we are performing recombination mapping in Xs^J mice. To map the locus, we needed to outbreed our Xs^J animals to castaneus mice to introduce a distinct chromosomal background, as we encountered substantial homozygosity in the candidate interval. Offspring from this outcross do not exhibit the Xs^J phenotype. When breeding carriers from the outcross to B6C3FeF1/J mice, we experienced a penetrance of 36%. These data show variable penetrance of the Xs^J phenotype that is greatly dependent upon mouse genetic background. We have mapped the Xs^J locus to a 322 kb region on mouse chromosome 7 and are currently evaluating candidate genes. Since $Gli3^{XtJ}$ and Xs^J mice overlap phenotypically, we hypothesize that the gene mutated in the Xs^J mice is a gene in the Gli3/Shh pathway. To test this hypothesis, we are evaluating by *in situ* hybridization the expression of *Shh*, *Gli3*, and other members of the Gli3/Shh pathway in Xs^J embryos. Preliminary data show no change in *Shh* expression in Xs^J embryos. Here we present our genetic analysis strategy, our phenotypic characterization, the mapping data, and our plan for developmental analysis of the animals.

Molecular Basis of Mendelian Disorders Session
Exhibit Hall E Wed 4:30PM-6:30PM, Thu 4:30PM-6:30PM, Fri 10:30AM-12:30PM

Poster 1264/F
Presentation Time: Fri, Oct 26, 2007, 10:30AM-12:30PM
SIX3 mutations in holoprosencephaly (HPE) are loss-of-function alleles. *S. Domene[1], K.B. El-Jaick[2], E. Roessler[1], F. Lacbawan[1], B. Feldman[1], M. Muenke[1]* 1) NHGRI/NIH, Bethesda, MD; 2) Laboratorio de Genetica Molecular, Brazil.

Holoprosencephaly (HPE) is the most common structural anomaly of human forebrain development, with a prevalence of ~1 in 250 conceptuses and ~1 in 16,000 at birth. Mutations in at least eight different genes have been identified in human HPE patients. We have previously shown that SIX3, a transcription factor known to be involved in midline forebrain and eye formation during early development in the mouse, is associated with HPE in humans. No functional studies have been performed to date. It consists of two highly conserved domains: a SIX domain needed for interaction with other proteins and a DNA-binding homeodomain. SIX3 interacts with groucho corepressor proteins through two eh1-like motifs located within the SIX domain. This interaction is required both for the autorepression of six3 itself and for the regulation of other early developmental genes.

In addition to 18 previously reported SIX3 mutations we describe here 29 novel mutations. The total of 47 mutations are located throughout the entire SIX3 gene and include 33 missense, 5 nonsense, 8 frameshift mutations and 1 in frame deletion. To demonstrate the function of these mutations we established several complementary approaches using the zebrafish as a model system: 1) overexpression of SIX3, 2) morpholino (MO) knockdown and rescue assay and 3) detection of marker changes using in situ hybridization. With these assays we have functionally characterized these SIX3 mutations for the first time as significant loss-of-function alleles. For example, single point mutations in the eh1-like motif result in loss of function suggesting that interaction with groucho is essential for SIX3 activity. In addition, several nonsense mutations located in the SIX domain and homeodomain which result in early termination of the protein result in loss of function. Our data elucidate how SIX3 functions during development and increase our understanding of its role in the pathogenesis of HPE. Furthermore, these results are crucial for genetic counselling of families with children with HPE.

Metabolic Disorders Session
Exhibit Hall E Wed 4:30PM-6:30PM, Thu 4:30PM-6:30PM, Fri 10:30AM-12:30PM

Poster 1527/F
Presentation Time: Fri, Oct 26, 2007, 10:30AM-12:30PM
Clinical and molecular characterization of Hermansky-Pudlak Syndrome type-6. *R. Hess[1], M. Huizing[1], A. Helip-Wooley[1], L. Vincent[1], R. Fischer[1], J. White[2], W.A. Gahl[1]* 1) Medical Genetics Branch, NHGRI/NIH, Bethesda, MD; 2) Univ Minnesota, Minneapolis, MN.

Hermansky-Pudlak syndrome (HPS) is a rare disorder of

vesicle formation characterized by oculocutaneous albinism, a bleeding diathesis and, in some patients, granulomatous colitis or pulmonary fibrosis. Eight autosomal human genes have been shown to cause various HPS phenotypes, and at least five additional genes correspond to murine models. We previously described clinical, molecular and cellular characteristics of HPS subtypes 1 through 5. Here we report our detailed clinical and genetic studies on patients with HPS-6. The human *HPS6* gene (murine *ruby-eye*) is located on 10q24.32 and consists of a single large exon coding for a protein of 775 aa. We screened 19 patients, without defects in other HPS causing genes, and identified 4 patients with 7 different novel *HPS6* mutations, including two frameshift (c.238dupG, c.1938delTG), three nonsense (Q305X, Q75X, Q412X), one large chromosomal deletion, and one missense mutation (T272I). Most nonsense and frameshift mutations generating premature termination codons cause nonsense mRNA mediated decay (NMD), while intronless genes, like *HPS6*, are usually not monitored by NMD. Expression analysis in two HPS-6 patients revealed no mRNA decay in fibroblasts; hence a truncated protein is most likely produced. Clinically, our HPS-6 patients exhibited a relatively mild HPS phenotype, including mild iris transillumination, variable hair and skin pigmentation, and absent platelet dense granules. Pulmonary fibrosis and granulomatous colitis were not observed in these patients, although they were all under 27, an age before which lung disease rarely develops in HPS. It is important to continue to follow adults with HPS-6 for the development of restrictive lung disease. The clinical features of HPS-6 resemble those of HPS-3 and HPS-5, presumably because HPS3, HPS5 and HPS6 interact with each other in BLOC-2 (biogenesis of lysosome-related organelles complex -2). These findings are important for the prognosis of newly diagnosed HPS-6 patients, and for studying the role of the HPS6 protein in the biogenesis of lysosome-related organelles.

Statistical Genetics and Genetic Epidemiology Session
Exhibit Hall E Wed 4:30PM-6:30PM, Thu 4:30PM-6:30PM, Fri 10:30AM-12:30PM
Poster 2003/F
Presentation Time: Fri, Oct 26, 2007, 10:30AM-12:30PM
Linkage study in Puerto Rican families with Endometriosis. *E.M. Ledet[1], R. Thouta[2], J.E. Bailey-Wilson[3], I. Flores[4], D. Mandal[1]* 1) Department of Genetics, Louisiana State University Health Sciences Center, New Orleans, LA; 2) Department of Pathology, Louisiana State University Health Sciences Center, New Orleans, LA; 3) NHGRI/NIH, Baltimore, MD; 4) Department of Microbiology, Ponce School of Medicine, Ponce, PR.

Endometriosis is a disease which has affected millions of women; yet, much is still unclear about this often misunderstood condition. Endometriosis is defined by the growth of endometrial tissue, both endometrial stroma and endometrial glands, outside of the uterine cavity. Currently, the exact number of women suffering with endometriosis is unknown, but some, epidemiological studies have indicated a prevalence of 5-20% in women of reproductive age.

Our previous linkage studies on 39 Puerto Rican families produced a LOD score of 1.75 at one of the candidate regions on chromosome 10. For this study, 41 Puerto Rican families with two or more patients with surgically diagnosed endometriosis were recruited; blood samples and patient histories were obtained. Marker genotypes were obtained on chromosomes 1, 3, 7, 8 and 10. Specifically, Mendelian inconsistencies were screened and cleaned from the data set using Sib-Pair and PedCheck, and allele frequencies were calculated utilizing Sib-Pair. Significant allelic association was revealed with an empiric p value of 0.0095 at one of the candidate regions. The marker allele frequencies have been estimated from the data though Sib-Pair. The data would be further utilized to do linkage analysis to identify any susceptibility loci. Additionally, utilizing patient histories, the presence and incidence of other conditions, namely ovarian, lymphoma, breast, and prostate cancers, within the families with respect to endometriosis will be assessed and analyzed. In this study we intend to identify any markers associated with endometriosis on chromosomes 1, 3, 7, 8, or 10, identify and document any correlation, especially with relation to cancer, between family disease history and endometriosis, and, in general, characterize the histories and disease symptoms within this Puerto Rican population.

Molecular Basis of Disorders With Complex Inheritance Session
Exhibit Hall E Wed 4:30PM-6:30PM, Thu 4:30PM-6:30PM, Fri 10:30AM-12:30PM

Poster 2431/F
Presentation Time: Fri, Oct 26, 2007, 10:30AM-12:30PM
Effect of lysososomal protein glucocerebrosidase on α-synuclein turnover. *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) LNG/NIA/NIH, Bethesda, MD; 3) Dept. of Pharmacology, UPenn, Philadelphia, PA.

The synucleinopathies which include Parkinson disease(PD), are characterized by aberrant α-synuclein fibrillization resulting in the formation of pathological inclusions. In PD, inclusions in neuronal cell bodies and processes are termed Lewy bodies(LBs) and Lewy neurites (LNs). Studies in familial PD implicate that abnormalities in protein clearance can lead to neurodegeneration. Although defects in the ubiquitin-proteosome system (UPS) may contribute to PD, alternate pathways such as lysosomal degradation are also involved in modulating α-synuclein accumulation. Recent evidence indicates an association between mutations in glucocerebrosidase(GBA), the lysosomal enzyme deficient in Gaucher disease, and PD as well as dementia with LB(DLB). We explored possible mechanisms to explain why α-synuclein might accumulate when GBA is mutated. To examine the effects of GBA mutations on the two pathways implicated in α-synuclein metabolism, brain samples from 7 subjects with PD or DLB carrying GBA mutations were studied with immunofluorescence. Ubiquitin and lysosomal markers were used with antibodies against glucocerebrosidase and α-synuclein. Although in some LBs, mutant glucocerebrosidase was present at the core, only 40-60 % of glucocerebrosidase positive LBs were ubiquinated. However, all LBs and LNs positive for both α-synuclein and glucocerebrosidase displayed antigenicity to the lysosomal markers. Proteosome function was examined using the small degron CL-1, which demonstrated no influence of either wild-type or mutant GBA on the UPS. α-synuclein solubility and turnover were studied in Cos-7 cell co-transfected with h-A53T α-synuclein and wild-type or mutant GBA. Detergent fractionation demonstrated higher levels of soluble α-synuclein in cell lines carrying wild-type GBA. In pulse-chase experiments, there was also more effective clearance of α-synuclein in the presence of wild-type GBA. These data suggest that glucocerebrosidase may affect α-synuclein catabolism, and when mutated, may interfere with the lysosomal clearance of α-synuclein aggregates.

Poster 2456/F
Presentation Time: Fri, Oct 26, 2007, 10:30AM-12:30PM
Functional analysis of a nonsynonymous coding variant (R325W) in the pancreatic β-cell specific zinc transporter, *SLC30A8*, associated with type 2 diabetes. *M.R. Erdos[1], L. Qin[2], L.L. Bonnycastle[1], A.J. Swift[1], A.G. Sprau[1], A.U. Jackson[3], C.W. Willer[3], C.L. Yang[4], S. Humphreys[4], D.H. Ellison[4], J. Tuomilehto[5], R.N. Bergman[6], M. Boehnke[3], K.L. Mohlke[2], F.S. Collins[1]* 1) GTB, NHGRI, NIH, Bethesda, MD; 2) UNC, Chapel Hill, NC; 3) U Mich, Ann Arbor, MI; 4) OHSU, Portland, OR; 5) National Public Health institute, Helsinki, Finland; 6) USC, Los Angeles, CA.

Genome wide association studies have identified several novel susceptibility genes for type 2 diabetes (T2D) including *SLC30A8*, a pancreatic β-cell specific zinc transporter. Type 2 diabetes association with the SNP (rs13266634) that marks a non-synonymous coding substitution (R325W) in *SLC30A8* achieves genome wide significance (OR= 1.12, p= $5.3x10^{-8}$) in the combined analysis of three major studies (DGI, UKT2D, and FUSION). We now report that quantitative trait analyses in ~2380 FUSION individuals also suggest association with systolic blood pressure (p= .028), pulse pressure (p= .004), triglycerides (p= .036, p=.009 in controls), fasting free fatty acids (p= .024) and BMI-related traits (BMI, waist, whr; p= .033-.05). In db/db diabetic mice, dietary zinc supplementation has been shown to attenuate hyperglycemia and hyperinsulinemia. In a pilot study, normal glucose tolerant Finns homozygous for the risk allele (C, n=16) had modestly lower, but not statistically different, plasma zinc levels (72.6 ug/dl, SD= 15.0) than those homozygous for the non-risk allele (T, n=19; 75.9 ug/dl, SD= 11.5). We have synthesized both alleles of the full length SLC30A8 cDNA and transfected these into HeLa cells. We observed similar expression levels and cellular localization for each allele, and we are now examining zinc uptake with each allele using the cell permeable zinc fluorophore, Fluozin-3. In a second model system, we are injecting *Xenopus laevis* oocytes with *in vitro* transcribed cRNA for each allele of *SLC30A8* in the presence of $^{65}Zn^{+2}$ supplemented media, and monitoring the zinc transporter activity by radioactivity uptake. These studies may define the mechanism for this newly discovered risk factor for type 2 diabetes, with the potential for future therapeutic insights.

Gene Structure and Function Session
Exhibit Hall E Wed 4:30PM-6:30PM, Thu 4:30PM-6:30PM, Fri 10:30AM-12:30PM

Poster 2775/F
Presentation Time: Fri, Oct 26, 2007, 10:30AM-12:30PM
Sequencing of PKHD1 in Autosomal Recessive Polycystic Kidney Disease/Congenital Hepatic Fibrosis (ARPKD/CHF). *D. Adams[1], H. Edwards[1], A. Garcia[1], E. Font-Montgomery[1], M. Huizing[1], P. Choyke[3], T. Heller[5], P. Mohan[6], K. Daryanani[7], L. Guay-Woodford[4], W. Gahl[1], M. Gunay-Aygun[1,2]* 1) Section on Human Biochemical Genetics, Medical Genetics Branch, NHGRI; 2) Intramural Office of Rare Diseases, NIH; 3) NCI, NIH; 4) Univ. of Alabama, Birmingham AL; 5) NIDDK, NIH; 6) CNMC, Wash., DC; 7) NIH Clinical Center.

ARPKD/CHF, a form of PKD with onset primarily in childhood, is typically associated with CHF complicated with portal hypertension (PH). ARPKD/CHF results from mutations in PKHD1, one of the largest genes in the human genome. PKHD1 exhibits a complex splicing pattern. The longest open reading frame, composed of 66 exons, encodes fibrocystin, a 4074 amino acid protein located on the primary cilia-basal body/centriole complex. PKHD1 also has 19 alternate exons. Although the diagnosis of ARPKD/CHF is still made clinically in most patients, confirmation of diagnosis with DNA analysis is increasingly employed, especially in atypical patients and for prenatal diagnosis. The current mutation detection rate ranges from 75-85%. To date, more than 300 PKHD1 mutations throughout the gene have been reported. As part of an ongoing NIH natural history study on ARPKD/CHF and other ciliopathies (www.clinicaltrials.gov, trial NCT00068224), we have sequenced the PKHD1 gene in a total of 66 patients, including 45 clinically typical ARPKD/CHF, 17 atypical/unknown PKD/CHF, 3 CHF/PH associated with ADPKD, and 1 Caroli's disease. The pathogenicity of the missense mutations was evaluated using existing databases, intraspecies sequence conservation, and the conservation of amino acid chemistry. In the 84 typical ARPKD/CHF proband alleles, 70 potentially pathogenic mutations were detected. Sixteen of these had not been previously reported. In the 34 atypical/unknown alleles, 10 potentially pathogenic mutations were detected. No pathogenic PKHD1 mutations were found in the 6 alleles of patients with CHF and PH associated with ADPKD or in the 2 with Caroli's disease. We continue to sequence DNA from more ARPKD/CHF and related ciliopathy patients, enrolling new patients in an effort to improve diagnostic accuracy and better characterizing these disorders.

DAY V SATURDAY, OCTOBER 27

Plenary and Platform Presentations

Platform Session 54 Hall H
Regulatory Element Discovery and Function
Sat Oct 27, 2007 08:00AM-10:30AM

Platform Presentation 221
Presentation Time: 09:00AM-09:15AM
Identification and characterization of cell type-specific and ubiquitous chromatin regulatory elements. *G.E. Crawford[1], H. Xi[2], H.P. Shulha[2], J.M. Lin[2], T.R. Vales[1], Y. Fu[2], D.M. Bodine[3], R.D.G. McKay[4], J.G. Chenoweth[4], P.J. Tesar[4], T.S. Furey[1], B. Ren[5], Z. Weng[2]* 1) Institute for Genome Sciences & Policy, Duke University, Durham, NC; 2) Boston University, Boston, MA; 3) National Human Genome Research Institute, Bethesda, MD; 4) National Institute of Neurological Disorders and Stroke, Bethesda, MD; 5) Ludwig Institute for Cancer Research, University of California San Diego, La Jolla, CA.

The identification of regulatory elements from different cell types is necessary for understanding the mechanisms controlling cell type-specific and housekeeping gene expression. Mapping DNaseI hypersensitive (HS) sites is an accurate method for identifying the location of functional regulatory elements. We have used a high throughput method, called DNase-chip, to identify 3904 DNaseI HS sites from six cell types across 1% of the human genome. A significant number (22%) of DNaseI HS sites from each cell type are ubiquitously present among all cell types studied. Surprisingly, nearly all of these ubiquitous DNaseI HS sites correspond to either promoters or insulator elements: 86% of them are located near annotated transcription start sites (TSS) and 10% are bound by CTCF, a protein with known enhancer blocking insulator activity. We also identified a large number of DNaseI HS sites that are cell type-specific (only present in one cell type); many of these regions do not map to promoters, are enriched for enhancer elements and correlate with cell type-specific gene expression as well as cell type-specific histone

modifications. Finally, we find that approximately 8% of the genome overlaps a DNaseI HS site in at least one the six cell lines studied, indicating that a large percentage of the genome is potentially functional. Collectively, these results show that ubiquitous chromatin structures are predominantly associated with promoters and insulators while enhancers tend to associate with cell type-specific chromatin structures.

Platform Session 59 Room 20D
Therapy for Genetic Disorders Session
Sat Oct 27, 2007 08:00AM-10:30AM

Platform Presentation 267
Presentation Time: 08:00AM-08:15AM
Long-term oral cysteamine therapy attenuates the morbidity and mortality of nephropathic cystinosis in adults. *W. Gahl[1], J.Z. Balog[1], K. O'Brien[1,2], G. Golas[1,2], R. Kleta[1,2], I. Bernardini[1]* 1) Section on Human Biochemical Genetics, Medical Genetics Branch, NHGRI, NIH, Bethesda, MD; 2) Office of Rare Diseases, NIH, Bethesda, MD.

Nephropathic cystinosis, a lysosomal storage disorder due to defective transport of cystine out of lysosomes, results from mutations in CTNS. Almost half the patients in North America and Europe are homozygous for a 57-kb deletion in CTNS. Without treatment, children with cystinosis suffer from renal Fanconi syndrome and its complications, growth retardation, photophobia, and end-stage renal failure requiring kidney transplantation. Treatment with oral cysteamine (Cystagon), which can reduce cellular cystine levels by 95%, dramatically slows glomerular deterioration and normalizes growth; Cystagon is approved by the FDA for use in pre-transplant cystinosis patients. Based upon our examinations of 100 adult cystinosis patients between 1985 and 2006, we report striking rates of mortality (33%; mean age 29 years) and morbidity (24-75% for each complication), specifically related to hypothyroidism, hypergonadotropic hypogonadism (in men), pulmonary insufficiency, swallowing abnormalities, myopathy, retinopathy, vascular calcifications, and diabetes. Homozygosity for the 57-kb CTNS deletion did not correlate with these individual complications, but did correlate with mortality and with the overall severity of the morbidity. In adults, long-term (>8years) oral cysteamine therapy was associated with significantly greater height and weight, older age at renal transplant, lower serum cholesterol levels, and lower rates of morbidity and mortality. In fact, as duration of cysteamine therapy increased, the frequencies of myopathy, diabetes mellitus, pulmonary dysfunction, swallowing abnormalities, vascular calcification, retinopathy and death decreased. We conclude that all cystinosis patient should receive oral cysteamine therapy, and the registration for Cystagon should be amended to include post-transplant cystinosis as an indication. In addition, we should redouble our efforts to diagnose and treat cystinosis early, including attempts at newborn screening.

Platform Presentation 275
Presentation Time: 10:00AM-10:15AM
A farnesyltransferase inhibitor prevents cardiovascular disease in a progeria mouse model. *B.C. Capell[1], M. Olive[1], M.R. Erdos[1], K. Cao[1], D.A. Faddah[1], K.N. Conneely[2], H. San[1], X. Qu[1], H. Avallone[3], F. Kolodgie[3], R. Virmani[3], E.G. Nabel[1, 4], F.S. Collins[1]* 1) NHGRI, NIH, Bethesda, MD; 2) University of Michigan School of Public Health, Ann Arbor, MI; 3) CVPath, Gaithersburg, MD; 4) NHLBI, NIH, Bethesda, MD.

Hutchinson-Gilford progeria syndrome (HGPS) is the most dramatic form of human premature aging. Death occurs at a mean age of 13, usually from heart attack or stroke. HGPS is almost always caused by a *de novo* point mutation in the *LMNA* gene that results in production of a mutant lamin A protein, termed “progerin”, that is permanently modified by a lipid farnesyl group. It is hypothesized that progerin remains associated with the nuclear membrane due to its farnesyl-anchor, thus acting as a dominant negative, disrupting the lamina, leading to the blebbed nuclei that are the cellular hallmark of the disease. Treatment with farnesyltransferase inhibitors (FTIs) has been shown to prevent and even reverse this nuclear abnormality in cultured HGPS fibroblasts. In a study extending over a year, we show that the dose-dependent administration of the FTI, tipifarnib (R115777, Zarnestra ®) to a transgenic mouse model of HGPS can ameliorate a cardiovascular phenotype (loss of vascular smooth muscle cells (VSMC) in the media of the large arteries) that is strikingly similar to the cardiovascular disease seen in HGPS. Twenty-eight mice were randomly assigned to receive oral administration of 450 mg/kg/d, 150 mg/kg/d, or vehicle-only beginning at one month of age. Following sacrifice at 9-12 months of age, five blinded observers scored pathology levels examining both VSMC loss and proteoglycan accumulation. Using levels of the biomarker non-farnesylated HDJ-2 as a measure of *in vivo* FTI activity, we found a highly significant association between FTI activity and the prevention of the cardiovascular phenotype. Experiments currently underway will determine whether this FTI can also reverse this cardiovascular disease in HGPS mice that are allowed to reach 6 months or 9 months of age before treatment is started. Our results provide encouraging evidence in support of a clinical trial of FTIs for this rare and devastating disease.
