Program Nr: 1 from the 2004 ASHG Annual Meeting

Mutations in a novel member of the chromodomain gene family cause CHARGE syndrome. *L.E.L.M. Vissers*[1], *C.M.A. van Ravenswaaij*[1], *R. Admiraal*[2], *J.A. Hurst*[3], *B.B.A. de Vries*[1], *I.M. Janssen*[1], *W.A. van der Vliet*[1], *E.H.L.P.G. Huys*[1], *P.J. de Jong*[4], *B.C.J. Hamel*[1], *E.F.P.M. Schoenmakers*[1], *H.G. Brunner*[1], *A. Geurts van Kessel*[1], *J.A. Veltman*[1]. 1) Dept Human Genetics, UMC Nijmegen, Nijmegen, Netherlands; 2) Dept Otorhinolaryngology, UMC Nijmegen, Nijmegen, Netherlands; 3) Dept Clinical Genetics, The Churchill Hospital, Oxford, United Kingdom; 4) Children's Hospital Oakland Research Institute, BACPAC Resources, Oakland, CA.

CHARGE association denotes the non-random occurrence of ocular coloboma, heart defects, choanal atresia, retarded growth and development, genital hypoplasia, ear anomalies and deafness (OMIM #214800). Almost all patients with CHARGE association are sporadic and its cause was unknown. We and others hypothesized that CHARGE association is due to a genomic microdeletion or to a mutation in a gene affecting early embryonic development. In this study array-based comparative genomic hybridization (array CGH) was used to screen patients with CHARGE association for submicroscopic DNA copy number alterations. De novo overlapping microdeletions in 8q12 were identified in two patients on a genome-wide 1 Mb resolution BAC array. A 2.3 Mb region of deletion overlap was defined using a tiling resolution chromosome 8 microarray. Sequence analysis of genes residing within this critical region revealed mutations in the CHD7 gene in 10 of the 17 CHARGE patients without microdeletions, including 7 heterozygous stop-codon mutations. These findings indicate that haploinsufficiency of this gene explains the majority of patients with CHARGE association. CHD7 encodes a novel member of the chromodomain helicase DNA protein family and is an essential gene in early embryonic development affecting multiple organ systems, including heart, inner ear and retina. Microdeletions encompassing the causative gene have been reported to occur at low frequencies in single gene disorders. We show that high-resolution genome-wide screening by array CGH is an effective new approach for the localization of such causative genes. This approach is of special interest for sporadic malformation syndromes that cannot be tackled by other mapping approaches because of reproductive lethality.

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Program Nr: 2 from the 2004 ASHG Annual Meeting

Disruption of the microRNA pathway by the targeted loss of eIF2C2 results in embryonic lethality and failure of neural tube closure. *R.S. Alisch, P. Jin, S.T. Warren.* Department of Human Genetics, Emory University School of Medicine, Atlanta, GA.

MicroRNAs are a class of noncoding RNAs that play a critical role in development by regulating the translation of target mRNAs and possibly through chromatin modifications. MicroRNA function requires the formation of a RNA-induced silencing complex (RISC). The Argonaute family of proteins are highly conserved members of the RISC and one member, Ago1 and its mammalian ortholog eIF2C2, have recently been shown to interact with FMRP, whose deficiency causes fragile X syndrome. In order to more fully understand the microRNA pathway in mammals, we disrupted the eIF2C2 locus in mice. Two independent eIF2C2 knockout lines were found to transmit the interrupted allele without an obvious heterozygous phenotype. Of the mice born from heterozygous crosses, none were homozygous for the eIF2C2 mutation suggesting the absence of eIF2C2 is a developmental lethal. While homozygous embryos were not observed at embryonic day (e)13.5, homozygous embryos were recovered at e9.5 in appropriate genetic ratios. Inspection of the homozygous embryos revealed a failure of neural tube closure resembling anencephaly. Using these eIF2C2 knockout lines, in which the -galactasidase gene was incorporated into the eIF2C2 locus, we observed strong expression of eIF2C2 in the brain and testes, which correlates well with the failure of neural tube closure. These data indicate the importance of the microRNA pathway in the development of the mammalian brain and are consistent with the widespread expression of microRNA loci in the brain. These eIF2C2 knockout mouse lines will further elucidate RISC function, help understand the role of microRNAs in FMRP function and allow testing of the hypothesis, derived from lower organisms, that the microRNA pathway is involved in targeted chromatin modification perhaps including genomic imprinting.

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Program Nr: 3 from the 2004 ASHG Annual Meeting

High Density SNP Map Reveals Interrupted and Interlaced Organization of Linkage Disequilibrium Among Markers. *J. Belmont[1], F. Yu[1], P. Hardenbol[2], X. Lu[1], M. Moorhead[2], G. Scott[1], S. Ghose[1], S. Pasternak[1], T. Willis[2], M. Faham[2], S.M. Leal[1], J. Taylor[3], R. Morris[4], N. Kaplan[3], R.A. Gibbs[1].* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) ParAllele Biosciences, South SanFransisco; 3) NIEHS; 4) Duke University, Durham, NC.

Dense SNP marker maps are expected to facilitate the interpretation of genetic association studies by identifying markers that optimally tag the most common haplotypes. Here we have analyzed genotype data from 17,528 SNP markers Chromosome 12 in a reference set of 30 Northern European parent-offspring trios to evaluate the performance of a novel approach to the construction of a linkage disequilibrium map. Applying matrix decomposition to the standardized composite linkage disequilibrium (AB) matrix identified subsets of markers that exhibit significant LD with one another. These subsets may be composed of strictly adjacent markers but often members of the groups are not contiguous in their sequence positions. Two or more subsets may overlap creating an interlaced pattern. The marker subsets correspond closely to the observed haplotypes. On chromosome 12 the mean number of markers per subset was 3.4-4.8 (depending on the parameters used to identify subsets of markers with LD), and the mean and median genomic distance encompassed by subsets were 88.3 Kb and 53.2 Kb, respectively. Marker assignment to a specific subset was found to be stable to marker density, minor allele frequency, analysis window, and to some extent analysis window length. Maps of marker subsets can be constructed from whole chromosome SNP data and such maps illustrate useful features of the underlying linkage disequilibrium relationships among markers. This approach overcomes some of the problems associated with haplotype block definitions based on confidence intervals alone.

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Program Nr: 4 from the 2004 ASHG Annual Meeting

The Correlation Structure of 1,586,383 SNPs Within and Between Diverse Human Populations Defines a Large Fraction of All Common Human DNA Variation. *D.R. Cox, D. Hinds, L. Stuve, W. Barrett, P. Starink, P. Hickey, D. Ballinger, K.A. Frazer.* Perlegen Sciences Inc., Mountain View, CA.

We have defined the allele frequencies and correlation structure between 1,586,383 SNPs determined to be polymorphic in one or more of 71 unrelated individuals (24 European American, 23 African American, and 24 Han Chinese), and have submitted these 112 million genotypes to dbSNP. The average distance between adjacent SNPs is 1.7 kb, with 95% of the genome covered with gap sizes of <50kb. A SNP is present within 10 kb of 93% of 22,904 protein-coding genes. A total of 20,165 SNPs are present in exons, with 10,795 SNPs resulting in synonomous and 9370 SNPs resulting in nonsynonomous amino acid changes. Of the 1,586,383 SNPs, 80% have a minor allele frequency greater than 5% in the African American sample, while 71% and 65% of SNPs have a minor allele frequency greater than 5% in the European American and the Chinese samples, respectively.

In an effort to determine the fraction of all common human DNA variation captured by this set of 1,586,383 SNPs, we have taken advantage of the fact that the African American and European samples used in our study have been completely re-sequenced for 152 genes by the Seattle SNP Project (http://www.pga.uwashington.edu). We calculated the fraction of all common SNP information present in the 152 genes that was defined by our SNP set by determining the coefficient of determination (r^2) between the genotype of each >10% minor allele SNP discovered by the Seattle SNP project and the most correlated SNP genotype in our data. The results reveal that even though our SNP set contains only 23% and 21% of all SNPs with a minor allele >10% in the European American sample and the African American sample, respectively, these SNPs have average r^2 values of 84% and 72% , respectively, when considering the complete set of >10% minor allele frequency SNPs present in these samples. These results indicate that the set of 1,586,383 SNPs defines a large fraction of common DNA variation in diverse human populations, providing a powerful reagent for assessing the causal role of common DNA variation in complex human traits.

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A common, sex-dependent *RET* enhancer mutation underlies Hirschsprung disease: use of comparative genomics and genetic association to interrogate a disease locus. *E.S. Emison[1], A.S. McCallion[1], C.S. Kashuk[1], R.T. Bush[1], E. Grice[1], S. Lin[1], M.E. Portnoy[2], D.J. Cutler[1], E.D. Green[2], A. Chakravarti[1], NIH Intramural Sequencing Center.* 1) Inst Genetic Medicine, Johns Hopkins Univ, Baltimore, MD; 2) Genome Technology Branch, National Human Genome Research Inst, National Institutes of Health, Bethesda, Maryland.

Comparative genomics reveals that 5% of the human genome is under selection; only 1.5% of the genome is protein-encoding while the function of the remaining 3.5% is unknown. We report the use of a novel, integrated strategy of comparative genomics and a family-based genetic association study (TDT) to identify putative functional sequences outside coding elements and refine the genetic interval associated with disease. The multifactorially inherited birth defect Hirschsprung disease (HSCR) represents a disorder in which mutations within non-coding elements are predicted. HSCR is a relatively common non-Mendelian disorder in which the receptor tyrosine kinase *RET* harbors rare coding sequence mutations in less than 50% of cases. We show that a common, non-coding variant at *RET* contributes significantly to risk and explains the remaining cases. Alignment of 350 kb of human genomic sequence at *RET* with the orthologous sequences from 12 vertebrates identified 120 conserved sequences (CS), 68 of which are non-coding. By overlaying the CS elements with the peak of transmission distortion observed among 30 SNP markers in 126 trios, we identified a common variant (>25% frequency) within a conserved, enhancer-like sequence of *RET* intron 1 which is significantly associated with HSCR. Intriguingly, transmission to affected boys is much greater than to affected girls, as predicted for a complex trait with a 4:1 sex bias. Consequently, the sex-dependence is a property of the specific *RET* mutation. We further demonstrate that the mutation significantly abrogates *in vitro* enhancer function in a neuroblastoma cell line, decreasing reporter gene expression 5-fold relative to the wild type allele.

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Program Nr: 6 from the 2004 ASHG Annual Meeting

A comprehensive review of the health and developmental outcomes of children conceived through Assisted Reproductive Technologies (ART). *K.L. Hudson[1,2], K.A. Robinson[3], A. Ananthakrishnan[4], D.E. Pritchard[2], S. Goodman[1,5]*. 1) ART Children's Health Panel, GPPC, SARM, AAP, coordinated by Johns Hopkins Univ., Washington, DC; 2) Genetics & Public Policy Ctr, Johns Hopkins Univ., Washington, DC; 3) Department of Medicine, Johns Hopkins School of Medicine, Baltimore, MD; 4) Bloomberg School of Public Health, Johns Hopkins Univ., Baltimore, MD; 5) Department of Oncology, Division of Biostatistics, Johns Hopkins School of Medicine, Baltimore, MD.

An increasing number of families are using Assisted Reproductive Technologies (ART). Some recent reports suggest a possible link between children conceived through ART and certain adverse health and developmental outcomes. The response to these reports by the popular press has caused concern and confusion among patients, providers, and policymakers. A panel of experts in pediatrics, obstetrics/gynecology, reproductive-endocrinology, biostatistics, epidemiology and genetics was assembled to systematically review the published literature addressing ART childrens health outcomes. The outcomes studied fell into five categories: genetic syndromes and malformations, postnatal growth, medical outcomes beyond one year, cancer, and psychosocial and developmental outcomes. We performed electronic and manual literature searching through January 2004 to identify English-language studies that compared outcomes for children of ART versus natural conception, and studies that compared outcomes between types of ART. Of over 2000 citations identified, data from approximately 200 studies were determined to be eligible for inclusion in our review. This systematic review did not find strong evidence of an increased risk of any adverse clinical outcomes associated with ART, although for some outcomes the evidence was of insufficient quantity, quality or consistency to make firm conclusions. We identified limitations in the existing studies, such as use of inappropriate controls and inconsistent data reporting. The report includes recommendations for additional research and database creation to generate higher quality evidence for future investigations.

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Program Nr: 7 from the 2004 ASHG Annual Meeting

Identification of mammalian target mRNAs for microRNAs. *M. Nakamoto, P. Jin, S.T. Warren.* Dept Human Genetics, Emory Univ Sch Medicine, Atlanta, GA.

MicroRNAs (miRNAs) are a recently recognized class of non-coding RNA that play a critical role in development and regulation of gene expression by mediating gene silencing in plants and animals. Much of the silencing is by translational suppression of mRNAs with sequence partially complementing the miRNA sequence. While there are hundreds of miRNA loci in humans, each potentially recognizing many mRNAs, the process of matching individual miRNAs with their target mRNAs is largely computational and not exceptionally robust. Here we have developed an *in vivo* approach to identify miRNA targets by identifying mRNAs exhibiting a translational shift in their polysome profile following siRNA knock down of individual endogenous miRNAs in the human HepG2 cell line. To test this approach, we transfected a luciferase reporter construct containing the 3' UTR of the *FMR1* gene within which the target sequence of the endogenous miRNA *mir30* was embedded. Following siRNA knock down of *mir30* RNA, we observed a shift in the target reporter mRNA to heavier polysome fractions, as expected from a release of translational suppression. A control mRNA without *mir30* target sequence exhibited no polysome shift with or without *mir30* knock down. By similarly knocking down individual endogenous miRNAs and using mRNA isolated from selected polysome fractions to interrogate oligonucleotide microarrays, we can now identify potential target mRNAs by their profile shift compared to control cells. By identifying the mRNA targets of miRNA loci, we can begin to assess the contribution of miRNA mutations to human disease.

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Program Nr: 8 from the 2004 ASHG Annual Meeting

An imprinted, non-coding transcript potentially encoding miRNAs in the mouse Prader-Willi syndrome region.

K. Yamasaki, M. Stefan, R.D. Nicholls. University of Pennsylvania, Philadelphia, PA.

Prader-Willi syndrome (PWS) is associated with neonatal failure-to-thrive, hyperphagia and obesity, and other clinical features. Loss of function of 10 known imprinted, paternally expressed genes in chromosome 15q11-q13 occur in PWS: 5 each encode proteins or box C/D snoRNAs. Nevertheless, the functions of PWS-genes remain unknown. Mouse models of PWS recapitulate the neonatal phenotype, with nine of the imprinted loci conserved. In both species, these genes split into two clusters, with 3 (in human) or 4 (in mouse) intronless, protein-coding genes separated by ~ 1 Mb from the *SNURF-SNRPN-snoRNA* polycistronic gene cluster. Although the region between these clusters in the mouse is not fully covered by genomic sequence, we examined whether additional imprinted genes occur in this interval. We first built a BAC contig spanning *Ndn* to the imprinting center (IC), a genetic element controlling imprinting across the entire domain, with just 2 small gaps. *In silico* analyses of mouse sequence databases identified several ESTs in this region, primarily from embryonic cDNA libraries. Subsequently, using EST, RT-PCR, and 5-RACE strategies, we assembled a long non-coding, alternatively spliced transcript spanning at least 59 exons, termed *Nccr* (for non-coding, clustered repeat). Fourteen additional exons map 3 but could not yet be definitely linked. *Nccr* showed imprinted, paternal only, brain-specific expression, primarily in the last half of embryogenesis. Analysis of genomic sequence from 3 *Nccr* identified 35 copies of an ~ 1.2 kb clustered repeat, and, using mFold, include two potential RNA hairpin structures (G=-19 and -31.4 kcal/mole) reminiscent of microRNAs (miRNA). This hypothesis is being examined by RNA expression studies. The putative promoter-exon 1 of *Nccr* and two intronic elements show differential allelic methylation in brain. One of the intronic elements and the promoter-exon 1 are highly conserved, suggesting that a similar imprinted transcript may occur in human. A potential role for the *Nccr* locus in the PWS phenotype in mouse and human may thus exist.

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Program Nr: 9 from the 2004 ASHG Annual Meeting

Nuclear respiratory factor-1 (NRF-1) is a master regulator of the Prader-Willi syndrome imprinted gene domain.

M. Stefan[1], K. Yamasaki[1], T. Ohta[1], S. Rodriguez[2], T.P. Yang[2], R.A. Simmons[1], R.D. Nicholls[1]. 1) University of Pennsylvania, Philadelphia, PA; 2) University of Florida, Gainesville, FL.

Prader-Willi syndrome (PWS) patients have neonatal hypotonia and failure to thrive followed by hyperphagia and severe obesity, due to loss of imprinted expression of 10 genes of unknown function. Imprinting at these loci is coordinated by an imprinting center (IC) and we recently identified *in vitro* binding for the transcription factors (TFs) NRF-1 and CTCF within alternative promoters of the *Snurf-Snprn* IC locus, correlating germ cell and embryo expression of alternative transcripts with the establishment of parental imprinting. However, the mechanistic relationship to somatic expression of PWS genes and the phenotypic basis of the syndrome were unknown. In a PWS-deletion mouse model with neonatal lethality we found embryonic deficits of - and -cells in pancreatic islets with postnatal impairment in glucose homeostasis, deficits in energy metabolism and significantly reduced liver mitochondrial complex I+III activity. To examine a potential broader role for NRF-1, a major TF for nuclear genes encoding mitochondrial functions, *in silico* analyses of mammalian genome sequence using permutations of the NRF-1 binding motif revealed that most PWS-imprinted genes possess conserved NRF-1 sites. Using allele-specific chromatin immunoprecipitation (ChIP), and other assays, we demonstrate that NRF-1 binds *in vivo* to an intronic activator and all 11 alternative promoters of the *Snurf-Snrpn*-snoRNA locus, the *Ndn* promoter, and a mini-CpG island near *Mkrn3* and *Magel2*. A conserved, non-coding cluster of 4 stereospecific NRF-1 sites within the PWS domain, hypothesized to act as a neuronal-specific regulatory element, is also under study. ChIP shows the NRF-1:DNA interactions are limited to open chromatin [acetylated H4 and di-methylated H3 (Lys4) histones] and unmethylated CpG-islands of the active, paternally-derived allele in mouse fibroblasts and brain tissue. In conclusion, NRF-1 acts as a master TF for PWS gene expression and establishment of imprinting, providing a molecular link to mitochondrial and energy pathways and explaining the pathophysiological basis of the PWS phenotype.

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Program Nr: 10 from the 2004 ASHG Annual Meeting

Alternative transcription start site and polyadenylation site usage in the FMR1 gene. *C. Carosi*[1,2], *A. beilina*[2], *C. Bagni*[1], *P.H. Schwartz*[3], *P.J. Hagerman*[2], *F. Tassone*[2]. 1) Department of Biology, University of Rome, Tor Vergata, Italy; 2) Molecular Medicine, University of California, Davis, CA, USA; 3) National Human Neural Stem Cell Resource, Childrens Hospital of Orange County, Orange, Ca.

We have recently identified three transcription start sites in the FMR1 promoter (site I, II and III), a result that has been observed for other TATA-less promoters. The remarkable finding is that the fractional utilization of the transcription start sites is a function of the CGG repeat number. Premutation alleles appear to preferentially express the longer FMR1 mRNA (upstream site longer transcript) compared to alleles in the normal range. In human brain tissue, including cerebellum and hippocampus, derived from both normal and premutation brains, an additional transcriptional start site located between Sites I and III in the FMR1 gene has been also identified. The current results indicate that the CGG repeat element in the FMR1 gene directly influences transcription initiation, and suggests that modification in the 5end of the FMR1 transcript could play a role in the reduced translation efficiency observed in premutation alleles. In addition, alternative usage appears to occur in different tissues. A polyadenylation site in the human FMR1 gene at about 2 kb downstream of the stop codon has been previously reported. We have now identified additional polyadenylation sites in the 3UTR of both the mouse and the human FMR1 gene. More importantly, alternative polyadenylation generate several mouse transcripts, and only the shorter form appears to be efficiently transported at the synapses. These results suggest that post-transcriptional events can modulate the subcellular localization of the FMR1 mRNA, suggesting the involvement of a specific localization signal. Thus, a differential usage of polyadenylation sites utilized for a specific sub-cellular localization could also occur in humans. This studies are currently under investigation.

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Program Nr: 11 from the 2004 ASHG Annual Meeting

Gene deserts flanking the Gdf6 and Bmp2 genes contain numerous conserved, long-range regulatory enhancers that function during embryonic development. *D.P. Mortlock, R.L. Chandler, K.J. McDermott, L. Ramirez.* Center for Human Genetics, Vanderbilt Univ, Nashville, TN.

Comparisons of mammalian genome sequences suggest that much noncoding DNA is conserved and likely contains functional elements, such as cis-acting enhancers that activate tissue-specific gene transcription. However, enhancers can be hundreds of kilobases from their cognate genes. We are analyzing the regulatory landscape around the Bmp2 and Gdf6 genes, two members of the murine BMP (Bone Morphogenetic Protein) family of secreted signaling proteins. Both genes are important for embryonic development of various soft tissues and skeletal elements. A large gene desert adjacent to Gdf6 is postulated to contain enhancers that drive Gdf6 transcription in embryonic limb, spine and skull joints, and paw interdigits. To verify this, we have tested BAC transgenes by using an in vivo transgenic reporter assay. Intergenic BACs were coinjected with a promoter/LacZ minigene to generate transgenic mouse embryos. This revealed distant Gdf6 cis-regulatory domains controlling expression in the interdigits, heart outflow tract, and developing wrist/ankle. The interdigit and heart enhancers were mapped over 130 kilobases from Gdf6 and a wrist/ankle enhancer is over 400 kb distant, thus they are among the farthest known cis-regulatory elements. Each of the regulatory domains contains highly conserved sequences. Like Gdf6, Bmp2 has a complex expression pattern in development and is also flanked by large intergenic regions. To assess for potential long-range regulatory elements flanking Bmp2, two Bmp2-LacZ BACs were tested in transgenic mice. This revealed complex Bmp2 expression in skeletal cartilages, digits, brain, kidney, mammary glands, hair follicles and other tissues. The two-BAC strategy allowed us to assay almost 400 kb of the Bmp2 locus and assigned many regulatory elements to specific, potentially distant intergenic regions. We suggest long-distance regulatory elements are not unusual, but instead are a hallmark of genes involved in developmental signaling pathways. These data confirm that gene deserts can contain distant, dispersed regulatory sequences for adjacent genes.

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Program Nr: 12 from the 2004 ASHG Annual Meeting

Homozygosity for a Dominant-negative Type I Collagen Mutation Attenuates the Type IV OI Phenotype of the Heterozygous Brtl Mouse: Insight into Disease Mechanism. *J.C. Marini[1], S. Leikin[2], C. Bergwitz[1], T.E. Uveges[1], K.M. Kozloff[3], A. Forlino[1], N.V. Kuznetsova[2], S.A. Goldstein[3], G. Gronowicz[4].* 1) BEMB, NICHD/NIH, Bethesda, MD; 2) Sect Phys Biochem, NICHD/NIH, Bethesda, MD; 3) 3Ortho Res Labs, U Mich, Ann Arbor, MI; 4) 4U Conn Health Ctr, Farmington, CT.

The Brtl mouse is a dominant-negative model for type IV osteogenesis imperfecta (OI) caused by a glycine substitution (G349C) knocked-into one col1a1 allele. This study characterizes the novel genetic situation in which homozygosity for a dominant mutation (Brtl/Brtl) attenuates the heterozygous phenotype. Given the 30% perinatal mortality and moderately severe bone disease of surviving Brtl/+ mice, we expected homozygosity to be lethal. Surprisingly, perinatal survival for Brtl/Brtl was equal to WT and their weight at 2 mos was intermediate to WT and Brtl/+. Brtl/Brtl skeletal staining revealed decreased osteoporosis, less flared thorax and fewer intrauterine rib fractures compared to Brtl/+. Brtl/Brtl 1 fibroblasts synthesize only mutant 1(I) mRNA and virtually all 1 chains form disulfide-linked homodimers. Brtl/Brtl procollagen is efficiently secreted from cells and well-incorporated into matrix while Brtl/+ demonstrates selective secretion of the mutant collagen. Type 1 collagen containing one ^{35}S Cys mutant 1(I) chain is selectively retained by Brtl/+ cells with only 30-50% of these heterotrimers secreted into media. This results in decreased collagen levels in Brtl/+ matrix. *In vivo* collagen molecules with one mutant chain are deficient in Brtl/+ skin and lung. Bone strength and histology of Brtl/Brtl femurs were studied. Areal and vBMD are normal in Brtl/Brtl while femoral cross sectional area, trabecular thickness, and BV/TV are intermediate between Brtl/+ and WT. Brtl/Brtl femurs withstand normal loading to fracture and are less brittle than Brtl/+. Histomophometry at 2 mos was similar in all genotypes. The attenuated bone phenotype in Brtl/Brtl, as compared to Brtl/+, suggests several potential mechanisms of OI: the direct dominant negative effect of mutant type I collagen, the extent of matrix insufficiency, and potential detrimental interactions between reactive-SH moieties and non-collagenous proteins.

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Program Nr: 13 from the 2004 ASHG Annual Meeting

Toxic effects of expanded polyalanine repeats in Drosophila. *D.C. Rubinsztein*[1], *Z. Berger*[1,2], *J.E. Davies*[1], *C.J. O'Kane*[2]. 1) Medical Gen, Cambridge Univ, Cambridge Inst Medical Res, Cambridge, United Kingdom; 2) Department of Genetics, Cambridge University, CB2 3EH, UK.

The expansion or duplication of polyalanine repeats is the primary mutation causing at least 9 diseases. In one of these diseases, oculopharyngeal muscular dystrophy, polyalanine expansions in the polyadenine binding protein 2 (PABP2) lead to the formation of intranuclear aggregates similar to those seen in polyglutamine diseases. Expression of polyalanine expansions has been previously shown to form aggregates and cause toxicity in mammalian cells, independent of the protein context. In order to study the effects of polyalanine expansion mutations in vivo, we have generated a Drosophila model expressing nuclear-targeted green fluorescent protein tagged with short and long polyalanine tracts. Expanded alanines form aggregates in vivo and cause toxic effects when expressed in a variety of different tissues in Drosophila. The pathogenic effects are alleviated by the aggregation inhibitor Congo red. These data suggest that the toxicity of polyalanine expansions is at least partially associated with their tendency to aggregate.

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Program Nr: 14 from the 2004 ASHG Annual Meeting

Determinants of CAG/CTG repeat instability: Identification of cis-acting elements and trans-acting factors. *R.T. Libby*[1], *K.A. Hagerman*[2,3], *S.L. Baccam*[1], *C.E. Pearson*[2,3], *A.R. La Spada*[1,4,5]. 1) Dept of Lab Medicine, Univ Washington Med Ctr, Seattle, WA; 2) Program of Genetics & Genomic Biology, Hospital for Sick Children, Toronto, ON, Canada; 3) Dept of Molecular & Medical Genetics, University of Toronto, Toronto, ON, Canada; 4) Division of Medical Genetics, Dept of Medicine, Univ Washington Med Ctr, Seattle, WA; 5) Division of Neurogenetics, Dept of Neurology, Univ Washington Med Ctr, Seattle, WA.

Mouse models of CAG/CTG trinucleotide repeat expansions have provided useful clues as to the factors that control repeat instability. We focused our studies on the spinocerebellar ataxia type 7 (SCA7) CAG/CTG repeat, as expanded SCA7 repeats display marked instability with a dramatic propensity for expansion in humans. Our analysis of SCA7 transgenic mice has revealed that genomic context is an important determinant of repeat instability: The human DNA region 3' of the SCA7 CAG repeat is required for instability in transgenic mice, as its deletion abrogates instability. One intriguing motif within the SCA7 locus 3' region is a binding site for CTCF, a DNA-binding protein with a variety of regulatory functions that likely stem from its ability to modulate DNA structure upon binding. Multiple disease loci associated with unstable CAG/CTG repeats, including HD, SCA2, SCA3 and DM1 contain adjacent CTCF binding sites. To test the hypothesis that CTCF binding is a determinant of CAG/CTG repeat instability, we mutated the CTCF binding site 3' of the SCA7 (CAG)92 repeat in a 13.5 kb SCA7 genomic construct (RL-SCA7 92R) previously shown to produce marked repeat expansions in transgenic mice. After demonstrating that the resulting construct (RL-SCA7 92R CTCF-) had lost its ability to bind CTCF, we used it to derive transgenic mice. Independent lines of RL-SCA7 92R CTCF- mice display a significant reduction in CAG/CTG repeat instability upon intergenerational transmission. These results suggest that an intact CTCF binding site is required for repeat instability at the SCA7 locus.

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Genetic determinants of quantitative variation in human long- and middle-wave photoreceptors. *S. Deeb[1], T. Kutyavin[2], A. Shafer[2], M. Hawrylycz[2], J. Stamatoyannopoulos[2].* 1) Dept Medicine/Med Genetics, Univ Washington, Seattle, WA; 2) Dept of Molecular Biology, Regulome, 551 N 34th St, Seattle, WA 98103.

The human retina contains three classes of cone photoreceptors, the short-wave (S), middle-wave (M) and long-wave (L) sensitive cones that allow trichromatic color vision. The genes encoding the L (OPN1LW) and M (OPN1MW) pigments are tandemly arrayed on the X-chromosome, where one L gene is followed by a variable number of M genes, of which only the first is functional. The L:M cone ratio in the retina varies widely (>10-fold) between individuals, and is strongly correlated with the ratios of OPN1LW:OPN1MW mRNA. We hypothesized that such variability results from genetic variation in cis-acting regulatory elements. To identify such elements comprehensively, we applied a novel technique - Quantitative Chromatin Profiling - in the context of human retinoblastoma cells (WERI)to effect high-resolution localization of functional non-coding elements throughout a 35kb region encompassing the OPN1LW gene and 20kb of upstream sequence. We readily identified the OPN1LW promoter and the previously described LCR, and show that the latter in fact comprises two distict elements which are differentially affected by previously described deletions that give rise to blue cone monochromacy. We also identified a novel element further upstream from the LCR, as well as one in the distal 4th intron of OPN1LW. We then resequenced the four upstream elements (OPN1LW promoter, proximal LCR element, distal LCR element, novel upstream element) in 31 males for whom the L:M mRNA ratios had been accuratly determined in retinal samples. Surprisingly, the promoter and LCR elements lacked sequence diversity, effectively eliminating primary lesions in these elements as the cause of interindividual variability in the L:M mRNA ratio. By contrast, we show that variation in the novel upstream element we identified is significantly associated with high L:M ratios ($p<0.0078$). The results provide insight into the mechanism of L and M gene regulation and suggest a general strategy for dissection of quantitative traits linked to specific candidate loci.

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Program Nr: 16 from the 2004 ASHG Annual Meeting

SLC19A3 encodes a novel transporter. *W. Zeng, J.F. Gusella.* Molecular Neurogenetics Unit, Massachusetts General Hospital, Harvard University, Charlestown, MA 02129.

Biotin-responsive basal ganglia disease (BBGD) is a autosomal recessive disorder characterized as early onset of a subacute encephalopathy with confusion, dysarthria and dysphagia, then progress to severe cogwheel rigidity, dystonia and quadriparesis. We have mapped the BBDG gene to 2q36.3 and identified two missense mutations in this gene, which has also been named *SLC19A3*. In order to examine the *SLC19A3* protein for its normal function, we use expression constructs using *SLC19A3* cDNAs and transfect into cultured cells. [3H] labeled biotin and [3H] thiamine, [14C] pantothenate, [3,5,7,9-3H] folic acid, D-[14C] pyridoxine and [3,5,7-3H] methotrexate have been used for uptake assays based on published procedures. Comparison of biotin and thiamine uptake capacity with normal *SLC19A3* and mutant, empty vectors showed a significantly higher uptake capacity in normal *SLC19A3* transfected cells. Confocal microscopy using V5 monoantibody reveals the SLC19A3 protein localizing on cell plasma membrane. These findings indicate that *SLC19A3* coding a novel membrane protein capable of transporting two substrates: biotin and thiamine.

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Association of the Organic Cation Transporter (OCTN) SLC22A4 and SLC22A5 genes with Psoriatic Arthritis.

A. Barton[1], P. Ho[1], J. Bowes[1], S. Eyre[1], B. Newman[2], I. Bruce[1], A. Silman[1], J. Worthington[1]. 1) ARC-EU, Univ Manchester, Manchester, United Kingdom; 2) Academic Dept of Medical Genetics, Univ Manchester, United Kingdom.

Two recent studies of complex diseases, Crohns disease (CD) and rheumatoid arthritis (RA), have reported association to genes mapping to the cytokine gene cluster on 5q31. In particular, a functional single nucleotide polymorphism (SNP) mapping to intron 1 of the OCTN1 (SLC22A4) gene has been associated with RA (1) whilst a haplotype of a different SNP in the same gene and one in an adjacent gene, OCTN2 (SLC22A5), has been associated with CD (2). As patients with CD have an increased risk of developing psoriatic arthritis (PsA) and because joint inflammation is common to both RA and PsA, we hypothesized that polymorphism in the OCTN gene cluster may also be important in the aetiology of PsA. We, therefore, investigated the association of 10 SNPs spanning the SLC22A4 and SLC22A5 genes in 471 Caucasian PsA patients and 605 population controls using 5 allelic discrimination assays (Taqman, ABI). Allele and haplotype frequencies, estimated using the EM algorithm, were compared between PsA patients and controls. Genotypes for all cases and controls were in Hardy-Weinberg equilibrium. Using single-point analysis, 2 SNPs (rs3763112 mapping to SLC22A4 and rs2631367 mapping to SLC22A5) were significantly associated with PsA ($p = 0.0074$ and $p = 0.0008$, respectively). Furthermore, a haplotype of 2 adjacent SNPs (rs1050152 and rs2631367) was strongly associated with PsA ($p=0.00025$). Interestingly, this is the same haplotype as is associated with CD, and has been shown to alter OCTN transcription and transporter function. In conclusion, a strong association to a functional haplotype of SNPs across the SLC22A4/5 locus implicates a role for these genes in susceptibility to PsA. Given that CD and PsA occur together more than would be expected by chance alone, the fact that the same haplotype has now been associated with both conditions suggests that common pathways may underlie these inflammatory diseases.
Refs:1.Tokuhiro S et al. Nat Genet 2003; 35: 341-8. 2.Peltekova VD et al. Nat Genet 2004; 36: 471-5.

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Program Nr: 18 from the 2004 ASHG Annual Meeting

A risk haplotype in the SLC22A4/SLC22A5 gene cluster influences phenotypic expression of Crohn's disease. *W. Newman[1,2], X. Gu[3], R.F. Wintle[4], D. Cescon[1], V.D. Peltekova[1,4], M. van Oene[4], C.I. Amos[3], K.A. Siminovitch[1,4].* 1) Samuel Lunenfeld and Toronto General Research Institutes, Mount Sinai Hospital and the University Health Network, Toronto, Canada; 2) Academic Department of Medical Genetics, St Mary's Hospital, Manchester, UK; 3) Department of Epidemiology, University of Texas, MD Anderson Cancer Centre, Houston, Tx; 4) Ellipsis Biotherapeutics Corporation, Toronto, Canada.

Previously we identified two functionally-relevant polymorphisms in the SLC22A4 and SLC22A5 genes at the IBD5 locus which alter gene/protein function and comprise a two allele haplotype (SLC22A-TC) associated with increased risk for Crohns disease. We examined the contribution of this susceptibility haplotype alone and in combination with CARD15 to Crohns disease subphenotypes and to susceptibility to ulcerative colitis.

Phenotype-genotype associations were evaluated in a Canadian cohort including 507 Crohns disease, 216 ulcerative colitis and 487 ethnically-matched controls genotyped for the SLC22A4 1672 C>T, SLC22A5 -207G>C and the major Crohns disease-associated CARD15 variants. The SLC22A-TC haplotype was strongly associated ($p<0.0001$) with Crohns disease in the non-Jewish subgroup of this cohort and the combination of SLC22A-TC homozygosity and one or more of the common CARD15 disease susceptibility alleles engendered a 7 fold increase in risk for Crohns disease ($p = 9 \times 10^{-8}$) and a 4 fold increase in risk for ileal disease ($p = 0.001$). The risk haplotype showed only a suggestive association with Crohns disease in the Jewish subgroup and no association with ulcerative colitis in the cohort or in subgroups stratified by CARD15 genotypes. The SLC22A-TC haplotype acts together with CARD15 disease susceptibility alleles to increase risk for Crohns disease and ileal disease among Crohns patients, but does not contribute to risk for ulcerative colitis in this Canadian cohort. The association of the SLC22A-TC haplotype and CARD15 alleles with ileal disease suggests that these variants have biologically intertwined effects in Crohns disease pathogenesis.

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Variation within NOS2A and susceptibility to multiple sclerosis (MS). *J.R. Oksenberg*[1], *L.F. Barcellos*[2, 1], *G. McNeill*[2], *S.J. Caillier*[1], *S. Schmidt*[3], *B.A. Cree*[1], *M.A. Pericak-Vance*[3], *J.L. Haines*[4], *S.L. Hauser*[1]. 1) Dept Neurology, UC San Francisco; 2) School of Public Health, UC Berkeley; 3) Center for Human Genetics, Duke University; 4) Center for Human Genetics Research, Vanderbilt University.

A large body of research supports a complex etiology in MS involving genetic contributions from both HLA and non-HLA loci. Our recent analysis of four polymorphisms located within NOS2A on ch.17q11 in 427 Caucasian-American families (1706 individuals; 427 affecteds) and an African-American case-control data set (442 and 292, respectively) revealed significant evidence for both single locus and extended haplotype disease associations (Ann Neurol 55:793;2004). This effect was most prominent in HLA-DR2 positive families. Extensive re-sequencing of NOS2A (27 exons and promoter region) in 20 individuals identified 39 additional variants. Pairwise measures of linkage disequilibrium were calculated across NOS2A revealing varied patterns of association. We selected four informative promoter SNPs (positions: -2477, -1026, -954, -277) for further study in the MS datasets. Disease associations were present for -277 -1026 and -2447 in the Caucasian-American families (p=0.003, 0.01, and 0.005, respectively), and for -954 in the African-American dataset (p=0.005). Haplotype analyses were extended to include the four original polymorphisms and the new promoter SNPs. The strongest evidence for over-transmission was observed for NOS2A haplotypes comprised of -1026, TAAA, -277, (p=0.0004), TAAA, -277, exon10 (p=0.0003) and -277, exon10, exon16 (p=0.0013) polymorphisms in the Caucasian families. In addition, we also performed linkage analysis in 184 multicase MS families (1104 individuals; 464 affecteds) for the NOS2A polymorphisms and seven flanking microsatellites. Our results revealed evidence for linkage to NOS2A only (HLOD=2.4), and were also restricted to HLA-DR2 positive families (HLOD=2.7). These findings provide strong support for both linkage and association to a new candidate disease gene on ch.17q11 in MS, and suggest that variation within NOS2A contributes to disease susceptibility, and may also interact with HLA-DR.

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Modeling the effects of HLA-DR and -A loci in multiple sclerosis (MS) families reveals a complex genetic contribution to susceptibility. *L.F. Barcellos[1,6], J.M. Penko[1], G. McNeill[1], S. Sawcer[2], A. Compston[2], A.B. Begovich[3], S. Schmidt[4], M.A. Pericak-Vance[4], J.L. Haines[5], S.L. Hauser[6], J.R. Oksenberg[6].* 1) School of Pub Hlth, UC Berkeley; 2) Univ of Cambridge, UK; 3) Roche Molecular Systems, Alameda, CA; 4) Center for Human Genetics, Duke Univ; 5) Center for Human Genetics Research, Vanderbilt Univ; 6) Dept Neurology, UC San Francisco.

The underlying etiology of MS is unknown, involving both genetic and environmental contributions. In a recent study of HLA-DR genotypes in 549 Caucasian American families, we demonstrated a dose effect of DR2 on disease risk. In these families, genotypes with one or two copies of DR2 conferred, respectively, 2.7 and 6.7 fold risks as compared to DR2-negative genotypes (AJHG 72:710;2003). Additional reports have suggested that DR3, as well as genes in the HLA class I region may also contribute independently, or in combination, respectively, with the HLA-DR2 associated susceptibility to MS. We performed similar analyses in an independent dataset of 492 MS families recruited in the UK. All family members were categorized by disease status and HLA-DR2 genotype (DR2/DR2, DR2/DRX and DRX/DRX, where X denotes other DR alleles), and analyzed using CLR modeling. Results for observed risks conferred by DR2/2 and DR2/X in the UK dataset were very similar to our previous report (OR=7.5, $p<10^{-4}$ and OR=3.4, $p<10^{-4}$, respectively), confirming a dominant dose effect (DR2/DR2 vs. DR2/X, OR=2.2, p=0.0003). Similarly, a dose effect was also detected for DR3 in the combined UK/US dataset (3856 individuals; 1300 cases); however, in contrast to DR2, the risk conferred by DR3 appears to be recessive, with two copies (DR3/DR3) increasing risk 2.0 fold (p=0.009) compared to no risk for DR3/DRX individuals (OR=0.9, p=ns). A comparison to DR2/DRX, of risk conferred by the DR2/DR3 genotype, further demonstrates that one copy of DR3 has no effect (OR=1.3,p=ns). Modeling of cis and trans effects of HLA-DR and -A loci in 472 Caucasian American families also indicates that particular genotypic combinations comprised of both loci confer greater disease risk. These results underscore the importance of considering mode of inheritance for genetic studies in complex diseases.

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The Haplotype HLA-B*0700, DRB1*1501, DQA1*0102, DQB1*0602 Augments the Risk for the Development of AS in Addition to HLA-B27. *L. Jin[1], G. Zhang[1], M.H. Weisman[2], J. Bruckel[3], H.R. Schumacher[4], M.A. Khan[5], R.B. Inman[6], W.P. Maksymowych[7], T.M. Martin[8], D.T.Y. Yu[2], J.T. Rosenbaum[8], J.D. Reveille[9]*. 1) Dept Environmental Health, Univ Cincinnati, Cincinnati, OH; 2) UCLA, Los Angeles, CA; 3) The Spondylitis Association of America, Sherman Oaks, CA; 4) The University of Pennsylvania, Philadelphia, OR; 5) Case Western Reserve University, Cleveland, OH; 6) The University of Toronto, Toronto, ON, Canada8The; 7) The University of Alberta, Edmonton, AB, Canada; 8) Oregon Health & Science University, Portland, OR; 9) The University of Texas-Houston Health Science Center, Houston, TX.

The overwhelming influence of HLA-B27 has proven a confounder in determining the contribution of other MHC genes to susceptibility to AS. Other studies also suggested that the HLA-B7 Cross Reactive Group(B7, B22(B55), B22(B56), B40 and B42) further influences susceptibility to AS. In order to clarify whether there exist other MHC loci or other allele(s) besides HLA-B27 associated with AS, we developed a model free conditional TDT analysis method to remove the effects of known causal allele(s)(i.e.HLA-B27). All patients met modified New York Criteria for AS (1984). MHC class I genotypes were determined by SSCP typing and class II alleles by oligotyping, with high resolution DRB1 typing by sequence analysis. The parents carrying HLA-B27 were removed in counting the number of transmissions. HLA-B*2705 was strongly associated with AS (p=10-35). After applying Bonferroni's correction only two other MHC alleles showed significant negative associations (HLA-B*1800-pc=0.005 and B*4400-pc=0.0009) After conditioning on the presence of the two major B27 alleles (*2705 and *2702), no individual HLA-B, DRB1, DQA1, DQB1 or DPB1 alleles were significantly associated with AS after Bonferroni's correction. Further multi-locus TDT analysis following the same conditional schema revealed one MHC haplotype to be highly unbalanced in transmission. Such unbalance was not observed in other HLA-DRB1 haplotypes containing HLA-B*0700. These results suggest the haplotype HLA-B*0700, HLA-DRB1*1501, DQA1*0102, DQB1*0602 augments the susceptibility to AS.

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Genetic dissection of Selenoprotein S reveals a quantitative trait nucleotide variant influencing plasma levels of pro-inflammatory cytokines. *J. Curran[1], K. Elliott[1], J. Jowett[1,2], Y. Gao[3], K. Gluschenko[1], G. Cai[4], A. Comuzzie[4], T. Dyer[4], K. Walder[2,3], P. Zimmet[1,2], G. Collier[2,3], A. Kissebah[5], J. Blangero[2,4].* 1) International Diabetes Institute, Caulfield, Au; 2) AGT Biosciences LTD, Victoria, Au; 3) Metabolic Research Unit, Deakin University, Geelong, Au; 4) Southwest Foundation for Biomedical Research, San Antonio, TX; 5) Medical College of Wisconsin, WI.

Recent studies heightened the importance of circulating cytokines in the pathogenesis of the metabolic syndromes chronic complications including diabetes and CVD. We recently identified Selenoprotein S (*SELS*) as a candidate influencing the pro-inflammatory events. *SELS* (formerly *Tanis*) was identified by differential gene expression in *Psammomys obesus*, a polygenic animal model of the metabolic syndrome. *SELS* is located on human chromosome 15q26.3, a region containing QTLs influencing pro-inflammatory disorders. To comprehensively assess the role of *SELS* genetic variation in inflammation, we resequenced 9.2kb of the gene identifying 13 SNPs and genotyped these in 522 individuals distributed over 92 families. As indicators of inflammation, we measured plasma levels of three pro-inflammatory markers (IL-6, IL-1 and TNF-). Bayesian quantitative trait nucleotide analysis revealed strong associations between genetic variation in *SELS* and all three cytokines. Four polymorphisms showed a significant association with these cytokines. In particular, one promoter polymorphism exhibited strong evidence for a major association with IL-1 levels (p=0.000016), IL-6 levels (p=0.0005), and TNF- levels (p=0.00089). We estimated a posterior probability of 0.94 that this variant is of direct functional consequence (or is highly correlated with a functional variant). To confirm our statistical prediction, we performed molecular functional analysis of this polymorphism. Results revealed this variant altered promoter activity and *SELS* expression when cells were exposed to ER stress agents (p=0.00006). These results suggest that *SELS* plays a direct functional role in mediation of the pro-inflammatory processes and consequently chronic complications of the metabolic syndrome.

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Gene-by-gene interaction effects on asthma: results of a genome-wide ordered subset linkage analysis. *D. Meyers, L.A. Lange, J. Xu, E. Bleecker, The Collaborative Study on the Genetics of Asthma (CSGA).* Ctr Human Genomics, Wake Forest Sch of Med, Winston Salem, NC.

Linkage analyses have shown inconsistent results for asthma in different populations, possibly due to epistatic effects(gene-gene interaction). Ordered subset analysis(OSA) (Hauser 2004) is a powerful new approach that may be used to determine if linkage at one location is independent of linkage at another. A genome-wide scan for asthma susceptibility has been performed on families in the NHLBI-funded Collaborative Study on the Genetics of Asthma(CSGA) (Xu 2001). 144 Caucasian families and 107 African-American families were ascertained through two sibs with asthma. We have performed a genome-by-genome OSA using the CSGA data. There was significant evidence for interaction between regions previously reported in genome scans, but not necessarily in the CSGA. The highest lod difference (the difference between the conditional, obtained by subsetting conditional on the reference location, and unconditional lod scores) in Caucasians was for chromosome 2 at 60.4cM conditional on 14 at 16.7cM (lod=4.21;p=0.0005) and for chromosome 15 at 65.4cM conditional on 5 at 30cM in African-Americans (lod=3.79;p=0.0001). For OSA, the p-value is the estimated significance obtained when testing the null hypothesis that the evidence for linkage at the target region is independent of the evidence for linkage at the reference region. The second highest lod difference both in the African-American and Caucasian families was for chromosome 20p conditional on chromosome 6 in the African-Americans (lod =3.63;p<0.0005) and conditional on chromosome 8 in the Caucasians (lod=4.19;p=0.0002). The 20p linkage is in the region of the asthma susceptibility gene, ADAM33 (VanEerdewegh 2002; Howard 2003), which has been replicated in a CSGA case-control population (Howard 2003). Identifying regions where evidence for linkage is not independent facilitates subsetting pedigrees into more homogenous groups, and should increase overall power to detect linkage at the target location. These results suggest that epistatic effects from previously identified chromosomal regions may contribute to asthma susceptibility.

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CD14 polymorphisms and endotoxin exposure interact in the development of allergic sensitisation. *S. John[1], A. Custovic[2], F. Jury[1], A. Woodcock[2], R. Niven[2], W. Ollier[1], A. Simpson[2].* 1) CIGMR, Univ Manchester, United Kingdom; 2) North West Lung Centre, Univ Manchester, United Kingdom.

Introduction: High exposure to endotoxin in the home is associated with reduced risk of allergic sensitisation. CD14 is a receptor for endotoxin, and a promoter polymorphism in the CD14 gene at position -159 (C to T) is associated with more severe allergy. Within the setting of an unselected birth cohort study, we investigated whether polymorphisms in CD14 interact with endotoxin exposure in modifying the risk of allergic sensitisation at age 5 years. Methods: 442 children were genotyped for the SNP at position 159 in CD14. We measured specific IgE to mite, cat, dog, grass, egg, milk and peanut and defined sensitisation as IgE>0.2 kU/L to at least one allergen. Endotoxin levels were measured in dust samples collected from homes, and the results expressed as total endotoxin recovered (EU/m2). Data were analysed using logistic regression. Results: Genotype frequencies were consistent with other populations (TT 25%;, CT 47%;, CC 28%;) and in Hardy-Weinberg equilibrium. The frequency of allergic sensitisation was 33% and was not associated with CD14 genotype (p=0.40). Endotoxin exposures covered a wide range (0.01-544,748 EU/m2). For the whole population, increasing endotoxin exposure was associated with a decreased risk of sensitisation (ODDS ratio 0.85 95%; confidence interval (CI) [0.76-0.95], p=0.005). However, for the TT and CT genotypes, there was no association between endotoxin exposure and sensitisation (ODDS 0.95 CI [0.71-1.23], p=0.71 and ODDS 0.90 CI [0.77-1.04], p=0.16 respectively). In children with the genotype CC, increasing endotoxin exposure was associated with a marked and significant reduction in the risk of allergic sensitisation (ODDS 0.70 CI [0.55-0.89], p=0.004. Conclusions: Increasing endotoxin exposures is associated with a reduced risk of allergic sensitisation, but only in children homozygous for the C allele in the SNP 159 in CD14. With respect to the design of intervention studies aimed at prevention of sensitisation, only individuals with CC genotype at position 159 in CD14 may benefit from high exposure to endotoxin.

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Identification and Analysis of Polymorphisms within *CFALR*, *CASP10*, and *CASP8*: Association with Asthma and Related Phenotypes. *A.K. Smith, L.A. Lange, E.J. Ampleford, D.A. Meyers, E.R. Bleecker, T.D. Howard.* Ctr Human Genomics, Wake Forest Univ Sch Med, Winston-Salem, NC.

Asthma is a complex disease characterized by bronchial hyperresponsiveness (BHR), inflammation, and intermittent airway obstruction, which occurs as a result of the interaction between host susceptibility and environmental exposures. *CFALR*, *Caspase 10*, and *Caspase 8* are clustered in a region on chromosome 2q which has been linked to asthma-related phenotypes in multiple studies. They are integral in the regulation of the immune response because of their involvement in eliminating activated T cells after initial expansion, thereby protecting against autoimmunity. We have performed association studies with novel and previously identified polymorphisms throughout these genes in three ethnically diverse asthma populations - African Americans (168 cases, 269 controls), US Caucasians (233 cases, 245 controls), and US Hispanics (116 cases, 130 controls). Asthma and total serum IgE levels were examined in both cases and controls while PC_{20} (the methacholine concentration that causes a 20% fall in pulmonary function measured by FEV_1) and FEV_1/FVC ratio were examined in cases only. Associations in each population were observed with asthma ($p = 0.0064$-0.027), PC_{20} ($p = 0.0004$-0.041) total serum IgE levels ($p = 0.0039$-0.046), and FEV_1/FVC ratio ($p = 0.0003$-0.048). Estimated haplotypes in each population were also associated with asthma ($p = 0.007$-0.038), PC_{20} ($p = 0.001$-0.037), total serum IgE levels ($p = 0.001$-0.034) and with FEV_1/FVC ratio ($p = 0.00049$). The majority of the single SNP and haplotype associations were in or near *CASP10*, suggesting its importance in these phenotypes.

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The SERPINE2 gene is associated with Chronic Obstructive Pulmonary Disease. *D.L. DeMeo[1,2], T. Mariani[2], C. Lange[3], A. Litonjua[1], J. Celedón[1], J. Reilly[2], H.A. Chapman[4], D. Sparrow[5], B.H. Mecham[2], S. Srisuma[2], A. Spira[6], J. Beane[6], V. Pinto-Plata[7], F.E. Speizer[1], S. Shapiro[2], S.T. Weiss[1], E.K. Silverman[1,2].* 1) Brigham & Women's Hospital,Channing Laboratory, Boston, MA; 2) Brigham & Women's Hospital, Dept Medicine,Pulmonary Division, Boston, MA; 3) Harvard School of Public Health, Boston, MA; 4) U of California at San Franscisco, Div Pulmonary & Critical Care, San Francisco,CA; 5) Veterans Affairs Medical Center, Boston, MA; 6) Boston University Medical Center, Boston, MA; 7) St. Elizabeth's Hospital, Boston, MA.

Innovative approaches are crucial for identifying novel susceptibility genes for complex diseases. We integrated results from murine and human gene expression microarray studies of lung tissue with whole genome scan linkage results to select a candidate gene (SERPINE2) for investigation in chronic obstructive pulmonary disease (COPD). SERPINE2 is located on chromosome 2q33-35, a genomic region linked to COPD phenotypes.We genotyped 18 SNPs in SERPINE2 and performed association analysis with spirometric phenotypes in 127 severe, early-onset COPD pedigrees in the Boston Early-Onset COPD Study. Using the family-based association test implemented in PBAT, we observed significant association of quantitative COPD phenotypes and five SNPs in SERPINE2 (p ranging between 0.0002 and 0.05). With models including a gene-by-smoking interaction term we observed significant association of COPD phenotypes with eight SERPINE2 SNPs (p ranging between 0.0001 and 0.02).Association of three of these eight SERPINE2 variants was replicated in a case-control association analysis of COPD cases from the National Emphysema Treatment Trial and smoking controls with normal spirometry from the Normative Aging Study; two additional SNPs demonstrated a trend for association. Using expression array analysis we have prioritized candidate gene selection, and we have demonstrated association and replication of association of SERPINE2 as a potential COPD susceptibility gene.

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Program Nr: 27 from the 2004 ASHG Annual Meeting

A 100 kb region in 3q13.31 is significantly associated with coronary artery disease: the power of genome-wide linkage combined with peak-wide association analysis. *J. Vance[1], L. Wang[1], C. Haynes[1], L. Huang[1], S. Gregory[1], W. Kraus[1,2], E. Hauser[1], P. Goldschmidt[1,2], GENECARD and AGENDA Investigators.* 1) Center for Human Genetics,; 2) Department of Medicine and Division of Cardiology, Duke University Medical Center, Durham, NC.

Epidemiology studies suggested that coronary artery disease (CAD) has a strong genetic component. A genome wide screening in 420 families with early-onset CAD disease (GENECARD study) found significant linkage evidence (multipoint lod score=3.5) in chromosome 3q13 spanning over 60 mega bases. Our goal is to narrow the critical region and identify genetic variants conferring susceptibility to CAD. Systematic association analysis using single nucleotide polymorphism (SNP) was performed in case-control sets from the CATHGEN study. Subjects were selected based on their CAD index (CADi), a validated angiographical measure of the extent of CAD. CATHGEN included 301 young affected (YA: age55, CADi>32), 168 older affected (OA: age>55, CADi>74), and 204 controls (ON: age >60, CADi<23). We took a two-stage approach: a preliminary screening in pooled DNA followed by individual genotyping around significant markers at higher density to define the boundaries of the linkage disequilibrium (LD) block. Initial screening of 16 SNPs by DNA pooling revealed that the frequency of the G allele of rs1875518 is significantly higher in OA than ON (OA-ON=12.2%, p=0.001), which is confirmed by individual genotyping (OA=57.2%; ON=45.5%). Additional genotyping around rs1875518 defined an LD block extending ~100 kb that is highly associated with OA in Caucasians. Moreover, preliminary evidence supports the association of this block in the GENECARD probands versus Cathgen ON. Finally, we identified a novel microsatellite marker (3M0238) within the block, which breaks the LD and formed a significant risk haplotype (P<.005) with rs1875518: rs1875518_G-3M0238_253 is twice as prevalent in OA (21.39%) as in ON (11.39%). In conclusion, we identified a 100 kb region in 3q13.31containing genetic susceptibility for CAD. Our data suggest that carriers of rs1875518_G-3M0238_253 are at higher risk of developing CAD.

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LD based GWS for Crohn's Susceptibility Genes using the Quebec Founder Population. *J. Raelson[1], P. Croteau[1], D. Ballinger[2], H. Fournier[1], N. Laplante[1], Q. Nguyen Huu[1], B. Paquin[1], R. Allard[1], R. Paulussen[1], J-M. Vidal[1], R. Govindaraju[1], J. Hooper[1], G. te Meerman[3], D. Cox[2], M. Belouchi[1].* 1) Galileo Genomics, Ville St-Laurent (Montreal), PQ, Canada; 2) Perlegen Sciences, Mountain View, CA; 3) University of Groningen, Netherlands.

A linkage disequilibrium (LD) based genome wide scan (GWS) for Crohn's disease genes was performed using 1500 individuals of French Canadian descent (500 trios) from the Quebec Founder Population (QFP). All individuals were genotyped for 248,000 SNP markers (average gap size, 15 kb) for a total of 372 million genotypes. Cases were either affected children (365 trios), for which parental non-transmitted chromosomes were used as controls, or affected parents (135 trios), for which spousal chromosomes served as controls. Phase and haplotype frequencies were deduced from trio genotypes with ambiguities resolved using the E-M algorithm. Case-Control association analysis for single markers and for multi-marker haplotypes was performed using a proprietary algorithm (LDSTATS). Two previously published Crohn's genes, NOD2/CARD15 (- log10 P value for best single marker association = 6.6; - log10 P for best 9 marker haplotype = 7.6) and OCTN/SLC22A4-5 (- log10 P for best single marker = 5.2; - log10 P for best 9 marker haplotype = 6.2) as well as over 10 additional disease associated candidate loci (- log10 P > 6.0 for 9 marker haplotypes), each narrow enough to contain 1 - 4 genes, were identified. Fine mapping in the NOD2 region at a density of 1 SNP per kb resolved the peak to a region of less than 20 kb containing the P268S mutation previously associated with Crohn's. Statistically significant LD was observed over distances ranging from 200 to over 1500 kb in differnt regions. Individual genotype data was used to make a fine scale recombination map accross the entire genome in order to develop a marker map reflecting the LD in the QFP (the Quebec LD Map) for use in future GWSs.

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Genomewide scan for epistasis in multiplex nonsyndromic cleft lip with or without palate families. *B.S. Maher*[1], *T.H. Goldstein*[1], *M.E. Cooper*[1], *M.A. Mansilla*[2], *S. Daack-Hirsch*[2], *M.L. Marazita*[1], *J.C. Murray*[2]. 1) Univ Pittsburgh; 2) Univ Iowa.

Nonsyndromic cleft lip with or without palate (CL/P) is one of the most common congenital anomalies affecting approximately 1 in 1000 live births. Although the inheritance of CL/P is believed to be complex in nature, much recent progress has been made in understanding some specific contributions of genes or genomic regions. Complex phenotypes, like CL/P, are likely due to the interaction of several genes. Recently, linkage methods for detecting epistasis have been developed, refined and discussed at length in the literature. As an approach for assessing gene-gene interactions in 220 (1676 genotyped pedigree members, 567 affected) Filipino multiplex CL/P families, we utilized a model-free linkage analysis method to assess multipoint linkage across 373 10 cM genome scan markers with the inclusion of 37 markers at candidate loci as covariates. The general conditional logistic model for linkage analysis (Olson, 1999) allows for the inclusion of covariates and all affected relative pair types in large multiplex pedigrees. Multipoint linkage parameters were first estimated under the base model (i.e. no covariates), then the genome scan analysis was repeated for each candidate locus as a covariate. Regions in which the LOD score with a covariate was significantly increased versus the base model are presumably regions with genes that interact with the candidate locus (covariate). To address concerns that the behavior of the test statistic when applied to large multiplex families is influenced by both family and marker characteristics, we performed genomewide simulations to determine the null distribution of the test statistic when no disease locus is present. Evidence for a genomewide-corrected significant interaction was detected between D15s816 (15q26.2) and a SNP in the WRN gene (p=0.0001). Additional moderately significant interactions were detected between markers at 6q21 and two candidate genes (MSX1: p=0.0005 and PTCH: p=0.0003), between 16q12.1 and NRG (p=0.0002), and between 1q23-q31 and ROR2. Grant support: CIDR N01-HG-65403; 5R01DE008559.

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Lod score and MCMC methods applied to a complex trait: LDL size. *G.P. Jarvik, M.D. Badzioch, R.P. Igo, F. Gagnon, J.D. Brunzell, R.M. Krauss, A.G. Motulsky, E.M. Wijsman.* University of Washington, Seattle, WA, USA.

Low density lipoprotein (LDL) size is associated with vascular disease and with familial combined hyperlipidemia (FCHL). We used 1 and 2 marker lod score and whole chromosome multipoint Bayesian Markov chain Monte Carlo (MCMC) oligogenic linkage analysis methods for a 10 cM genome scan of LDL peak particle diameter and adjusted for age, sex, body mass index, and triglycerides (PPD_{asbt}), in 4 large families with FCHL (N=185). Complex segregation analyses (CSA) found a codominant PPD_{asbt} model with the portion of the total variance attributable to the major locus (Vg/Vt) =0.6, vs. 0.36 without triglyceride (TG) adjustment. CSA models were used for the lod score analyses. MCMC analyses used joint segregation/linkage analyses with an oligogenic model. We identified significant evidence of PPD_{asbt} linkage to a chromosome (chr) 9p locus (multipoint lod_{max}=3.7, MCMC intensity ratio, IR=21) in family 1, and across all 4 families to chrs 16q23 (lod_{max}=3.0, IR=43) near *CETP* and 11q22 (lod_{max}=3.7, IR=120). Chr 14q24-31, a region with prior suggestive PPD_{asbt} linkage evidence in another sample, yielded an IR=71 and lod_{max}=1.8 in the combined families and IR=24 and lod_{max}=2.4 in family 4. Without TG adjustment, no significant lod scores were obtained. These large FCHL pedigrees demonstrate that LDL size is a trait influenced by multiple loci, and is confounded by TG. This underscores the use of relatively homogeneous families enriched for the trait. Lod score methods performed well, despite locus heterogeneity. However, oligogenic MCMC joint segregation/linkage analysis methods were required to detect the previously reported 14q locus. These results also demonstrate the applicability of MCMC methods to single large families.

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Program Nr: 31 from the 2004 ASHG Annual Meeting

Guidelines for genotyping in genome wide linkage studies: SNP versus micro-satellite maps. *D. Evans, L. Cardon.* Wellcome Trust Centre for Human Genetics, Oxford University, Oxford, Oxfordshire, United Kingdom.

Genome wide linkage scans have traditionally employed panels of micro-satellite markers spaced at intervals of ~10 cM across the genome. However, there is growing realization that a map of closely spaced SNPs may offer equal or superior power to detect linkage than low density micro-satellite maps. We performed a series of simulations to calculate the information content associated with micro-satellite and SNP maps across a range of different marker densities, heterozygosities and pedigree structures. In the case of micro-satellite markers we varied density across 11 levels (1 marker every 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 cM) and marker heterozygosity across 6 levels (2, 3, 4, 5, 10 or 20 equally frequent alleles), whilst in the case of SNPs we varied marker density across 4 levels (1 marker every 0.1, 0.2, 0.5 or 1 cM) and minor allele frequency across 7 levels (0.5, 0.4, 0.3, 0.2, 0.1, 0.05, and 0.01). When parental genotypes were available, a map consisting of micro-satellites spaced every 2 cM, or a relatively sparse map of SNPs (i.e. at least 1 SNP per cM) was sufficient to extract the majority of inheritance information from the map (i.e. > 95% in most cases). However, when parental genotypes were unavailable, it was important to use as dense a map of markers as possible in order to extract the greatest amount of inheritance information. Importantly, the information content associated with a traditional map of micro-satellite markers (i.e. 1 marker every ~10 cM) was significantly lower than the information content associated with a dense map of SNPs or micro-satellites. These results strongly suggest that previous linkage studies that have employed sparse micro-satellite maps could benefit substantially from reanalysis using a denser map of markers.

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Program Nr: 32 from the 2004 ASHG Annual Meeting

Decision diagram based multipoint linkage analysis; ALLEGRO2. *D. Gudbjartsson[1], T.A. Thorvaldsson[1], G. Gunnarsson[2], A. Kong[1], A. Ingolfsdottir[3].* 1) Dept Statistics, DeCode Genetics, Reykjavik, Iceland; 2) Mathematics Department, University of California Santa Barbara, CA; 3) BRICS, Dept of Computer Science, Aalborg University, Denmark.

We have developed a multipoint linkage analysis algorithm based on Algebraic Decision Diagrams (ADDs). ADDs are an extension of Binary Decision Diagrams (BDDs). The ADD data structure allows for compact storage of probability distributions over inheritance vectors, and allows for reuse of repeated calculations. Our new implementation is based on the CUDD package.

In addition to the savings offered directly by using ADDs, our algorithm incorporates improvements that increase the speed of the implementation. Among these are delaying the convolution of uninformative bits at the locus to which a distribution is being convolved, and only calculating convolution probabilities that are needed at the locus to which a distribution is being convolved.

Our implementation takes advantage of genetic information and is therefore able to handle almost arbitrarily large pedigrees if genetic information is close to being complete, but it is also a significant improvement when genetic information is not complete. As an example we take a real pedigree with 25 non-founding and 11 founding members (39 bits) with data available for 19 microsatellite markers. Our implementation is able to analyze this pedigree in 5 minutes, while neither the current publicly available version of MERLIN (version 0.10.2) or GENEHUNTER (version 2.1_r5) are able to make any progress. By removing family members until complexity level of the pedigree is down to 25 bits MERLIN was able to analyze it in just over two minutes while GENEHUNTER needed 33 minutes, and our implementation took a second. If the complexity was moved back up to 27 bits MERLIN ran out of memory (needed over 3.5Gb), while GENEHUNTER did not run because of foreseeable lack of memory, and our implementation took a second.

This new multipoint linkage analysis algorithm has been implemented into a new version of our linkage analysis package, ALLEGRO2, and will be made publicly available.

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A method for targeted marker selection. *C.L. Simpson*[1], *V. Hansen*[1], *P.C. Sham*[3], *J.F. Powell*[2], *A. Al-Chalabi*[1]. 1) Department of Neurology, Institute of Psychiatry, London, United Kingdom; 2) Department of Neuroscience, Institute of Psychiatry, London, United Kingdom; 3) Statistical Genetics, Institute of Psychiatry, London, United Kingdom.

Genome-wide and other large-scale association studies must balance the need for the densest possible marker set with the problems of limiting cost and effort required. Here we present a program (MaGIC), which we have designed as part of a genome wide search for susceptibility genes for amyotrophic lateral sclerosis. This exploits genome assembly data to create lists of markers correlated with genes or other genomic features at a user-specified density and spacing.Publicly available assembly data was used to generate a comprehensive database of SNPs and microsatellite markers. The genome was treated as comprising bins of user-defined width. Genomic features were treated as point sources based on start position. From each bin containing a target feature, a specified number of markers were chosen. For targeting at a given density, rank ordering of genomic features per bin was used to select the threshold. Commercial marker sets were imported and used as the source marker set to assess the method for targeting and the set for relevance.A targeted marker set typically contains a fraction of the markers of an evenly spaced marker set with little loss of information. For example, a marker set evenly spaced at 50Kb intervals would require 60,000 markers but using MaGIC produces a set of 41,771 markers with no loss of power. Analysis of the Affymetrix human 100K GeneChip microarray showed that about 44% of exons were targeted, whereas the same number of markers can target nearly 100% of exons when selected using MaGIC. Targeted markers sets are therefore more efficient and cost-effective than an evenly spaced marker set.

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Linkage disequilibrium inflates Type I error rates in multipoint linkage analysis when parental genotypes are missing. *A.L. Boyles, W.K. Scott, E.R. Martin, S. Schmidt, Y.J. Li, A. Ashley-Koch, M.P. Bass, M.A. Pericak-Vance, M.C. Speer, E.R. Hauser.* Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC.

The effect of linkage disequilibrium (LD) between markers on linkage analysis has been an issue of much concern to statistical geneticists, but few studies have examined the magnitude of its effects. Using simulated nuclear families and affected sibling pairs, we identified a dramatic increase in the false positive rate in multipoint linkage analysis when LD was present but unaccounted for, when parental genotypes were missing. Even at low levels of LD, such as a Dof 0.6, multipoint linkage analysis produced LOD scores over 2 in 5% of the replicates when there was no true linkage to disease. In the extreme case, a D of 1 produced a multipoint LOD score over 3 in 76% of replicates under the recessive model. Type I error rates were not inflated in two-point LOD scores or when parents were fully typed in the simulated data. Increased sample size also increased the proportion of false positives observed. When there was a mixture of markers in strong LD and markers in equilibrium, the increase in false positives was reduced, but not eliminated. Misspecification of the population haplotype frequencies by assuming linkage equilibrium is the likely cause of the inflated multipoint LOD scores. This strongly supports the evaluation of LD in all multipoint linkage analyses, particularly when parental genotypes are missing, and further suggests that unrecognized LD may be suspected when two-point and multipoint linkage analyses show a marked disparity. Given the higher density and increasing popularity of genome-wide SNP screens, it is likely that LD will be problematic in future linkage analysis studies.

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Program Nr: 35 from the 2004 ASHG Annual Meeting

Enhanced linkage maps from family-based genetics studies. *C. He[1], G. Abecasis[2], X. Kong[1], P. Concannon[3], X. Xu[4], S. Buyske[1], D.E. Weeks[5], T. Matise[1].* 1) Rutgers Univ, Piscataway, NJ; 2) University of Michigan, Ann Arbor, MI; 3) Benaroya Research Institute, Seattle, WA; 4) Harvard School of Public Health, Boston, MA; 5) University of Pittsburgh, PA.

Meiotic linkage maps are the foundation of both linkage and linkage disequilibrium studies for mapping disease genes. Existing genome-wide linkage maps were built using only small collections of pedigrees, and so have wide confidence intervals surrounding estimates of map distance. Incorrect marker order and map distances can lead to incorrect or imprecise results from linkage analyses, so there is a clear need for more accurate genetic maps. Accurate estimates of meiotic map distance cannot be obtained by any means other than by linkage analysis using genotype data. We have collected a very large sample of genotype data from disease-mapping studies genotyped by the Mammalian Genotyping Service in Marshfield, IL. This sample includes genotypes for more than 15,000 individuals from over 3,000 pedigrees for markers on the Marshfield screening sets 8-11. These sets have average map resolutions of 8-10 cM. Several ethnic groups are represented in this sample. We have cleaned numerous pedigree structure and genotyping errors in these data, as well as verified the marker orders. Using a novel error modeling feature in the Merlin program, we are using this dataset to a) test for population-specific distribution of recombination; and b) re-estimate the distances on these screening set maps, both sex-averaged and sex-specific. Comparison of a large Caucasian sample (956 meioses) with a large Chinese sample (1626 meioses) showed that these two linkage maps are in quite good agreement with each other, with only one map interval showing significantly different estimates of meiotic distance. The variances of most distance estimates are smaller for the larger sample than for the smaller sample, supporting our hypothesis that larger datasets lead to better estimates of map distance. Our analyses result in screening set maps with improved accuracy, which can in turn be used to improve the accuracy of results of disease-mapping studies.

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The Effect of Ethnicity on the Construction of Human Genetic Linkage Maps. *E. Jorgenson[1,2], H. Tang[3], M. Gadde[2], M. Province[4], M. Leppert[5], S. Kardia[6], N. Schork[7], R. Cooper[8], DC. Rao[4], E. Boerwinkle[9], N. Risch[2], Family Blood Pressure Program (FBPP), which is supported by the NHLBI.* 1) Epidemiology and Biostatistics, UCSF, San Francisco, CA; 2) Genetics, Stanford University, Stanford, CA; 3) Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 4) Biostatistics, Washington University School of Medicine, St. Louis, MO; 5) EIHG, University of Utah,Salt Lake City, UT; 6) Dept. of Epidemiology, University of Michigan School of Public Health, Ann Arbor, MI; 7) Psychiatry, University of California, San Diego,La Jolla, CA; 8) Loyola University Medical Center,Maywood, IL; 9) Human Genetics, University of Texas-Houston Health Science Center, Houston, TX.

Human genetic linkage maps are based on rates of recombination across the genome. These rates in humans vary by the sex of the parent from which alleles are inherited, chromosomal position and genomic features such as GC content and repeat density. We demonstrate for the first time that ethnicity affects genetic map length in humans. We constructed genetics maps in four racial/ethnic groups: Caucasians, African-Americans, Mexican-Americans, and East Asians (Chinese and Japanese) based on 353 microsatellite markers. These maps were generated from the largest number of human subjects of any map constructed to date. We identified regional and genome-wide differences across ethnic groups. Some, but not all, of this variation was explained by the presence of null alleles at many of the loci and ethnic differences in null allele frequencies. The results of our investigation are instructive both for inferences of possible genetic influences on human recombination as well as for future linkage studies, especially those involving populations of non-Caucasian ethnicity.

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Genotype-phenotype correlation at the PHOX2B locus allows prediction of tumour risk in patients with congenital central hypoventilation syndrome (CCHS). *J. Amiel[1], D. Trochet[1], L.M. O'Brien[2], D. Gozal[2], H. Trang[3], A. Munnich[1], C. Gaultier[3], O. Delattre[4], S. Lyonnet[1]*. 1) Genetics, INSERM U-393, Necker Hospital, Paris, France; 2) Department of Pediatrics, Kosair Children's Hospital, Louisville, Kentucky, USA; 3) Physiology, Robert Debre Hospital, Paris, France; 4) INSERM U-509, Institut Curie, Paris, France.

CCHS (Ondine's curse) is a complex dysautonomic disorder primarily manifesting as sleep-associated respiratory insufficiency and markedly impaired ventilatory responses to hypercarbia and hypoxaemia. CCHS patients present Hirschsprung disease (HSCR) and tumours of the sympathetic nervous system (TSNS), such as neuroblastoma, in 20% and 5% of cases respectively. We undertook a candidate gene approach and screened genes involved in the developmental cascade of neural crest cell progenitors. We identified PHOX2B as the disease causing gene with an autosomal dominant mode of inheritance and de novo mutation at the first generation. We have subsequently shown that heterozygous mutations of PHOX2B may also account for TSNS either familial or syndromic (+ HSCR).We report on the clinical and molecular assessments of a multicentric series of 188 CCHS patients, either isolated or associated with HSCR (21%) and/or TSNS (5%). The mutation detection rate was 90% in our series and the most prevalent mutation was an in-frame duplication resulting in an expansion of + 5 to + 13 alanines in the 20 alanine stretch at the carboxy terminal of the protein homeodomain. Somatic mosacism in parents could be detected in 8% of families with important consequences regarding genetic counselling. In a minority of patients (11 cases) the PHOX2B heterozygous mutation was either a missense or a frameshifting mutation. Analysis of genotype-phenotype interactions strongly supports the contention that CCHS patients who developed malignant TSNS will harbor either a missense or a frameshift heterozygous mutation of the PHOX2B gene. Thus, PHOX2B molecular testing points to a subset of CCHS patients who are at very high risk for developing tumours, and therefore justify a very careful clinical follow-up, at least during the first 2 years of life. .

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Program Nr: 38 from the 2004 ASHG Annual Meeting

Tumor Formation Observed in a Model for the Carney Complex. *L. Kirschner[1], W. Towns[1], D. Kusewitt[2], C. Stratakis[3].* 1) Dept Human Cancer Genetics, Ohio State Univ, Columbus, OH; 2) Dept of Vet Biosciences, Ohio State Univ, Columbus, OH; 3) Sect. Genetics and Endocrinology, NIH, Bethesda, MD.

Carney Complex (CNC, MIM #160980) is a multiple endocrine neoplasia syndrome characterized by spotty skin pigmentation, myxomas, schwannomas, and endocrine tumors. We have demonstrated that this autosomal dominant condition is caused by inactivating mutations of *PRKAR1A*, the gene that encodes the type 1A regulatory subunit of Protein Kinase A (PKA, cyclic-AMP dependent protein kinase). In order to better understand the molecular mechanisms by which loss of *PRKAR1A* causes tumors in patients, we have attempted to create a mouse model for this condition. Using homologous gene targeting, we generated mice carrying an allele of this gene that lacks exon 2, which encodes the initiator ATG codon. Previous studies from human patients had indicated that mutation of exon 2 was sufficient to produce a null allele of the gene, and we have confirmed that the mouse allele is also null. In this abstract, we report the preliminary characterization of the phenotype of our *Prkar1a*$^{+/-}$ mice in a mixed genetic background. Mice that are homozygous null for *Prkar1a* die in utero by day e9.5, in agreement with prior studies. Heterozygous null mice are born with the expected frequency, and exhibit no increase in early mortality. Mice begin to develop tumors in the 7th month, and the incidence increases as the mice age. Although endocrine tumors have not been detected, spindle-cell neoplasms have been observed, as well as a malignant thymoma. In addition to these tumors, the majority of mice develop multiple tumors on the tail after the age of 1 year. These tumors appear to originate in the vertebral bones and destroy the normal architecture. Interestingly, these lesions have not been observed in vertebral bodies proximal to the pelvis. Molecular analysis of the tumors observed in *Prkar1a*$^{+/-}$ mice is at an early phase, but analysis to date suggests that there is loss of heterozygosity in the tumors. These mice should provide an excellent model system for understanding the tumorigenesis associated with heterozygous loss of the *Prkar1a* gene as observed in patients with the Carney Complex.

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Program Nr: 39 from the 2004 ASHG Annual Meeting

Molecular classification of gastrointestinal hamartomatous polyposis as a diagnostic adjunct to genetic counseling. *K. Sweet[1], X.P. Zhou[1], J. Heinz[1], R. Pilarski[1], J. Willis[2], T. Prior[1], T. Frebourg[3], B. Teh[4], L. Aaltonen[5], C. Eng[1]*. 1) Ohio State Univ, USA; 2) Case Western Reserve Univ, USA; 3) INSERM EMI-9906, Facultè de Mèdecine, France; 4) Van Andel Research Inst, USA; 5) Univ Helsinki, Finland.

The elucidation of the genetic etiology of the inherited hamartoma polyps syndromes (HPS) only began in 1997 when germline mutations in PTEN were found in Cowden and Bannayan-Riley-Ruvalcaba syndromes, and in LKB1/STK11 in Peutz-Jegher syndrome (PJS). Subsequently, germline mutations in MADH4 and BMPR1A were found in juvenile polyposis syndrome (JPS). Although classic presentations of each are easily recognized, the number and distribution of polyps differ among these syndromes, and there can be considerable variation in the determination of histologic subtype by pathologists. Subsets of patients with HPS remain molecularly unexplained, as do families with serrated adenomas, hyperplastic polyposis and admixed polyps, thus making genetic counseling a challenge. We sought to determine if molecular classification of various HPS was possible and useful for genetic counseling. We accrued 46 probands with hamartomatous or mixed polyps who met 1 of the following 4 criteria: I, JPS (N=10) or PJS (3) but mutation negative at their respective susceptibility genes; II, undefined HPS (9); III, combination of hyperplastic, serrated adenomas or adenomatous polyps (19), ranging from 5-40 polyps (mean 18); IV mixed polyposis, each with hamartoma, adenoma and hyperplastic polyps (5). Germline mutation and deletion analysis at the 4 known susceptibility genes in addition to BHD, BRAF and MYH were performed. Among the first 27 probands, a hemizygous deletion encompassing BMPR1A and PTEN was found in 1 (8%) member of Group I, and PTEN mutations (612insC and IVS3-7 delCTTT, respectively) in 2 (10%) from Group III. Review of the clinical phenotype for the 3 individuals revealed features reminiscent of, but not classic for, Cowden syndrome. One, an Ashkenazim, from Group IV was found to have linkage to the CRAC2 locus on 15q. Thus, genetic classification of HPS may be helpful in genetic counseling as each has different component neoplasias.

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Expression and modulation of the U-box type Ubiquitin Ligase UBE4A in human cancers. *G. Contino, S. Pucci, F. Pichiorri, F. Sesti, L. Baghermajad Salhei, L.G. Spagnoli, G. Novelli.* Biopathology, Univ. of Rome "Tor Vergata", Rome, Italy.

The UBE4A gene is human homologue of S.Cerevisiae Ufd2 and encodes for an U-box type Ubiquitin Ligase (E3). Ubiquitin represents the degradation signal for the proteasome, a multi subunit ATP-dependent protease responsible for degradation of protein whose levels are regulated either constitutively or in response it changes in cellular environment. Ubiquitin-Proteasome Pathway (UPP) is a basic cellular process involved in cell cycle progression, proliferation and differentiation, apoptosis, DNA repair and stress response. Particularly, interactions with VCP/p97, ataxin-3, HSC-70 have been reported in many U-box proteins. We first analyzed the expression of UBE4A gene both at mRNA and protein level in several human and fetal tissues and then examined UBE4A expression in different pathological conditions including 17 different type of tumors. Results showed that UBE4A expression and subcellular localization was significantly altered in all the desmoplastic reactions and modulated in some specific tumors such as ovarian, gastric and colon cancer and lymphomas. Particularly, gastric and colon cancer showed that UBE4A over-expression was associated with tumor size, lymphatic permeation, lymph node metastasis, and proliferative activity. We then analyzed the in vitro expression and subcellular localization of the UBE4A protein in human colonic carcinoma cell line CaCo2, after apoptotic stimuli. Immunocitochemistry and Western blot analysis showed a relocalization of UBE4A during oxidative stress and apoptosis. These data suggest that UBE4A may play a role in oxidative stress and apoptosis and possibly be a marker of tumors with enterocytic differentiation. Furthermore these data confirm a role of UPP in progression of tumorigenesis.

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Cowden syndrome susceptibility gene, *PTEN*, regulates phospholipase-C (PLC) and phospholipaseD (PLD). *C. Alvarez-Breckenridge, K. Waite, C. Eng.* Hum Cancer Genet, Ohio State Univ, Columbus.

Germline mutations in *PTEN* cause 85% of Cowden syndrome (CS), characterized by hamartomas and a risk of breast and thyroid cancer, and 65% of Bannayan-Riley-Ruvalcaba syndrome (BRRS) characterized by lipomatosis, hemangiomas and speckled penis. Often, BRRS patients have a lipid myopathy believed to be due to long-chain hydroxyacyl-coA dehydrogenase (LCHAD) deficiency although this is controversial. Somatic PTEN alterations are found in sporadic breast cancer. PTEN modulates cellular levels of phosphatidylinositol-4,5-bisphosphate (PIP2) and phosphatidylinositol-3,4,5-triphosphate (PIP3) and is an antagonist of the PI3K/AKT pathway. Beyond this apoptotic pathway, little is known about the role of PTEN in regulating other lipid signaling events. Since phospholipids have been shown to play a critical role in signal transduction and cellular proliferation and PTEN to control cellular phospholipid levels, we hypothesized that PTEN functions as a regulator of lipid signaling and homeostasis. PLD and PLC activation are regulated by PIP2/3 levels and have been shown to play an important role in cell division. Thus, we examined the ability of PTEN to regulate PLD activity and PLC translocation (as a measure of its activity). Increased PTEN expression in unstimulated MCF-7 breast cancer cells results in 51% increase in phosphatidic acid with a decrease in phosphatidylcholine, suggesting that PTEN may regulate PLD. PTEN overexpression results in 30% increase in basal PLD activity. Since PLC is involved in PLD activation and is regulated by PIP2/3 levels, we investigated the role of PTEN on PLC activation. Estrogen stimulation resulted in PTEN-dependent translocation of PLC to the plasma membrane. PLC translocation did not occur in cells expressing dominant negative C124S PTEN mutation. PLC translocation is accompanied by an increase in PLD activity in response to estrogen. Our data suggest that PTEN plays a role in modulating the PLC:PLD activation pathways and indicates that the pathogenesis of CS/BRRS has a more complex biochemical basis beyond simple over-activation of the PI3K pathway, and may also begin to explain the etiology of lipid myopathy in BRRS.

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Genetic mapping of a third Li-Fraumeni syndrome (LFS) predisposition locus to human chromosome 1q23. *L.L. Bachinski[1], S.E. Olufemi[1], X. Zhou[2], C.-C. Wu[2], S. Shete[2], G. Lozano[1], C.I. Amos[2], L.C. Strong[3], R. Krahe[1,4]*. 1) Section of Cancer Genetics, University of Texas M. D. Anderson Cancer Center, Houston, TX; 2) Department of Epidemiology University of Texas M. D. Anderson Cancer Center, Houston, TX; 3) Clinical Cancer Genetics, Department of Molecular Genetics University of Texas M. D. Anderson Cancer Center, Houston, TX; 4) Human Cancer Genetics Program, Department of Molecular Virology, Immunology and Medical Genetics, Comprehensive Cancer Center, Ohio State University, Columbus, OH.

Li-Fraumeni syndrome (LFS) is a clinically and genetically heterogeneous inherited cancer syndrome. Most cases (~70%) identified and characterized to date are associated with dominant germline mutations in the tumor suppressor gene TP53 (p53) located in 17p13.1. Based on segregating germline mutations, CHEK2 in 22q12.2 was also recently implicated as a minor predisposing LFS locus. Out of a large cohort of LFS kindreds, our initial genome-wide scan focused on four extended, non-p53 LFS pedigrees. We have shown that there is additional genetic heterogeneity in LFS kindreds with inherited predisposition at loci other than p53 and CHEK2. Using a genome-wide scan for linkage with complementing parametric and non-parametric analysis methods, we have identified linkage to a region of about 4 cM in 1q23, a genomic region not previously implicated in this disease. We obtained definitive evidence for linkage with multi-point LOD scores >3.00 between D1S2635/154.28 cM and D1S1677/161.50 cM (SIMWALK, HLOD = 3.57). The same region appeared to be excluded in one of the 4 families with LOD scores <-2.00 throughout, indicating possible additional genetic heterogeneity. Identification of the major non-p53 predisposing gene and its underlying mutations should provide insight into other genetic events that predispose to the genesis of the diverse tumor types associated with LFS and its variants.

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The true story of Gastrointestinal Neurofibromatosis. *T. De Raedt[1], H. Brems[1], J. Cools[1], R. Sciot[2], J. Himpens[3], P. Marynen[1], E. Legius[1].* 1) Dept Human Genetics, Catholic Univ Leuven, Leuven, Belgium; 2) Department of Pathology, KULeuven, Leuven, Belgium; 3) AZ Sint Blasius, Dendermonde, Belgium.

The neurofibromatoses are a heterogeneous set of conditions. One of these is gastrointestinal neurofibromatosis. At present very few families with gastrointestinal NF have been published (Lipton and Zuckerbrod, 1966 and Heimann et al., 1988). Gastrointestinal neurofibromatosis is phenotypically described as: neurofibromas strictly limited to the intestine, adult onset with incomplete penetrance, variable expression, autosomal dominant inheritance pattern and absence of other features of NF1 or NF2. We reanalysed the Belgian family with intestinal neurofibromatosis published by Heimann et al. (1988) and found an inherited mutation in the juxtamembrane region of PDGFRa. Somatic activating mutations in KIT and PDGFRa are often found in sporadic GIST (gastrointestinal stromal tumours). These activating mutations result in downstream activation of the JNK/STAT, MAPK and Akt pathways. GIST is also one of the tumours that occur at an increased frequency in NF1 patients and is defined as a non-epithelial gastro-intestinal tumour expressing c-KIT. It is thought that they arise from the interstitial cells of Cajal or their precursor cells. Recently a mutation in PDGFRa was found in a family with familial GIST (Chompret et al. 2004). We also reanalysed the pathology of the gastrointestinal tumors in our family and conclude that they show the typical features of neurofibromas with infiltrating mast cells. The proliferating cells are c-KIT negative. The presence of S-100 expression and scattered neurofilament positive axons further supports the diagnosis of neurofibroma. We argue that the lesions described as intestinal neurofibromas are related to GISTs, and that gastrointestinal neurofibromatosis is allelic to familial GIST. Sporadic GISTs with mutations in c-KIT or PDGFRa and familial GISTs with a constitutional c-KIT mutation are usually responsive to treatment with Gleevec (STI-571). It remains to be seen if tumors in families with a constitutional PDGFRa mutation are equally sensitive to Gleevec therapy.

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Program Nr: 44 from the 2004 ASHG Annual Meeting

The Y-Located TSPY Gene Co-Expresses with Biological Markers for Testicular Germ Cell Tumors in Gonadoblastoma. *Y.-F.C. Lau[1], Y.M. Li[1], P.M. Chou[2], E. Vilain[3].* 1) Dept of Medicine, VA Med. Center, University of California, San Francisco, CA; 2) Dept of Pathology, Northwestern University, Chicago, IL; 3) Dept. of Hum Genetics, University of California, Los Angeles, CA.

The gonadoblastoma on the Y chromosome (GBY) is the only oncogenic locus on this male-specific chromosome. Recent complete sequencing of the human Y chromosome revealed that the testis-specific protein Y-encoded (TSPY) gene is the only functional gene residing on the critical region for GBY, thereby establishing this gene to be a strong candidate for this oncogenic locus. TSPY is a tandemly repeated gene that encodes a protein with significant homology to the oncoprotein, SET. TSPY binds to cyclin B and possibly plays a role in cell cycle regulation. The GBY gene(s) predisposes the dysgenetic gonads of XY sex reversed patients to develop gonadoblastoma that shares significant morphological and pathological properties with the more common testicular germ cell tumors (TGCTs). TGCTs are hypothesized to arise from mutational events during the differentiation of primordial germ cells and gonocytes since many of the early germ cell markers, such as Oct3/4, c-kit and PLAP, are expressed in TGCTs. Using various immunofluorescence techniques, we have demonstrated the co-expression of TSPY and early germ cell markers in two cases of gonadoblastoma. TSPY was first detected in minute foci of tumor precursor cells localized at the epithelial cell layer of empty and dysfunctional follicles. TSPY persisted in the tumor cells that grew from these minute foci and gradually and completely filled up the empty follicles. The follicular tumors grew in size, but in confined morphology. At late stages, such confined growth could progress into a network of more loosely organized tumor cell clusters that, in turn, could take on more aggressive properties. Since TSPY is involved in cell cycle regulation, its abnormal expression in the germ cells of dysgenetic gonads suggests that it might play a critical role in the initiation and progression of gonadoblastoma. Co-expression of TSPY and early germ cell markers in both gonadoblastoma and TGCTs supports a common etiological origin for both germ cell cancers.

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Somatic mosaicism and cancer: An examination into the role of the androgen receptor gene in prostate cancer. *B. Gottlieb*[1], *C. Alvarado*[1], *L.K. Beitel*[1], *K. Sircar*[4], *A. Aprikian*[3], *M.A. Trifiro*[1,2]. 1) Dept. Cell Genetics, Lady Davis Inst Medical Res, Montreal, PQ, Canada; 2) Dept. Medicine, McGill University, Montreal, Quebec, PQ, Canada; 3) Dept. Urology, MUHC, Montreal General Hospital, Montreal, PQ, Canada; 4) Dept. Pathology, MUHC, Montreal General Hospital, Montreal, PQ, Canada.

The ontology of most cancers is unknown as in very few cases is a single genetic or environmental event implicated as the sole causal agent. To understand the origin of prostate cancer we have investigated the tissue heterogeneity of tumors, i.e. that they consist of normal (benign), pre-cancerous and cancerous cells, by examining whether tissue heterozygosity is an indication of genetic heterozygosity. We have used laser capture microdissection (LCM) to dissect and then sequence different areas of prostate tumors that had been identified as homozygous according to their cancer pathology. All of these dissected tissues showed a remarkable degree of somatic mosaicism in the androgen receptor (AR) gene, with the presence of several different lengths of the exon 1 CAG repeat, a well-known polymorphism, already implicated as a possible risk factor in prostate cancer. Overall, there was a clear trend of tissues with higher Gleason scores to have shorter tract lengths, sometimes even shorter than the shortest reported in normal tissues. It should be noted that while benign tissue did show some somatic mosaicism the tract length changes tended to be limited to one or two CAG repeats from the normal mean length of 21, and that only cancerous tissues exhibited significantly shorter length repeats. Based on the inverse relationship between CAG repeat length and AR gene responsiveness, this somatic mosaicism would likely result in cells that are considerably more responsive to androgens. The high degree of somatic instability of the AR gene in prostate, as compared to other tissues, is likely to be extremely significant. Our results indicate that the somatic mosaicism of the AR gene exhibited in prostate cancer tissue may serve as an important marker for early detection of onset and subsequent development of the disease.

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Program Nr: 46 from the 2004 ASHG Annual Meeting

A germline DNA polymorphism enhances alternative splicing of the KLF6 tumor suppressor gene and is associated with increased prostate cancer risk. *J. Martignetti[1], G. Narla[1], A. DiFeo[1], S. Friedman[1], D. Schaid[2], W. Isaacs[3], S. Hebbring[2], A. Komiya[3], S. Jacobsen[2], D. Friedrichsen[4], J. Stanford[4], E. Ostrander[4], A. Chinnaiyan[5], M. Rubin[6], J. Xu[7], S. Thibodeau[2].* 1) Mount Sinai School of Medicine, New York, NY; 2) Mayo Clinic/Foundation, Rochester, MN; 3) Johns Hopkins Medical Institution, Baltimore, MD; 4) Fred Hutchinson Cancer Research Center, Seattle, WA; 5) University of Michigan Medical School, Ann Arbor, MI; 6) Harvard Medical School, Brigham and Womens Hospital, Boston, MA; 7) Wake Forest University School of Medicine, Winston-Salem, NC.

Prostate cancer (PCa) is a leading and increasingly prevalent cause of cancer death in men. While family history of disease is one of the strongest PCa risk factors and suggests a hereditary component, the predisposing genetic factors remain unknown. We first demonstrated that KLF6 is a tumor suppressor inactivated in PCa, and since then its functional loss has been further established in other human cancers. Wild type KLF6, but not patient-derived mutants, suppresses cell growth through p53-independent transactivation of p21. We now demonstrate the existence of three alternatively spliced, dominant negative KLF6 isoforms which are upregulated in tumor versus normal prostatic tissue. Two KLF6 variant proteins, KLF6-SV1 and SV2, are mislocalized to the cytoplasm and antagonize wtKLF6 function, leading to decreased p21 expression and increased cell growth. Interestingly, alternative splicing is significantly upregulated by the presence of a relatively common single nucleotide polymorphism (SNP) which generates a novel functional SRp40 DNA binding site. Moreover, in a tri-institutional study of 3,411 men, we identified a significant association of this single KLF6 SNP with an increased relative risk of prostate cancer in men with both hereditary and sporadic forms of the disease. Thus, these results are the first to identify a novel mechanism of self-encoded tumor suppressor gene inactivation and then link a relatively common SNP to both regulation of alternative splicing and an increased risk in a major human cancer.

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Mutations in the EP300 gene cause Rubinstein/Taybi Syndrome. *M.H. Breuning[1], J.H. Roelfsema[1], S. White[1], Y. Ariyrek[1], D. Bartholdi[2], D. Niedrist[2], F. Carnavale[3], J.T. den Dunnen[1], G.-J.B. van Ommen[1], R.C. Hennekam[4], D.J.M. Peters[1]*. 1) Center for Human and Clinical Genetics, Leiden Univ, Leiden, Netherlands; 2) Inst. Medical Genetics, Univ. Zurich, Switzerland; 3) Dept. Obst. & Gynaekol. Bari Univ, Italy; 4) Dept. Clinical Genetics, Acad. Med. Center, Amsterdam, Netherlands.

CBP and p300 function as transcriptional coactivators in the regulation of gene expression by various signal transduction pathways. The level of CBP is vital for normal development, as inactivation of one allele causes Rubinstein-Taybi syndrome. We now present evidence that mutations in EP300 also cause this disorder. EP300 was a potential candidate, being a close homologue of CBP, with both proteins serving in similar fashion as transcriptional coactivators. No mutations, however, had been found in the gene. After screening the entire CBP gene we found 29 mutations in 91 patients. We subsequently screened these patients for p300 mutations. Indeed, 3 inactivating mutations were detected in the EP300 gene. Two mutations were found using Denaturing Gradient Gel Electrophoresis (DGGE): one mutation, in exon 10, is a transition (c.1942C>T) that converts the triplet coding for the arginine at position 648 into a stop codon. The other mutation, in exon 15, is a deletion of 8 nucleotides that predicts a frameshift from codon 959 with a stop codon after 7 amino acids. The exact location of the 8bp deletion (c.2877_2884 del) was confirmed with an allele specific PCR. We analyzed DNA from the healthy parents of both patients with DGGE and sequencing and confirmed that the mutations occurred de novo as expected. Both mutations predict proteins less than half their normal size. The HAT domain will be entirely removed and it is known that loss of HAT activity of CBP is sufficient to cause RSTS. The third mutation, a deletion of the first exon, was found using Multiplex Ligation-dependent Probe Amplification (MLPA). Two probes, one within exon 1 and a second in intron 1, close to the first exon, revealed this deletion, whereas a probe in exon 2 showed a normal dosage. The effect of this deletion will most likely be no expression from the affected allele.

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Program Nr: 48 from the 2004 ASHG Annual Meeting

Array-based CGH and FISH fail to confirm duplication of 8p22-p23.1 in association with Kabuki syndrome. *J.E. Ming*[1], *J.D. Hoffman*[1], *Y. Zhang*[1], *J. Greshock*[2], *K.L. Ciprero*[1], *B.S. Emanuel*[1], *E.H. Zackai*[1], *B.L. Weber*[2]. 1) Division of Human Genetics, The Children's Hospital of Philadelphia and The University of Pennsylvania School of Medicine, Philadelphia, PA; 2) Abramson Family Cancer Research Institute, The University of Pennsylvania, Philadelphia, PA.

Kabuki (Niikawa-Kuroki) syndrome is associated with a characteristic facial appearance, cleft palate, congenital heart disease, and developmental delay. Although a variety of cytogenetically visible chromosomal rearrangements have been reported in single cases, the molecular genetic basis of the condition has not been conclusively established. A recent report described a duplication of 8p22-23.1 in 13 out of 13 Kabuki syndrome patients. We sought to determine the frequency of this duplication in our cohort of patients with Kabuki syndrome. Using two independent methods, array-based comparative genomic hybridization and fluorescence in situ hybridization, we assessed for this duplication in 15 patients with a definitive clinical diagnosis of Kabuki syndrome. No evidence for a duplication of 8p was obtained in this patient group by either method. We conclude that the 8p22-23.1 duplication may not be a common mechanism for Kabuki syndrome and that another genetic abnormality is likely to be responsible for the etiology of Kabuki syndrome in many patients.

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Program Nr: 49 from the 2004 ASHG Annual Meeting

A prospective analysis of the inheritance, pathogenesis, and natural history of bicuspid aortic valve with thoracic aortic aneurysm. *M.L. Loscalzo, D. Goh, P.J. Spevak, H.C. Dietz.* HHMI, Inst of Genet Med, and Dept Ped, Johns Hopkins Univ Sch Med, Baltimore, MD.

The autosomal dominant inheritance of bicommissural aortic valve (BAV) is well documented. However, the inheritance of BAV with thoracic aortic aneurysm (TAA) is less clear. Whether aneurysm is secondary to hemodynamic perturbation due to the BAV or a primary manifestation of the disorder remains controversial. Guidelines are needed regarding the follow-up and treatment of patients and their families. Here we present prospective analysis of eleven families with BAV and TAA. Affected status was determined by the echocardiographic presence of BAV and/or TAA or a history of aortic dissection, rupture, or surgical repair. Six of eleven families had at least two family members with both BAV and TAA, often in successive generations. Either partial penetrance (BAV alone) or complete nonpenetrance was observed in obligate carriers. Informatively, all 11 families had at least one family member with TAA in the absence of BAV. Of the 96 comprehensively evaluated relatives of affected probands, 45 (47%) had TAA with or without BAV. Among individuals with combined BAV/TAA, the majority had no hemodynamic consequence of the valve abnormality. In addition, many individuals with TAA also had dilatation of the main pulmonary artery (MPA). In the majority of families (9 of 11) aortic dilatation was either restricted to or maximal above the sinotubular junction (STJ), allowing distinction from Marfan syndrome and other described forms of familial TAA where dilatation is generally confined to the aortic root. Vascular dissection was observed in 6 of 11 families and occurred in individuals with or without BAV. These data document that BAV/TAA is an autosomal dominant disorder with variable expressivity and incomplete penetrance. The component features are both primary manifestations of the underlying gene defect. All family members, including those without BAV, require follow-up using imaging protocols that specifically assess aortic segments beyond the STJ. Aggressive medical and surgical management is required to modify the significant risk of sudden death due to aortic dissection and rupture.

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Program Nr: 50 from the 2004 ASHG Annual Meeting

A novel common and clinically aggressive autosomal dominant aortic aneurysm syndrome. *B. Loeys, H. Dietz.* McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins Univ, Baltimore, MD.

Aortic aneurysm and dissection is the cause of death in about 2% of individuals in industrialized countries. While abdominal aortic aneurysm is most commonly observed in old age and in association with risk factors that include atherosclerosis and smoking, ascending aortic aneurysm commonly occurs in young individuals in the absence of known risk factors, suggesting a major genetic contribution to predisposition. Familial ascending aortic aneurysm can occur in the context of defined heritable disorders of connective tissue (eg. Marfan syndrome) or as an isolated cardiovascular manifestation (eg. familial thoracic aortic aneurysm). Here we describe a novel aortic aneurysm syndrome characterized by the triad of hypertelorism, bifid uvula/cleft palate, and arterial tortuosity with ascending aortic aneurysm/dissection. In the absence of dedicated recruitment, over the past two years we have identified 15 patients from 7 unrelated families among a population of individuals presenting with aortic aneurysm, suggesting that this is a relatively common condition. The disease is characterized by autosomal dominant inheritance with high albeit incomplete penetrance. Other variable manifestations include malar hypoplasia, joint hypermobility, scoliosis, and patent ductus arteriosus. All adults with this condition have died from acute ascending aortic dissection before age 40. Importantly, in the one case that was followed prospectively, aortic dissection occurred in association with a maximal aortic dimension of 4.0 cm, far below the critical threshold of 5.5 cm that has been established for other forms of ascending aortic aneurysm. Light- and electronmicroscopic study of the aortic wall demonstrated a profound decrease in elastin content and loss of intimate spatial association between elastic lamellae and vascular smooth muscle cells, suggesting a severe defect in elastogenesis. We are currently examining candidate genes involved in elastic fiber formation and expanding selected pedigrees for use in positional cloning. Importantly, given the atypical behavior of this phenotype, early diagnosis and aggressive surgical intervention are mandatory to optimize outcome.

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Mutations in the beta subunit of the epithelial sodium channel in patients presenting with a cystic fibrosis-like syndrome. *M.B. Sheridan[1], J.D. Groman[1], P. Fong[1], C. Conrad[2], P. Flume[3], R. Diaz[4], C. Harris[5], M. Knowles[6], G.R. Cutting[1]*. 1) Johns Hopkins Univ., Baltimore, MD; 2) Stanford Univ., Palo Alto, CA; 3) Medical Univ. of SC, Charleston, SC; 4) Univ. of NV, Las Vegas, NV; 5) Vanderbilt Univ., Nashville, TN; 6) Univ. of NC, Chapel Hill, NC.

Cystic fibrosis (CF) is an autosomal recessive disorder caused by mutations in *CFTR* that manifests as elevated sweat electrolytes, respiratory disease, and exocrine pancreatic insufficiency. There are some patients with CF-like disease in only a subset of these organ systems who do not have mutations in *CFTR*. The epithelial sodium channel (ENaC), a protein regulated by CFTR, is a key component of epithelial ion transport in the lung and sweat gland. To determine if mutations in *ENaC* can produce CF-like disease, we sequenced the *ENaC* subunit genes (, , and) in 23 patients. Two previously observed benign mutations in *ENaC* (A334T and T663A) were found in four and 12 patients, respectively. We identified novel mutations in *ENaC* in three patients: two compound heterozygotes (P267L and 1670-2 AG; G294S and E539K) and one patient with a single mutation (S82C). Each substitution changes an amino acid highly conserved in *ENaC* orthologues and paralogues, or the canonical splice junction sequence, suggesting functional significance. P267L and G294S were not found in 176 and 324 ethnic specific control alleles, respectively, indicating they are not common polymorphisms. E539K causes loss of ENaC function when expressed in an oocyte expression system. Thus, the two compound heterozygotes carry putative deleterious mutations in each *ENaC* gene. Both patients have elevated sweat Cl^- and pulmonary disease, yet do not exhibit the salt wasting or elevated aldosterone and renin characteristic of pseudohypoaldosteronism, a recessive condition primarily associated with null mutations in , , or *ENaC*. These findings suggest that these patients have sufficient ENaC function for sodium absorption in the kidney, but not in the sweat gland or lungs. Thus, defects in *ENaC* can give rise to a novel phenotype with clinical features that overlap with CF.
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Craniofrontonasal Syndrome is caused by mutations of the Ephrin-B1 gene. *P.F. Wieacker[1], I. Wieland[1], S. Jakubiczka[1], P. Muschke[1], M. Cohen[2], H. Thiele[3], K.L. Gerlach[4], R.H. Adams[5], W. Reardon[6], B. Franco[7], P. Thierry[8], R. Koenig[9].* 1) Inst Human Genetics, Otto-von-Guericke Univ, Magdeburg, Germany; 2) Medizinische Genetik, Kinderzentrum, Muenchen, Germany; 3) Inst Human Genetics, Martin-Luther Univ, Halle, Germany; 4) Klinik MKG Chirurgie, Otto-von-Guericke Univ, Magdeburg, Germany; 5) London Research Institute, Cancer Research UK, London, United Kingdom; 6) National Centre for Medical Genetics, Dublin, Ireland; 7) Telethon Institute of Genetics and Medicine, Naples, Italy; 8) Service de Pediatrie, Centre Hospitalier Vesoul, France; 9) Inst Human Genetics, Johann-Wolfgang Univ, Frankfurt, Germany.

Craniofrontonasal syndrome (CFNS [MIM 304110]) is an X-linked disorder with unusual manifestation pattern because affected females show skeletal malformations including orbital asymmetry and hypertelorism as well as midline defects whereas male carriers are not or only mildly affected. We have mapped a CFNS gene in a region including the EFNB1 gene (MIM 300035). Since Efnb1 mutant mice display a spectrum of malformations and unusual inheritance reminiscent of CFNS, we analysed the EFNB1 gene in three CFNS families. A deletion of exons 2 to 5 and two missense mutations located in multimerization and receptor interaction motifs were found. Mutations were consistently found in obligate male carriers, affected males and affected heterozygous females. We conclude that mutations in EFNB1 cause CFNS. Meanwhile, 16 further mutations (11 missense mutations, 4 frameshift and 1 splice site mutations) in sporadic (12)and familial (4) cases. Most mutations were found in exons 2 and 3 corresponding to the extracellular domain. EFNB1 mutations were detected in all cases analysed so far. These results show for the first time that mutations of the ephrin receptor/ephrin signal transduction system are associated with a Mendelian disorder and that ephrin signaling is involved in human skeletal development. The sex-dependent manifestation of CFNS may be explained by the promiscuity of ephrin receptors and their ligands and as the consequence of random X inactivation. For this mechanism we propose the term cellular interference.

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Craniofrontonasal syndrome: mutations, mosaicism and X-inactivation. *S.R.F. Twigg[1], K. Matsumoto[1], A.M.J. Kidd[2], J. Dixon[2], J.B. Mulliken[3], H.G. Brunner[4], I.M.J. Mathijssen[5], J.M. Vaandrager[5], J. Morton[6], S.A. Wall[7], A.O.M. Wilkie[1,7]*. 1) Weatherall Institute of Molecular Medicine, Oxford, UK; 2) Central Regional Genetics Services, Wellington, New Zealand; 3) Dept Plastic Surgery, Children's Hospital, Boston, MA; 4) Dept Human Genetics, Nijmegen, The Netherlands; 5) Inst Plastic Surgery, Erasmus University Rotterdam, The Netherlands; 6) Clinical Genetics Unit, Birmingham, UK; 7) Craniofacial Unit, Oxford, UK.

Craniofrontonasal syndrome (CFNS) is an X-linked dominant disorder characterised by coronal synostosis, hypertelorism, bifid nasal tip, clavicular and thoracic abnormalities, digital webbing and longitudinally grooved nails. Unusually, females are more severely affected than males, who may only manifest hypertelorism and occasional cleft lip. Mutations of ephrin-B1 (*EFNB1*) were recently shown to cause CFNS. We describe the mutations found in 34 unrelated families that include 43 affected females and 3 mildly affected males. We found 31 distinct mutations: 6 frameshifting, 4 splicing, 4 nonsense, 16 missense and 1 large deletion. This is consistent with loss or reduction of ephrin-B1 function; almost half of the mutations are predicted to disrupt the protein, while many of the missense mutations are in regions of secondary structure or receptor binding. We did not observe any genotype-phenotype correlation. However in one family with 2 affected siblings (male and female) and a phenotypically normal mother, we demonstrated, by analysing samples from blood, hair roots and buccal mucosa, that the mother is a high level gonosomal mosaic. This has important implications for genetic counselling. Although *EFNB1* is normally subject to X-inactivation, extreme skewing is not observed in the blood of CFNS females. As the X-inactivation status may differ between tissues, we analysed sections of cranial periosteum overlying the coronal sutures from a familial case. Although extreme skewing was not present, there was marked variation in the ratio of X-inactivation along the periosteum. This is consistent with the proposal that the manifestation of *EFNB1* mutation in females results from ephrin B1-mediated sorting of cells, into patches expressing the normal and mutant alleles.

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Program Nr: 54 from the 2004 ASHG Annual Meeting

Warning: Hear Hoofbeats - Think Zebras In The 22q11.2 Deletion! *D.M. McDonald-McGinn, M. Maisenbacher, K. Ciprero, T. Hoffman, J. Catanzaro, C. Armeli, E. Goldmuntz, M. Gleason, L. Gillis, M. Mascarenhas, B. Porter, H. Hedricks, P. Nance, S. Adzick, D. Drummond, P. Pasquariello, D. Driscoll, B. Emanuel, E.H. Zackai.* Children's Hospital of Philadelphia, Philadelphia, PA.

It has become routine to provide anticipatory care for the associated features of the 22q11.2 deletion. However, weve observed a number of medical problems that are rare in the general population within our cohort of 600 patients. Most presented with chronic worsening symptomatology attributed to common childhood illnesses. Awareness of these problems may increase the rapidity of treatment & avoid prolonged illness. Findings include: vascular ring(36); intestinal malrotation(9), Hirshsprungs (5), late presentation of diaphragmatic hernia(4), cervical spine compression due to vertebral anomalies(3), Graves disease & hepatoblastoma. These abnormalities were identified due to respiratory distress & stridor in most patients with a vascular ring, chronic constipation from Hirshsprungs & dysphagia due to intestinal malrotation. The D-hernias were ascertained due to abdominal pain in a 3 1/2 yo & incidentally in 3 others (2, 9, & 10 months). Cervical spine compression was found due to toe-walking, hyperreflexia & seizure-like behavior in a 2 1/2 yo & incidentally in 2 others (5 & 8 years).Graves disease with unusually early presentation was diagnosed following unexplained tachycardia & irritability in a 31 mo. The patient failed medical therapy & required total thyroidectomy. Of note, Kawame reported Graves disease in 4 other patients. Hepatoblastoma was found as an abdominal mass in a 4 yo. The link with neoplastic disease is emerging as Scattone recently reported hepatoblastoma & renal cell carcinoma. Additional tumors include: hepatoblastoma (Adams M, p.c.), Wilms (Wallgren C, p.c.) & neuroblastoma. The latter neoplasm was found at autopsy in a newborn with DiGeorge syndrome whom we diagnosed prior to deletion studies (Patrone et al. 1990). Thus, it is important to be aware of these complications & recognize that patients with a 22q11.2 deletion will require careful medical surveillance in order to ascertain the occasional patient with significant morbidity.

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Program Nr: 55 from the 2004 ASHG Annual Meeting

Lifetime and current psychosocial and occupational functioning in adults with 22q11 Deletion Syndrome. *E. Chow*[1,2], *M. Watson*[2], *V. Wong*[2], *A.S. Bassett*[1,2]. 1) Dept Psychiatry, Univ Toronto, Toronto, ON, Canada; 2) Centre for Addiction and Mental Health, Toronto, ON, Canada.

Background: Little is known about the level of functioning in adults with 22q11 Deletion Syndrome (22qDS), although mental retardation and psychiatric disorders are commonly associated with the syndrome. Objective: To assess lifetime and current psychosocial, occupational, and adaptive functioning levels in a large group of adults with 22qDS. Methods: Lifetime and current marital/relationship status, housing situation, education/training history, occupational level, psychosocial functioning (Global Assessment of Functioning, GAF), and Vineland Adaptive Behavior Scale (VABS) scores were assessed in 79 adults (mean age=30.8y, SD=10.4; 38 M 41 F). 38 subjects had DSM-IV schizophrenia (SZ), and 41 subjects had no history of psychosis (NP). IQ scores were obtained using WAIS-R or WAIS-III. Results: There was a wide range of levels of functioning in subjects. Overall, 22qDS subjects had a mean IQ of 70.6 (SD=9.2, range=95 to 45), a GAF score of 57.5 (SD=15.6, range=85 to 10) and on average functioned at an age-equivalent level of 11y 9m on VABS (SD=3y 9m, range= 18y to 2y 1m). NP and SZ subjects did not differ significantly in sex distribution or mean IQ, but the SZ subjects were significantly older (mean age 34.0y v 27.8y, $p= 0.007$), had a lower VABS score (mean age equivalent (10y v 13y 2m, $p<0.001$), a lower GAF score (47.7 v 66.9, $p<0.001$), a lower education level (11.2y v 12.2y, $p=0.03$) and were less likely to be married (7.7% v 24.4%, $p=0.04$), had children (2.6% v 19.5%, $p=0.02$), lived independently (7.7% v 26.8%, $p=0.02$) and had gainful employment (25.6% v 53.7%, $p=0.01$). Conclusions: The level of functioning in adults with 22qDS is highly variable, with individuals with a psychotic illness functioning at a lower level.

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Antenatal presentation of Bardet-Biedl : the so-called Meckel-like syndrome. *H. KARMOUS-BENAILLY[1], J. MARTINOVIC[1], M.C. GUBLER[2], C. OZILOU[1], J. AUGE[1], S. AUDOLLENT[1], F. RAZAVI[1], M. VEKEMANS[1], T. ATTIE-BITACH[1].* 1) Department of Genetics and INSERM U-393, Hopital Necker, Paris, France; 2) INSERM U-574, Hopital Necker, Paris, France.

Bardet-Biedl syndrome (BBS, OMIM 209900) is a multisystemic disorder characterized by progressive retinal dystrophy, postaxial polydactyly, obesity, hypogonadism, learning difficulty and renal dysfunction. Other manifestations include diabetes mellitus, neurological signs, heart disease, and hepatic fibrosis. The condition is genetically heterogeneous and 5 genes are identified among the 6 mapped loci (BBS1-BBS6). Based on their homology to BBS genes, two more genes have been uncovered namely BBS7 and BBS8. A mutation of BBS1 on chromosome 11q13 is responsible for 40-50 % of BBS cases. In addition, a complex triallelic inheritance has been established in this disorder, i.e. in some families, three mutations at two BBS loci are necessary for the expression of the disease. The only clinical features that can be observed prenatally include polydactyly, a kidney anomaly and hepatic fibrosis. Cystic kidney dysplasia, polydactyly, occipital encephalocele and liver anomalies (hepatic fibrosis and bile duct proliferation) also characterise Meckel syndrome (MKS). Based on these observations, we have decided to sequence 4 BBS genes (BBS1, BBS2, BBS4 and BBS6) in a series of 13 antenatal cases mostly referred as Meckel or Meckel-like (because of association of a kidney anomaly, polydactyly and/or hepatic fibrosis to a brain anomaly, or the familial history or the kidney histology). In 6 cases, we identified a recessive mutation in a BBS gene (respectively BBS2: 3 cases, BBS4: 2 cases and BBS6: 1 case. We also found heterozygous BBS6 mutations in 3 additional cases. No BBS1 mutation could be identified in our series. These results extend the clinical spectrum of BBS with possible brain anomalies or severe cystic kidney dysplasia and strongly suggest that MKS and BBS are overlapping conditions.

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Mutations in the fibroblast growth factor 10 (*FGF10*) gene in autosomal dominant aplasia of lacrimal- and salivary glands. *M. Entesarian[1], H. Matsson[1], J. Klar[1], B. Bergendal[2], L. Olson[3], B. Falahat[4], N. Dahl[1].* 1) Department of Genetics & Pathology, Uppsala University, Uppsala, Sweden; 2) National Oral Disability Centre, The Institute for Postgraduate Dental Education, Jönköping, Sweden; 3) Department of Pediatric Dentistry, The Institute for Postgraduate Dental Education, Jönköping, Sweden; 4) Department of Dentomaxillofacial Radiology, The Institute for Postgraduate Dental Education, Jönköping, Sweden.

We have identified two families from Sweden with autosomal dominant aplasia of lacrimal and salivary glands (LSGA). The disorder is rare with a variable expressivity. Affected individuals show a combination of aplasia/hypoplasia of the lacrimal-, parotid-, submandibular- and sublingual glands. The patients present with irritable eyes and dryness of the mouth. No abnormalities in addition to aplasia/hypoplasia of lacrimal and major salivary glands were observed in the families investigated. A genome wide linkage screen using the two families revealed linkage to a single 22 cM region on chromosome 5p13.2-q13.1 flanked by markers D5395 and D5S2046. We obtained a maximum lod score of 5.72 (theta=0.00) with the marker locus D5S398. The linked region coincides with the fibroblast growth factor 10 (*FGF10*) gene locus. Previous studies in mouse have shown that *FGF10* is involved in multiorgan development including the formation of lacrimal- and salivary glands. We analyzed the *FGF10* gene in samples from affected individuals and we identified gene alterations with a predicted loss of function in both families. One family segregates for a deletion spanning part of the *FGF10* gene and the second family have a single nucleotide substitution in exon 3 resulting in a premature stop codon. The penetrance for LSGA among carriers of the mutations is 100% in the two families analyzed. We show for the first time that LSGA may be inherited as an autosomal dominant trait and associated with *FGF10* mutations.

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Program Nr: 58 from the 2004 ASHG Annual Meeting

Homozygous Mutations in *LPIN2* are Responsible for the Syndrome of Chronic Recurrent Multifocal Osteomyelitis and Congenital Dyserythropoietic Anemia (Majeed Syndrome). *H. El-Shanti[1], P. Ferguson[1], H. Majeed[4], J. Alami[4], S. Chen[1], M. Tayeh[1], L. Ochoa[1], S. Leal[2], A. Pelet[3], S. Lyonnet[3], A. Munnich[3].* 1) Dept Ped, Univ Iowa Hosps & Clinics, Iowa City, IA; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Department of Medical Genetics, INSERM U393, Hopital Necker-Enfants Malades, Paris, France; 4) Department of Pediatrics, University of Jordan, Amman, Jordan.

The syndrome of chronic recurrent multifocal osteomyelitis and congenital dyserythropoietic anemia is a rare autosomal recessive disorder of unknown etiology. Patients with this syndrome have chronic recurrent multifocal osteomyelitis that starts early in life and is sometimes associated with Sweet syndrome, a neutrophilic dermatosis. The congenital dyserythropoietic anemia is characterized by microcytosis, both peripherally and in the bone marrow. We identified six individuals with the syndrome of chronic recurrent multifocal osteomyelitis and congenital dyserythropoietic anemia from two unrelated families. We utilized homozygosity mapping and parametric linkage analysis as an approach to localize the gene responsible for this complex syndrome. The gene was mapped to a 5.5 cM interval (1.8 million base pairs) on the short arm of chromosome 18. On the examination of the genes in this interval, homozygous mutations were identified in *LPIN2* in affected individuals from the two families. We examined the pattern of expression of *LPIN2* and found it to be expressed in almost all tissues. The function of *LPIN2* remains unknown. We conclude that mutations in *LPIN2* result in an autoinflammatory condition of the bone and probably the skin, as well. Understanding the abberant immune response in this condition may shed light on the etiology of other inflammatory disorders including isolated chronic recurrent multifocal osteomyelitis, Sweet syndrome, SAPHO syndrome and psoriasis.

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Program Nr: 59 from the 2004 ASHG Annual Meeting

Mapping of sudden infant death with dysgenesis of the testes syndrome (SIDDT) by a high-density SNP genome scan and identification of TSPYL loss-of-function. *D.H. Lince[1], E.G. Puffenberger[2], J.M. Parod[1], D.W. Craig[1], S.E. Dobrin[1], A.R. Conway[3], E.A. Donarum[4], K.A. Strauss[2], T. Dunckley[1], J.F. Cardenas[1], K.R. Melmed[1], C.A. Wright[1], W. Liang[1], P.N.M.I. Stafford[1], C.R. Flynn[5], D.H. Morton[2], D.A. Stephan[1].* 1) Neurogenomics, TGEN, Phoenix, AZ; 2) Clinic for Special Children, Strasburg, PA; 3) Silicon Genetics, Redwood City, CA; 4) Neurodevelopmental Genetics, Barrow Neurological Institute, Phoenix, AZ; 5) Bioengineering, Arizona BioDesign Institute, Tempe, AZ.

We have identified a novel, lethal phenotype characterized by sudden infant death (from cardiac and respiratory arrest) with dysgenesis of the testes in males (SIDDT; OMIM # pending). Twenty-one affected individuals with this autosomal recessive syndrome were ascertained in nine separate sibships among the Old Order Amish. High-density single nucleotide polymorphism (SNP) genotyping arrays containing 11,555 SNPs evenly distributed across the human genome were employed to map the disease locus. A genome-wide autozygosity scan localized the disease gene to a 3.6 Mb interval on chromosome 6q22.1-q22.31. This interval contained 27 genes, including two testis-specific Y-like genes (TSPYL and TSPYL4) of unknown function. Sequence analysis of the TSPYL gene in affected individuals identified a homozygous frameshift mutation (457_458insG) at codon 153 resulting in truncation of translation at codon 169. Truncation leads to loss of a peptide domain with strong homology to the nucleosome assembly protein family. GFP-fusion expression constructs were constructed and illustrated loss of nuclear localization of truncated TSPYL, suggesting loss of a nuclear localization patch in addition to loss of the nucleosome assembly domain. These results shed light on the pathogenesis of a new disorder of sexual differentiation and brain stem-mediated sudden death, as well as give insight into a new mechanism of transcriptional regulation.

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Program Nr: 60 from the 2004 ASHG Annual Meeting

Interaction of the Cerebral Cavernous Malformations type 1 and 2 gene products. *J.S. Zawistowski*[1], *M.T. Uhlik*[2], *G.L. Johnson*[2], *D.A. Marchuk*[1]. 1) Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC; 2) Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Cerebral cavernous malformations are vascular lesions of the central nervous system consisting of clusters of dilated thin-walled blood vessels without intervening brain parenchyma. Hemorrhaging of these lesions can result in migraines, seizures, or lethal stroke. Three forms of autosomal dominant cerebral cavernous malformations have been mapped, and the disease gene products are known for two of the mapped loci. *CCM1* is caused by truncating mutations in *KRIT1*. *CCM2* results from mutations in *MGC4607* encoding the phosphotyrosine binding (PTB) domain protein malcavernin, the murine ortholog of which was concurrently characterized as osmosensing scaffold for MEKK3 (OSM). Here we provide three lines of evidence that suggest the *CCM1* gene product KRIT1 interacts with the *CCM2* gene product malcavernin. First, co-immunoprecipitation experiments with epitope-tagged molecules indicate strong KRIT1/malcavernin binding. To assess KRIT1/malcavernin binding in live cells, we employed micro-FRET analysis and show YFP-KRIT1and CFP-malcavernin associate in COS7 cells. Thirdly, YFP-KRIT1 exhibits both nuclear and cytoplasmic localization in COS7 cells, but when co-expressed with CFP-malcavernin, remains exclusively cytoplasmic--suggesting that malcavernin is capable of sequestering KRIT1. The interaction of PTB protein domains with NPxY amino acid motifs of beta integrin cytoplasmic tails is well characterized. To this end, we show here that mutation of the KRIT1 NPxY motif reduces the strength of the KRIT1/malcavernin interaction, as does a malcavernin point mutation in a PTB residue critical for structure of the domain. Taken together, these data provide evidence that the *CCM1* and *CCM2* gene products associate and may be involved in a complex integrin signaling network. It is therefore possible that cerebral cavernous malformations genetic heterogeneity may, at least in part, reflect different molecular components of the same signaling pathway.

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Program Nr: 61 from the 2004 ASHG Annual Meeting

Pathogenesis of cerebral cavernous malformation: Depletion of Krit1 leads to perturbation of 1 integrin-mediated endothelial cell mobility and survival. *J. Zhang[1], R.E. Clatterbuck[1], S. Basu[1], D. Rigamonti[1], H.C. Dietz[1,2]*. 1) Dept of Neurosurgery, Johns Hopkins Univ Sch Med, Baltimore, MD; 2) HHMI and Inst Genet Med, Johns Hopkins Univ Sch Med, Baltimore, MD.

Cerebral cavernous malformation (CCM) is an autosomal dominant microvascular disease characterized by vascular sinusoids that predispose to intracranial hemorrhage and seizure. Mutations in Krit1 (CCM1) and malcavernin (CCM2) account for the majority of familial CCM cases; however, little is known about the cellular functions of either protein. We have previously shown that full-length krit1 interacts with icap1, a modulator of 1 integrin signaling. Using RNA interference technology (RNAi), we demonstrate that krit1 plays a critical role in 1 integrin-mediated cell mobility, cell survival and ultimately angiogenesis. RNAi-mediated depletion of krit1 or icap1 reduces endothelial cell migration and invasion via the Rac1 signaling pathway. Krit1 modulates this pathway through positive regulation of GTP binding to Rac1. Depletion of krit1 or icap1 also inhibits 1 integrin-mediated cell survival via the ILK-AKT-BAD signaling pathway, increasing endothelial cell apoptosis in a caspase cleavage-dependent fashion. Krit1 influences this pathway through positive regulation of serine phosphorylation of ILK. Furthermore, depletion of krit1 or icap1 leads to an increased number of disorganized focal adhesions with decreased interaction of ILK and FAK with the focal adhesion docking protein paxillin, providing a potential mechanism for regulation of phosphorylation along these signaling pathways. The concordant effects of krit1 and icap1 on all signaling pathways can be explained by our observation that upon depletion of krit1, icap1 decreases in the cytoplasm and is no longer detected in the nucleus, suggesting that krit1 both stabilizes and shuttles icap1 and thus modulates its regulation of 1 integrin-mediated signaling. Further elucidation of the detailed molecular and cellular significance of these interactions will better define the role of krit1 in angiogenesis and yield more information about the molecular pathogenesis of CCM, thus facilitating the development of rational therapeutic strategies.

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Mutations of SGM1 cause Klippel-Feil syndrome - Segmental patterning in development of the spine, face, mouth and larynx. *R.A. Clarke, Z.M. Fang.* School of Medicine, St George Hospital, University of NSW, Sydney, NSW, Australia.

Klippel-Feil syndrome (KFS) is characterised by vertebral fusions that appear to arise from errors in axial segmentation. The resulting spinal deformities range in severity from slight restrictions to severe scoliosis and lethality. There are four classes of KFS (KF1-4) affecting 1 in 5000 births with very few KFS families reported. The KF2 class is the most prevalent form of KFS. KF2 can usually be distinguished from skeletal dysplasias, having the odd occurrence of vertebral fusion, by the minimum dominant expression of the fusion of the 2nd and 3rd cervical (C) vertebrae in common association with Sprengles Shoulder and/or some degree of craniofacial involvement.

We have identified KFS segregating with a paracentric inversion inv(8)(q22.2q23.3) within a five generation autosomal dominant KF2 family (KF2-01). FISH analysis using YAC, BAC and candidate gene probes positioned the inversion breakpoint within the 3rd intron of the SGM1 gene. Further screening identified a second autosomal dominant KF2 family (KF2-02) with a point mutation / nucleotide substitution within the 2nd exon of the SGM1 gene causing a putative missense proline to threonine transition. SGM1 is the first KFS gene to be identified.

Overlap between the complex phenotypes of these two KF2 families provides invaluable insight into SGM1s regulation of axial segmentation and spinal development. The sequential segmentation of the axial mesoderm into pre-vertebral somites proceeds in a cranio-caudal direction during embryogenesis. Within both the KF2 families described here the incidence of fusion was always cumulative in a cranio-caudal direction. And the overall frequency of vertebral fusion diminished cranio-caudally along the spine in gradient fashion. And vertebral fusion only recurred at identical periodic positions along the spine in skipping fashion. This proof of overlapping periodicities with skipped and graded patterns of control in multiple KF2 families with SGM1 mutations confirms SGM1s role in axial patterning, segmentation and spinal development.

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Loss of function mutations in *MAN1* result in osteopoikilosis, Buschke-Ollendorff syndrome and melorheostosis.

J. Hellemans[1], L. Preobrazhenska[4], A. Willaert[1], P. Debeer[5], P. Verdonk[2], B. Menten[1], N. Van Roy[1], S. Vermeulen[1], T. Costa[6], R. Savarirayan[7], W. Van Hul[8], F. Vanhoenacker[9], D. Huylebroeck[4], A. De Paepe[1], JM. Naeyaert[3], F. Speleman[1], K. Verschueren[4], P. Coucke[1], G. Mortier[1]. 1) Dept of Medical Genetics, Ghent Univ Hosp, Belgium; 2) Dept of Orthopedic Surgery, Ghent Univ Hosp, Belgium; 3) Dept of Dermatology, Ghent Univ Hosp, Belgium; 4) Dept of Developmental Biology, Univ of Leuven, Belgium; 5) Center for Human Genetics, Univ of Leuven, Belgium; 6) Medical Genetics Service, Sainte-Justine Hospital and University of Montral, Canada; 7) Genetic Health Services Victoria, Murdoch Childrens Research Institute and University of Melbourne, Australia; 8) Dept of Medical Genetics, Univ Hosp and University of Antwerp, Belgium; 9) Dept of Radiology, Univ Hosp and University of Antwerp, Belgium.

Osteopoikilosis, Buschke-Ollendorff syndrome (BOS) and melorheostosis are disorders characterized by increased bone density. The occurrence of one or more of these phenotypes in the same individual or family suggested that these entities might be allelic. We have collected three families in which affected individuals had osteopoikilosis with or without manifestations of BOS or melorheostosis. A genome wide linkage analysis in these families linked the locus to 12q12-q14.3 with a combined maximum two-point LOD score of 6.7 at =0 for markers D12S1661 and D12S1691. The identification of a microdeletion in an unrelated patient with osteopoikilois, short stature and microcephaly allowed us to reduce the linkage interval to a 3.07 Mb critical region. Sequencing of *MAN1* (candidate gene approach) showed heterozygosity for a loss of function mutation in all affected individuals of the 3 families and in 3 unrelated patients with osteopoikilosis. MAN1 is an integral protein of the inner nuclear membrane. Xenopus homologues of MAN1 specifically interact with BMP receptor activated Smads and antagonize BMP signaling during embryogenesis. Defects in the BMP/TGF signaling pathway have been shown to cause hyperostotic bone dysplasias in humans. We are currently investigating the specific role and interactions of MAN1 in the human BMP/TGF signaling pathway.

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Cartilage Hair Hypoplasia - New Pathogenetic Aspects. *P. Hermanns[1,4], J.G. Leroy[1,2], A. Bertuch[1], T. Bertin[1], B. Dawson[1,5], A. Tran[1], M. Schmitt[3], B. Zabel[4], B. Lee[1,5].* 1) Dept Molecular & Human Gen, Baylor College of Medicine, Houston, TX; 2) Ghent University Medical School, Ghent, Belgium; 3) Upstate University Syracuse, NY; 4) Children's Hospital, University of Mainz, Germany; 5) Howard Hughes Medical Institute, Houston, TX.

Cartilage Hair Hypoplasia (CHH) or metaphyseal chondrodysplasia McKusick type (MIM #250250) is an autosomal recessive multisystemic disease with osteochondrodysplasia, dwarfism, fine and sparse hair, deficient cellular immunity and a predisposition to malignancy. CHH is one of the few Mendelian diseases caused by mutation in a non-coding RNA gene, *RMRP*. *RMRP* is a nuclear encoded RNA that is the RNA component of a ribonucleoprotein complex that functions as an endonuclease. Yeast studies suggest its involvement in processing of pre-rRNA in the nucleolus, cleavage of mitochondrial RNA priming mitochondrial DNA replication, and progression of the cell cycle at the end of mitosis. Mutation analysis was performed in 32 patients, referred with the clinical diagnosis of probable CHH. *RMRP* mutations were found in 24 subjects. The combination of significantly reduced birth length, subsequent severe growth failure and deficient hair growth appeared to be highly correlated with the detection of mutations in the *RMRP* gene. A study of the PolIII *RMRP* promoter suggests that promoter duplications found in CHH patients abolish transcription of the *RMRP* gene, a finding confirmed by quantitative RT-PCR analysis of patient lymphoblasts. *RMRP* mutations introduced into the yeast ortholog NME1 neither altered mitochondrial function nor, affected mitochondrial depletion in a CHH patient fibroblast cell line. Interestingly, the most commonly found mutation 70A>G causes an alteration in the ribosomal processing and microarray studies performed with two patients suggest that *RMRP* mutation is associated with significant up-regulation of several cytokine family and cell cycle regulatory genes. These data suggest that alteration of ribosomal processing leads to altered cytokine signaling and cell cycle progression in terminally differentiated cell types involved in CHH pathogenesis, i.e., lymphocytic and chondrocytic lineages.

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Mutations in the *NODAL* signaling pathway are associated with human heterotaxy. *S.M. Ware[1], B. Mohapatra[2], T. Ho-Dawson[1], E. Weihe[2], H. Li[2], L. Smith[2], S.D. Fernbach[1], L. Molinari[1], B. Casey[3], N. Kaplan[4], K.L. McBride[1], J.A. Towbin[2], J.W. Belmont[1]*. 1) Dept Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Dept of Pediatrics (Cardiology), Baylor College of Medicine, Houston, TX; 3) Children's & Women's Health Centre, Vancouver BC V6H 3V4; 4) Biostatistics Branch, National Institute for Environmental Health Sciences, Research Triangle Park, NC.

Most occurrences of heterotaxy and mechanistically related non-syndromic cardiovascular malformations are sporadic and epidemiological features are consistent with complex genetic mechanisms. Data from animal models indicate that the nodal signaling pathway is critical for proper left-right patterning and cardiac development, and mutations in three genes within this pathway have been identified in a small minority of patients with heterotaxy. In order to test the hypothesis that multiple genes within the nodal pathway are causally associated with congenital heart defects, nine genes within the pathway, *NODAL*, *CRIPTO*, *ACVR2B*, *ALK4*, *ALK7*, *SMAD2*, *SMAD3*, *FOXH1* and *PITX2*, have been analyzed in patients with heterotaxy and mechanistically related congenital heart defects. A comprehensive mutation scanning and sequencing approach was used to examine 21.8 Kb of coding sequence in 227 sporadic cases and 298 controls (128 European-Americans, 17 African Americans, and 82 Hispanics). Non-synonymous rare variants were identified in 7 of 9 NODAL pathway genes. Logistic regression controlling for ethnicity demonstrated a significant association of non-synonymous rare variants in these genes with heart malformation (OR= 4.1; 95% CI 2.2-7.6; p.0001). Thus, testing the association of rare non-synonymous variants as a class allows definitive assessment of the impact of the NODAL pathway on this type of congenital heart disease. This study represents the first systematic characterization of the role of rare genetic variants in an entire developmental pathway and it establishes a novel paradigm for elaborating the molecular underpinnings of congenital heart defects.

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Familial short stature associated with a mutation in the growth hormone secretagogue receptor (*GHSR*). *J. Pantel[1], S. Cabrol[2], M. Legendre[1], L. Hilal[3], S. Nivot[4], Y. Hajaji[3], M.P. Vie-Luton[1], S. Morisset[5], D. Grouselle[6], M. de Kerdanet[7], A. Kadiri[8], J. Epelbaum[6], Y. Le Bouc[2], S. Amselem[1].* 1) INSERM U468, Creteil, France; 2) Hopital Armand-Trousseau, Paris, France; 3) Universite Ibn Tofail, Morroco; 4) CHU de Caen, France; 5) INSERM U573, Paris, France; 6) INSERM U549, Paris, France; 7) CHR de Rennes, France; 8) CHU Ibn Sina, Morroco.

Idiopathic short stature (ISS) is a disease condition particularly common in pediatric endocrinology. The term ISS is actually used for children in whom the etiology of the short stature is undefined. Therefore, the diagnosis of this heterogeneous disorder is currently based on exclusion criteria, including normal plasma GH values in response to classical pharmacological provocative tests. In very rare instances, patients with a diagnosis of ISS have secondarily been shown to carry a gene abnormality. The first endogenous ligand of the GH secretagogue receptor (GHSR) has been discovered in 1999. This ligand, called Ghrelin, is a hormone that stimulates GH secretion, induces appetite and weight gain. Consistent with these biological properties, transgenic rats with GHSR deficiency have been found to be smaller and leaner than controls. On the basis of these observations, we postulated that children with ISS may carry mutations in the *GHSR* gene, in spite of a normal response to classical pharmacological provocative tests. To test the possible involvement of the *GHSR* in the disease phenotype of patients with ISS, we screened its coding sequence for mutations in a family in which several members had a short stature of unknown origin. Sequence analysis of the *GHSR* gene within this family revealed a missense mutation that segregates with the growth retardation phenotype. If translated, this *GHSR* mutation would generate a charge change at a highly conserved position of the extracellular region of this seven transmembrane G protein-coupled receptor. As shown by transient expression studies, the binding of this mutant receptor to ^{125}I-Ghrelin is severely impaired. Overall, this first deleterious *GHSR* mutation, which elucidates the molecular basis of the ISS phenotype in this family, highlights the role of normal secretagogue signaling in the human.

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Program Nr: 67 from the 2004 ASHG Annual Meeting

Mice heterozygous for the GTF2I transcription factors exhibit behaviours seen Williams-Beuren syndrome. *T. Onay*[1,2], *E. Young*[2], *T. Lipina*[3], *A. Mandel*[2], *Z. Jia*[1], *J. Roder*[3], *L.C. Tsui*[4], *L.R. Osborne*[2]. 1) Hospital for Sick Children, Toronto, Canada; 2) Medicine, University of Toronto, Toronto, Canada; 3) Samuel Lunenfeld Research Institute, Toronto, Canada; 4) University of Hong Kong, Hong Kong, China.

Williams-Beuren syndrome (WBS) is a complex disorder, caused by the hemizygous deletion of genes on chromosome 7q11.23. The elastin gene has been shown to contribute to the phenotype, but no other gene has been unequivocally linked to this disorder. A family of general transcription factor genes (GTF2I) lie at the telomeric end of the WBS deletion, and since haploinsufficiency of transcription factors has been shown to underlie a number of developmental disorders, they make attractive candidates.

In order to dissect the role of hemizygosity for these genes in WBS, we have used gene targeting of Gtf2i and Gtf2ird1 in mice. Gtf2ird1 homozygous animals are viable and grossly normal, in agreement with a previous insertional Gtf2ird1 mutant, whilst Gtf2i homozygous mutant embryos are present at 7.5 dpc but resorb by 9.5 dpc. Preliminary analysis has revealed no gross anatomical abnormalities in either Gtf2i +/-, Gtf2ird1 +/- or double heterozygous mice. Behavioural analyses have revealed abnormalities associated with the mutation of both genes. Gtf2i +/- mice show normal motor activity but reduced anxiety and attentional deficits, while Gtf2ird1 -/- mice show increased motor activity and acoustic startle response. In addition, the Gtf2i +/- (but not the Gtf2ird1 -/- mice) show changes in basal synaptic function as measured by excitatory postsynaptic potential after stimulation of the CA1 region of the hippocampus.

The finding of behaviours similar to those seen in individuals with WBS (hyperactivity, attention deficit, hypersensitivity) in these mice suggests that the GTF2I transcription factor genes may contribute to the clinical symptoms seen in WBS. Further characterization of these mice, and of double heterozygous animals, will be important in determining the role of these genes in WBS.

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Inducible transgenic mouse model of oculopharyngeal muscular dystrophy (OPMD). *A. Mankodi[1], M. Becher[2], C.A. Thornton[1]*. 1) Neurology, University of Rochester, Rochester, NY; 2) Pathology, University of New Mexico, Albuquerque, NM.

OPMD is an adult onset neurodegenerative disease with worldwide prevalence. The genetic basis is a short expansion of alanine codons (normal: A_{10}; mutant: A_{12-17}) in the first exon of *PABPN1*. PABPN1 is a ubiquitous nuclear protein that regulates formation of poly(A) tails on mRNA. Mutant PABPN1 protein forms intranuclear aggregates and inclusions in patients with OPMD. To investigate the molecular pathogenesis of OPMD, we derived an inducible transgenic mouse model, i.e., one in which the disease gene can be turned on and off. First, we derived lines of transgenic mice expressing the RU486-regulable transactivator, GLVP, under the control of the CMV/ actin enhancer/promoter. Second, we derived target lines in which expression of human wild-type (hPABwt, 10 alanine codons) or mutant (hPABmut, 16 alanine codons) PABPN1 is controlled by GLVP. Next, we determined that administration of RU486 to hPAB/GLVP bitransgenic mice induces hPABwt or hPABmut mRNA. Long-term induction of hPABmut by RU486 led to progressive multi-system disease characterized by muscle wasting, axial myoclonus, impaired rotorod performance and cardiomyopathy. The phenotype was consistent in 3 of 4 hPABmut founder lines. The neurological impairment began at 2 months and reached survival endpoints in more than 70% of mice after 10 months of induction. Histologic analysis showed degeneration of skeletal and cardiac muscle fibers. Cytoplasmic rimmed vacuoles and intranuclear inclusions of ubiquitinated PABPN1, which are hallmarks of OPMD histopathology, were present in mice that expressed hPABmut. Uninduced bitransgenic mice remained healthy. Preliminary data indicate that neurologic impairment is reversible if induction of hPABmut is discontinued. To our knowledge, this is the first inducible mouse model of a muscular dystrophy. The consistency of the phenotype and the rapid progression of disease suggest that this transgenic model may be suitable for testing therapeutic strategies to reduce PABPN1 aggregation.

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The chromatin remodeling protein ATRX is critical for neuronal survival during corticogenesis. *D.J. Picketts[1,3], M. Jagla[1], J. Vanderluit[2], D. Garrick[4], R.J. Gibbons[4], D.R. Higgs[4], R.S. Slack[4], N.G. Bérubé[5].* 1) Molecular Medicine Program, Ottawa Health Research Inst, Ottawa, ON, Canada; 2) Neuroscience Program, Ottawa Health Research Inst, Ottawa, ON, Canada; 3) Departments of Medicine and Biochemistry, Microbiology, and Immunology, University of Ottawa, Ottawa, ON, Canada; 4) Weatherall Institute of Molecular Medicine John Radcliffe Hospital, Oxford, OX3 9DS; 5) Present Address: Departments of Biochemistry and Paediatrics, University of Western Ontario and Scientist, Child Health Research Institute, London, ON, N6C 6B5.

Mutations in chromatin remodeling genes, such as *ATRX*, underlie a number of genetic disorders including several X-linked mental retardation syndromes, yet their role in normal CNS development is unknown. Here, we used a conditional gene-targeting approach to inactivate *ATRX* specifically in the forebrain. Loss of *ATRX* causes widespread hypocellularity in the neocortex and hippocampus, and a pronounced reduction in forebrain size. Neuronal birthdating confirmed that fewer neurons reach the superficial cortical layers, despite normal progenitor cell proliferation. The loss of cortical mass results from an 8-fold increase in neuronal apoptosis during early stages of corticogenesis in the mutant animals. Moreover, cortical progenitors isolated from *ATRX*-null mice undergo enhanced apoptosis upon differentiation. Taken together, our results indicate that *ATRX* is a critical mediator of cell survival during early neuronal differentiation. As such, increased neuronal loss may contribute to the severe mental retardation observed in human patients.

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Program Nr: 70 from the 2004 ASHG Annual Meeting

Glial expression of polyglutamine-expanded ataxin-7 is sufficient to produce neuronal dysfunction in GFAP-SCA7 transgenic mice. *S.K. Grote[1], B.L. Sopher[1], A.R. La Spada[1,2,3]*. 1) Department of Laboratory Medicine, University of Washington, Seattle, WA; 2) Division of Medical Genetics, Department of Medicine, University of Washington, Seattle, WA; 3) Division of Neurogenetics, Department of Neurology, University of Washington, Seattle, WA.

Spinocerebellar ataxia type 7 (SCA7) is an autosomal dominant disorder characterized by gait ataxia and retinal degeneration, and is caused by a CAG / polyglutamine repeat expansion. We recently generated a transgenic mouse model of SCA7, and documented a non-cell autonomous degeneration of Purkinje cell neurons in the cerebellum of mice expressing ataxin-7 with 92 glutamines. One possible explanation for the non-cell autonomous Purkinje cell degeneration in the SCA7 mouse model is dysfunction of a population of glia (the so-called Bergmann glia) that surround, support, and sustain Purkinje cells. To determine if Bergmann glia dysfunction plays a role in the non-cell autonomous Purkinje cell degeneration seen in our SCA7 model, we obtained a modified version of the human glial fibrillary acidic protein (GFAP) promoter to target expression of polyglutamine-expanded ataxin-7 to Bergmann glia. Three lines of GFAP-SCA7-92Q transgenic mice have been produced, and display an age-dependent neurological phenotype of tremor, clasping, and gait instability. One line of GFAP-SCA7-10Q transgenic mice thus far appears normal. Ataxin-7 IHC of GFAP-SCA7-92Q mice reveals intense immunostaining of Bergmann glia soma and processes, but without detectable neuronal immunoreactivity in the cerebella of such mice. Our preliminary analysis of the GFAP-SCA7-92Q mice suggests that expression of mutant ataxin-7 restricted to glia of the cerebellum is sufficient to induce neuronal dysfunction.

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Molecular Basis of Pituitary Dysfunction: Mechanism of PROP1 Action. *S.A. Camper, R.D. Ward, M.L. Brinkmeier, I.O. Nasonkin, A.H. Vesper, M.A. Potok, B.M. Stone, F. Beuschlein, H. Suh, G.D. Hammer, L.T. Raetzman.* Depts. Human Genetics and Internal Medicine, 4301 MSRB III, Univ Michigan Medical Sch, Ann Arbor, MI.

Lesions in the homeodomain transcription factor PROP1 are the most common known cause of autosomal recessive hypopituitarism in humans. GH, TSH, PRL, LH and FSH are usually deficient, and hypocortisolism occasionally develops. Some patients initially have pituitary hyperplasia that resolves to hypoplasia. We investigated the mechanism of PROP1 action using mice with altered PROP1 levels, including a putative hypomorph, a novel, null mutant, and a transgenic over-expressor. Many features of the human patients are mimicked in PROP1 deficient mice. Adult mutants are consistently deficient in GH, TSH, PRL, LH and FSH, although there is no evidence of hypocortisolism. Genetic background profoundly influences the mutant phenotype, which is lethal respiratory distress syndrome, juvenile wasting associated with hypoglycemia, or viable adult dwarfism. *Prop1* is expressed transiently in early fetal development, but the total mass of the mutant pituitary prirmordium is indistinguishable from normal mice until a week after birth. Undifferentiated mutant progenitor cells appear unable to migrate to the anterior lobe, causing dorsal overgrowth. The lack of progenitors and enhanced apoptosis in young mice causes hypoplasia, analogous to the pituitary hyperplasia and degeneration of some human patients. Prop1 mutants have persistent, mislocalized expression of the HESX1, TLE3 repressor complex and are unable to activate expression of NOTCH2 and PIT1. Deficiency of AES, an antagonist of the repressor complex, causes pituitary anomalies, misexpression of NOTCH2 is sufficient for disruption of gonadotrope differentiation, and PIT1 is required for specification of cells expressing GH, TSH and PRL. Gonadotrope function is disrupted by reduced or elevated Prop1 expression, suggesting that the concentration of PROP1 is critical for normal pituitary function. In summary, *Prop1* mutant mice are valuable tools for understanding the mechanism of PROP1 action.

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Program Nr: 72 from the 2004 ASHG Annual Meeting

***Mitf* repression by the microphthalmia-associated transcription factor Chx10 is required for the maintenance of mammalian neuroretinal identity.** *D.J. Horsford*[1], *M.-T. Nguyen*[2], *G.C. Sellar*[3], *R. Kothary*[4], *H. Arnheiter*[2], *R.R. McInnes*[1]. 1) Prog Develop Biol, Hosp Sick Children, Toronto, ON, Canada; 2) Lab of Develop Neurogenetics, NINDS, NIH, Bethesda, MD, USA; 3) MRC Human Genetics Unit, Edinburgh, UK; 4) Ottawa Health Res Inst, Ottawa, ON, Canada.

In mammalian retinal development, a neuroectodermal cell undergoes a cell fate decision to become either a presumptive neuroretinal cell (under the influence of FGF) that expresses the homeodomain transcription factor Chx10 or a presumptive retinal pigment epithelium (RPE) cell that expresses the basic helix-loop-helix protein Mitf. *Chx10* loss-of-function mutations in human and mouse result in the orthologous phenotype of microphthalmia due to defects in neuroretinal progenitor cell proliferation. *Mitf* mutants have a dorsal RPE-to-neuroretinal phenotypic transformation, indicating that *Mitf* is a determinant of RPE identity. We report here that *Mitf* is expressed ectopically in the $Chx10^{or\text{-}J/or\text{-}J}$ neuroretina (NR), demonstrating that Chx10 normally represses the neuroretinal expression of *Mitf*. The ectopic expression of *Mitf* in the $Chx10^{or\text{-}J/or\text{-}J}$ NR deflects it towards an RPE-like identity; this phenotype results from from a partial loss of neuroretinal maintenance. Using *Chx10* and *Mitf* transgenic and mutant mice we identified an antagonistic interaction between *Chx10* and *Mitf* in regulating retinal cell identity. High Chx10:Mitf levels are essential for neuroretinal cell identity, while elevated Mitf:Chx10 levels ensure RPE cell identity. Double *Chx10;Mitf* mutant mice have a dramatically improved retinal phenotype, confirming that *Mitf* is necessary for the *Chx10* retinal phenotype and vice versa. Importantly, we also isolated a small number of *Chx10* mutant individuals of a mixed genetic background with a dramatic NR-to-RPE-like change in cell identity. *Chx10* is thus the first gene identified that is necessary for mammalian neuroretinal cell identity. Finally, FGF exposure in a developing OV has also been shown to repress *Mitf* expression. We demonstrate that the repression of *Mitf* by FGF is *Chx10*-dependent, indicating that FGF, Chx10 and Mitf are components of a pathway that determines and maintains the identity of the NR.

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Generation of mouse models of human congenital malformation syndromes using ENU mutagenesis. *D.R. Beier, K.G. Ackerman, B.C. Bjork, T.L. Harboe, J.L. Moran, P. Tran.* Genetics Division, Brigham and Womens Hospital, Harvard Medical School, Boston, MA.

The characterization of mouse developmental mutants can be useful for understanding human dysmorphologies and disease. We are screening embryos from mice mutagenized with N-ethyl-N-nitrosourea (ENU) for recessive phenotypes similar to human congenital defects. Third generation progeny are evaluated for developmental anomalies at embryonic d18.5; we thus identify defects in organogenesis that are consistent with survival *in utero* to late gestation but may cause post-natal lethality. Affected progeny are used directly for genetic mapping utilizing a 400-marker SNP panel we have developed that allows a rapid genome-wide analysis. With this strategy, we are able to localize the mutation to a recombinant interval by genotyping 8-10 affected mice. The main component of the mutation screen is an inspection of major organs after embryo dissection, followed by a more detailed analysis for craniofacial, kidney, brain and skeletal abnormalities. We have generated over 25 monogenic developmental mutants and we have identified the causal gene for 9 of these. The abnormalities observed are highly varied and many are similar to human malformation syndromes. Examples of models we have generated include congenital diaphragmatic defect, non-syndromic cleft palate, Robin sequence, spondylocostal dysostosis, omphalocele, non-bullous congenital icthyosiform erythroderma, epidermal bullosa, polycystic kidney disease, and structural heart disease. In several cases the mutated locus corresponds to a known human disease-associated gene. More importantly, for syndromes in which the molecular basis is less well understood (e.g., CDH and cleft palate), analysis of affected patients has revealed missense and nonsense mutations in the orthologs of genes identified in the analysis of our mutant models. Our results demonstrate that a recessive screen can be extremely productive for the purpose of generating abnormal developmental phenotypes. The utilization of a phenotype-driven analysis has proven useful for the identification of candidate loci that can be studied in human populations.

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Program Nr: 74 from the 2004 ASHG Annual Meeting

Identification of a Genetic Cause of Congenital Diaphragmatic Defect and Pulmonary Hypoplasia Through Characterization of an ENU-induced Mutant Mouse Model. *K.G. Ackerman[1], S.O. Vargas[2], M. Russell[3], K. Parker[1], B.R. Pober[3], P.K. Donahoe[3], D.R. Beier[1].* 1) Genetics, Brig & Wmns Hosp, Boston, MA; 2) Pathology, Children's Hosp, Boston, MA; 3) Surgery, Mass Gen Hosp, Boston, MA.

We use an ENU-mutagenesis screen to identify mutations causing developmental defects in late term mouse embryos. From our mutagenesis screen, we identified a mutant line with bilateral pulmonary hypoplasia and a diaphragmatic muscularization defect (eventration). Positional cloning revealed that this line carries a hypomorphic mutation of the transcriptional co-factor Fog2 (Zfpm2). As pulmonary hypoplasia may be secondary to a diaphragmatic defect in other models, characterization of Fog2 mutants included the evaluation of lung growth in culture. Lung explants performed prior to diaphragmatic muscularization demonstrated bilateral hypoplasia with lack of accessory lobe development. Thus, Fog2 plays a primary role in pulmonary development. The similarity of these defects to the human spectrum of diaphragmatic defects makes FOG2 an excellent a candidate gene for congenital diaphragmatic hernia (CDH). In addition, human FOG2 maps to 8q22.3 and translocations or deletions involving this region have been associated with CDH. To evaluate FOG2 as a candidate gene for CDH, sequence analysis was done on autopsy tissue from 30 patients with diaphragmatic defect and pulmonary hypoplasia. In this cohort, a heterozygote base change resulting in a premature stop codon (R112X) was identified in one case. Analysis of the parents revealed that this was a de novo mutation in the affected child, which strongly implicates the FOG2 mutation as causal for CDH in this patient. SNP genotyping in a control population of 400 healthy adults revealed no changes at this site. Our identification of FOG2 as the first causal gene for developmental diaphragmatic defects and congenital pulmonary hypoplasia very clearly demonstrates the utility of ENU mutagenesis as a tool for finding candidate genes for human developmental defects. This model is the first to demonstrate that the pulmonary hypoplasia associated with human diaphragmatic defects can result from a primary developmental defect in lung development.

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TGF-dependent pathogenesis of mitral valve prolapse in a mouse model of Marfan syndrome. *C.M. Ng*[1], *A. Cheng*[2], *D.P. Judge*[2], *H.C. Dietz*[1,2]. 1) HHMI and Inst of Genet Med, Johns Hopkins Univ Sch Med, Baltimore, MD; 2) Dept of Medicine, Johns Hopkins Univ Sch Med, Baltimore, MD.

Mitral valve prolapse (MVP) is a common human phenotype, yet little is known regarding disease pathogenesis. MVP can occur in the context of genetic syndromes including Marfan syndrome (MFS), an autosomal dominant condition caused by mutations in fibrillin-1. Myxomatous degeneration of the mitral valve with progressive dysfunction represents the leading indication for cardiovascular surgery and cause of death in children with MFS. Here we validate *Fbn1*-targeted mice as a genetically defined model of postnatally acquired myxomatous degeneration of the atrioventricular (AV) valves. Our prior use of fibrillin-1 deficient mice revealed that fibrillin-1 regulates the activation of the cytokine transforming growth factor beta (TGF) in the developing lung and that excessive signaling contributes directly to failed distal alveolar septation and the evolution of emphysema. We hypothesized that a similar mechanism is relevant to the multisystem pathogenesis of MFS, including the development of myxomatous changes of the AV valves. Mutant valves exhibited alterations in leaflet length, cellularity, and matrix content that correlated both temporally and spatially with excess TGF activation and signaling. Moreover, TGF antagonism *in vivo* rescued the valve phenotype in mouse models of MFS, suggesting a cause and effect relationship. Importantly, these studies also demonstrated that TGF is a physiologic regulator of AV valve morphogenesis. Expression analyses of developing fibrillin-1 deficient AV valve leaflets demonstrated upregulation of multiple TGF-responsive genes including igh3, Edn1, and Timp1. These are excellent candidate effectors of the valve phenotype due to their known influence on cellular proliferation and survival. In keeping with this hypothesis, we have observed a dramatic increase in cellular proliferation and suppression of apoptosis in the developing valve leaflets of *Fbn1*-targeted mice. These data provide critical insight into the pathogenesis of myxomatous mitral valve changes in MFS and perhaps in more common, nonsyndromic variants of mitral valve prolapse.

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Apoptosis in glycerol kinase deficiency (GKD): investigations in *Drosophila melanogaster*. *J.A. Martinez, E.R.B. McCabe.* Department of Pediatrics, David Geffen School of Medicine at UCLA, Los Angeles, CA.

Mutations in the glycerol kinase (GK) gene of plant and animal organisms reveal that the glycerol metabolic pathway affects a variety of biological processes, including growth, energy metabolism, and stress resistance. In humans, mutations in GK produce a variable clinical presentation with clear evidence that modifiers influence phenotypic variability. We generated GK deficient (GKD) *Drosophila melanogaster* in order to identify candidate modifier loci mediating this phenotypic variability. Combining classical genetic and genome-wide methods, we confirmed the existence of several glycerol kinase loci in *Drosophila*, with Gyk and CG7995 having closest similarity to human Xp21 GK. We isolated flies with recessive lethal loss-of-function or hypomorphic alleles. Loss-of-function alleles caused recessive lethality in the homozygous state. Ectopic bristle formation, consistent with a block in apoptosis, was observed in flies with heterozygous null or homozygous hypomorphic alleles. Conversely, ectopic expression of glycerol kinase in the *Drosophila* eye and mechanosensory neuron precursors led to photoreceptor cell death and cuticular bristle loss. Based on a candidate gene approach, we identified several modifiers of these phenotypes: cytoplasmic and mitochondrial glycerol 3-phosphate dehydrogenase (*cGpd, Gpo*), voltage dependent anion channel (VDAC/*porin*), and apoptotic protease-activating factor-1 (Apaf-1/*dArk*). Immunostaining confirmed mitochondrial co-localization of GK with Apaf-1 and VDAC. We further carried out a modifier screen using these phenotypes and identified additional members of the mitochondrial apoptosis cascade: an adenine nucleotide translocator, mitochondrial respiratory chain complex I, and the microRNA *mir-14*, a key regulator of cell death and fat metabolism in *Drosophila*. Investigations in the fly model of GKD identified modifier loci of the GKD phenotype as members of the apoptotic cascade, indicating that GK is involved in apoptosis. We speculate that studies in the fly and other model organisms will give us insight into novel pathogenic mechanisms and unanticipated modifier genes.

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Program Nr: 77 from the 2004 ASHG Annual Meeting

Microcephalin: linking human brain size and chromosome condensation. *A.P. Jackson[1], M. Trimborn[2], S.M. Bell[1], K.H. Surinya[1], C. Felix[3], L.M. Neumann[2], K. Sperling[2], H. Neitzel[2].* 1) Molec Medicine Unit, CSB, Univ Leeds, Leeds, United Kingdom; 2) Institute of Human Genetics, Charit Universitary Medicine Berlin, Campus Virchow, Humboldt University, Berlin, Germany; 3) Regional Cytogenetics Unit, St. Jamess University Hospital, Leeds, UK.

Microcephalin (MCPH1) is a gene mutated in primary microcephaly, an autosomal recessive neurodevelopmental disorder in which there is a marked reduction in brain volume to a size comparable with that of early hominids. PCC syndrome is a recently described disorder of microcephaly, short stature and misregulated chromosome condensation. Here, we report the finding that MCPH1 primary microcephaly and PCC syndrome are allelic disorders, both having mutations in the *microcephalin* gene. We establish that the two conditions share a common cellular phenotype of premature chromosome condensation (PCC) in the G2 phase of the cell cycle. This phenotype is identifiable on routine cytogenetic preparations and therefore will be a useful diagnostic marker for identifying individuals with *MCPH1* gene mutations.Furthermore neuronal heterotopias are present in some patients with microcephalin mutations, raising interesting questions regarding the possible extension of the MCPH1 primary microcephaly phenotype into neuronal migration disorders.We demonstrate that microcephalin is a nuclear protein by transient expression of a GFP-tagged expression construct and by siRNA that knockdown of MCPH1 is sufficient to reproduce the PCC phenotype. We also show that MCPH1-deficient cells exhibit delayed decondensation post-mitosis. These findings implicate microcephalin as a novel regulator of chromosome condensation and link the apparently disparate fields of neurogenesis and chromosome biology. Further characterisation of MCPH1 is thus likely to lead to fundamental insights into both the regulation of chromosome condensation and neurodevelopment.

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Program Nr: 78 from the 2004 ASHG Annual Meeting

Mutation in the telomeric regulator PIP1 causes urogenital and caudal dysgenesis in *acd* mutant mice. *C. Keegan*[1], *J. Hutz*[1], *T. Else*[1], *M. Adamska*[1], *S. Shah*[1], *A. Kent*[1], *J. Howes*[1], *W. Beamer*[2], *G. Hammer*[1]. 1) Univ Michigan, Ann Arbor, MI; 2) Jackson Laboratory, Bar Harbor, ME.

Adrenocortical dysplasia (*acd*) is a spontaneous autosomal recessive mouse mutant with developmental defects in organs derived from the urogenital ridge. In surviving adult mutants, adrenocortical dysplasia and hypofunction are predominant features. Adults are infertile due to lack of mature germ cells, and fifty percent develop hydronephrosis due to ureteral hyperplasia. In addition, mutants exhibit growth retardation from birth, and many have decreased fur and flaky skin. We report that the *acd* phenotype is caused by a splice donor mutation in *Pip1* (POT-1 interacting protein, also known as *Ptop*) in *acd* mice. This gene has recently been characterized by others as a novel component of the protein complex that controls telomere elongation by telomerase. Characterization of *Pip1* transcripts in *acd* mutants reveals two abnormal transcripts, consistent with a splicing defect. Expression of a wild-type *Pip1* transgene in *acd* mutants rescues the observed phenotype. Most mutants die within 1-2 days of life on the original genetic background. Analysis of these mutant embryos reveals variable, yet striking defects in caudal specification, limb patterning and axial skeleton formation. In the tail bud, reduced expression of *Wnt3a* and *Dll1* correlates with phenotypic severity of caudal regression. In the limbs, expression of *Fgf8* is expanded in the dorsal-ventral axis of the apical ectodermal ridge and shortened in the anterior-posterior axis, consistent with the observed loss of anterior digits in older embryos. The axial skeleton of mutant embryos shows abnormal vertebral fusions in the cervical, lumbar, and caudal regions. The growth retardation, skin, and germ cell abnormalities observed in adult *acd* mutants are reminiscent of premature aging syndromes caused by genomic instability in humans. However, this is the first report to show that a telomeric regulator is required for proper urogenital ridge differentiation, axial skeleton specification, and limb patterning in the mouse. Further studies are in progress to evaluate evidence of genomic instability in *acd* mutant animals.

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Program Nr: 79 from the 2004 ASHG Annual Meeting

Genetic interaction of MEN1 with ATM in a Drosophila model. *L.R. Marek, V. Busygina, A.E. Bale.* Dept Genetics, Yale Univ, New Haven, CT.

MEN1 is a cancer predisposition syndrome caused by mutations in a novel transcriptional co-repressor (menin). The MEN1 gene is conserved from humans to *Drosophila*, and our laboratory has previously shown that MEN1 mutant flies are hypersensitive to ionizing radiation and DNA cross-linking agents. The mechanism of action of MEN1 in response to DNA damage is unknown. To place the gene in a context of known DNA repair pathways, we are performing genetic interaction studies with key genes involved in response to DNA damage.

A genetic interaction with ATM was shown in both transgenic flies overexpressing MEN1 and in MEN1 loss-of-function mutants. *Drosophila* heterozygous for ATM and overexpressing MEN1 in the dorsal ectoderm had loss of mechanosensory organs (microchaete) in the thoracic region. This finding suggests that the two genes may act together in neuronal cells. Homozygous loss of the ATM gene in humans is associated with dropout of cerebellar neurons. A role for MEN1 in the nervous system has not been previously reported, but homozygous loss of this gene in mammals is lethal and heterozygosity may not be sufficient to cause a neurologic phenotype.

The combination of homozygous MEN1 loss-of-function and heterozygous ATM deletion resulted in increased gene conversion, not seen with either mutation, alone. This finding probably correlates with the high rates of spontaneous recombination found in A-T cells and indicates that MEN1 may cooperate in this phenomenon. Heterozygosity for ATM and MEN1 did not significantly enhance or suppress the radiation-sensitive phenotype of MEN1 mutant flies as measured by survival. However, there appeared to be a synergistic effect on a rough eye phenotype as well as an increased number of flies with a cleft in the eye. These eye phenotypes are associated with arrest of proliferation or increased apoptosis in the developing eye and probably indicate that MEN1 and ATM interact in the radiation response in eye tissue.

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Program Nr: 80 from the 2004 ASHG Annual Meeting

Investigation of the role of the *Drosophila* MEN1 homolog in DNA repair through gene expression analysis. *V. Busygina, L.R. Marek, A.E. Bale.* Dept Genetics, Yale Univ Sch Medicine, New Haven, CT.

Multiple Endocrine Neoplasia type 1 (MEN1) is a familial cancer predisposition disorder characterized primarily by tumors of the pituitary, the parathyroid, and the pancreatic islets. The MEN1 gene encodes a nuclear protein, menin, which is a transcriptional co-modulator and regulates the activity of several transcription factors such as JunD, Smad3, NF-kB and others.
MEN1 is conserved from *Drosophila* to mammals. We previously reported that homozygous loss of menin function in *Drosophila* causes hypersensitivity to ionizing radiation and DNA cross-linkers (nitrogen mustard and cisplatin). Here we report that MEN1 mutant *Drosophila* are also hypermutable. The rate of sporadic and radiation induced mutations was increased 2-3 times in MEN1 mutants compared to controls. After treatment with nitrogen mustard the mutants showed a 7 fold increase in mutation frequency. These data indicate that menin participates in DNA damage sensing or repair.
Since menin has been shown to function in regulation of transcription, it is possible that its effect on DNA repair is mediated through regulation of gene expression. We used Affymetrix microarrays to compare the changes in gene expression of control and MEN1 mutant flies in response to ionizing radiation. We identified 95 genes that were differentially expressed in this experiment (p0.05). Among these genes, two were also identified in a genetic screen for potential MEN1 interactors: Kismet, a DNA helicase, and LK6, a homolog of a yeast serine-threonine kinase that participates in DNA damage response. Genetic interaction studies are in progress to determine if there is an epistatic relationship between these two genes and Men1 in DNA damage response.

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Fanconi anemia proteins associate with telomeric proteins in ALT-immortalized human cells. *H. Root*[1, 2], *M.S. Meyn*[1, 2, 3]. 1) Molecular and Medical Genetics, University of Toronto, Toronto, ON; 2) Genetics and Genomic Biology, Hospital for Sick Children, Toronto, ON; 3) Paediatrics, University of Toronto, Toronto, ON.

Fanconi anemia (FA) homozygotes have short telomeres, an abnormality which correlates with genomic instability and risk of bone marrow failure. FA proteins have been implicated in genetic recombination, a process thought to be involved in the Alternative Lengthening of Telomeres (ALT) pathways that function in the ~10% of human tumors that lack telomerase. We now report data supporting a role for FA proteins in ALT telomere maintenance.
FANCD2, FANCA, and FANCG form discrete nuclear foci that localize to telomeric foci (TRF1) and PML bodies in ALT human fibroblasts, but not in telomerase-positive cells. Colocalization of FANCD2 and TRF1 predominately occurs within PML bodies in late S/G2. Colocalization of FANCD2 with TRF1 is not dependent on the continuing presence of BRCA1, as only one third of FANCD2 foci that colocalize with TRF1 also colocalize with BRCA1.
In late S/G2 ALT cells, ~90% of those FANCD2 foci that colocalize with TRF1 also colocalize with the BLM DNA helicase. We previously reported that BLM associates with the telomeric binding protein TRF2 in ALT cells. We now find that FANCD2 co-immunoprecipitates with TRF2 and with BLM in late S/G2 ALT cells. None of these interactions are seen in telomerase-positive cells, suggesting that, in ALT cells, FANCD2, BLM and TRF2 form an interacting complex at telomeres during DNA replication.
We are currently using siRNA to investigate the function of the FA proteins in ALT cells. Clones expressing shRNA targeting FANCD2 were isolated and 0 of 82 ALT clones had reduced FANCD2 expression. This differed from telomerase-positive clones, where 8 of 30 clones had reduced FANCD2 expression, suggesting that FANCD2 may be required for ALT function. Together, our results support a model in which FA proteins, along with BLM, facilitate recombinationdriven replication of telomeres in ALT cells.

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Program Nr: 82 from the 2004 ASHG Annual Meeting

Embryonic lethality in *Snm1/Fancd2* double mutant mice. *A. Hemphill*[1], *D. Bruun*[1], *Y. Akkari*[1], *Y. Torimaru*[1], *L. Thrun*[1], *J. Hejna*[1], *M. Grompe*[1,2], *S.B. Olson*[1], *S. Jones*[3], *R.E. Moses*[1]. 1) Department of Molecular & Medical Genetics, Oregon Health & Science Univ, Portland, OR; 2) Department of Pediatrics, Oregon Health & Science Univ, Portland, OR; 3) Department of Cell Biology, UMass, Worcester, MA.

The protein encoded by *SNM1* in *Saccharomyces cerevisiae* has been shown to be involved specifically in the repair of interstrand crosslinks (ICLs). Mice with a disruption in the murine *SNM1* homolog are sensitive to the ICL agent mitomycin C (MMC). Fanconi anemia (FA) is an autosomal recessive disorder characterized by sensitivity to ICL agents, as manifest by cell death and chromosome breaks and radials, indicating genome instability. To study the relationship between *Snm1* and the FA pathway, we constructed mice with disruptions in both *Snm1* and *Fancd2*. There is a significant decrease from predicted ratios for the number of mice born with the genotype *Snm1* -/-, *Fancd2* -/- (4 vs. 14 expected) in a mixed background, indicating embryonic lethality, not found in the *Fancd2*-/- or *Snm1*-/- mice. Interestingly, we also find a decrease in the number of mice born with the genotype *Snm1*+/-, Fancd2 -/- (2 vs. 10 expected), giving a $P<0.01$ for both sets. These results suggest that a defect in *Snm1* is additive to a defect in *Fancd2* alone, indicating non-epistasis, and that the effect appears in animals hemizygous for *Snm1* . To evaluate this in human cells, we used siRNA to deplete SNM1. Depletion of SNM1 in normal cells produces increased cell death and radial formation after MMC. Depletion of SNM1 in FA cells leads to an increase in radial formation and cell death with MMC, indicating additivity of the FA and SNM1 pathways. The breast cancer susceptibility gene, *BRCA1*, is known to act in the FA pathway, plus at least one other pathway for repair of ICLs. Depletion of both SNM1 and BRCA1 in a normal cell line results in no increase in sensitivity over that of BRCA1 alone, indicating that SNM1 functions in a pathway for ICL repair, dependent on BRCA1 and separate from the FA pathway. Thus both siRNA depletion and mouse models argue for a non-epistatic relationship of SNM1 and FANC proteins in ICL repair.

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Program Nr: 83 from the 2004 ASHG Annual Meeting

Fanconi anemia (FA) and oxidative stress; The FA protein complex regulates the activity and cleavage of the major mitochondrial peroxidase, peroxiredoxin 3. *S. Plon[1, 2], S.S. Mukhopadhyay[1], K.S. Leung[1], P.J. Hastings[2], H. Youssoufian[3].* 1) Dept Pediatrics, Baylor Col Medicine, Houston, TX; 2) Dept Molecular and Human Genetics, Baylor Col Medicine, Houston, TX; 3) Aventis Pharmaceuticals, Bridgewater, NJ.

Fanconi anemia (FA) is an inherited disorder of bone marrow failure, congenital anomalies and predisposition to cancer. Hematopoietic progenitor cells experience apoptosis-mediated death and FA cells are reported to have increased sensitivity to oxidative stress. Here, we show, through yeast two-hybrid interaction, co-immunoprecipitation and co-localization, that the FA group G protein (FANCG) interacts with the mitochondrial protein peroxiredoxin 3 (PRDX3). PRDX3 is a 2-cysteine protein of the peroxiredoxin family, which catalyzes the reduction of mitochondrial H_2O_2 and inhibits apoptosis. In FA-G lymphoblasts (but not FANCG-corrected cells), endogenous mitochondrial PRDX3 is cleaved by a calpain-like cysteine protease. A similar cleavage product (PRDX3-S) is seen in corrected FA-G cells after exposure to 1 mM H_2O_2. In vitro analyses demonstrate cleavage of PRDX3 by recombinant calpain II, which is blocked by addition of in vitro translated FANCG protein. In vivo immunoflourescence studies demonstrate that unlike normal fibroblasts, PRDX3 is not fully localized to the mitochondria of FA-G fibroblasts. Consistent with the similar clinical phenotype of patients in different FA subgroups, we find the PRDX3-S cleavage product in lymphoblasts from FA patients subtypes G, C and A. Structural changes of PRDX3 are associated with significant functional deficits. Mitochondrial extracts from FA-G, FA-C and FA-A lymphoblasts have a nine-fold decrease in peroxidase activity. These data are consistent with a model of FA pathogenesis in which defective mitochondrial peroxidase activity results in increased reactive oxygen species, apoptosis, DNA damage, cancer susceptibility and bone marrow failure. Strategies to decrease oxidative stress may improve the FA phenotype.

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Bloom syndrome protein is a required part of the Fanconi anemia pathway for crosslink repair. *R.E. Moses*[1], *A. Hemphill*[1], *Y. Akkari*[1], *Y. Torimaru*[1], *A. Hanlon Newell*[1], *R. Schultz*[2], *M. Grompe*[1,3], *S.B. Olson*[1]. 1) Department of Molecular and Medical Genetics, Oregon Health & Sci Univ, Portland, OR; 2) Department of Pathology, UT Southwestern, Dallas, TX; 3) Department of Pediatrics, Oregon Health & Sci Univ, Portland, OR.

Bloom syndrome (BS), characterized by growth defects, immunodeficiency and increased risk of cancers, is caused by defects in the BLM RecQ helicase. BS cells demonstrate genome instability, manifest as sister chromatid exchanges (SCE). BLM protein co-purifies in cell extracts with RPA and TOPO III along with the Fanconi anemia (FA) core complex of proteins. This observation led us to investigate whether the BLM protein functions in the FA pathway for genome stability following formation of interstrand crosslinks (ICLs). FA cells manifest genome instability by chromosomal breaks and radial formation following ICL damage. We used siRNA to deplete BLM in normal human fibroblasts, and following exposure to mitomycin C (MMC), they showed decreased cell survival compared to untreated cells and an increase in radials and breaks, closely resembling FA cells. The pattern seen was very similar to that of a BS cell line (GM08505) treated with MMC. Additionally, increased SCE formation was observed in normal fibroblasts following BLM depletion. However, depletion of BLM did not alter formation of FANCD2-mono-ubiquitin, a modification required for FA pathway function. Depletion of BLM in FA cells produced no increase in breaks or radial formation. When BS cells were depleted for FANCA protein, once again, no increase in radial formation was noted. These findings indicate that BLM is required for normal ICL repair and functions exclusively in the FA pathway, downstream from the formation of FANCD2-Ub. In agreement with the hypothesis that BLM and FANC proteins function epistatically, a mouse deficient in *Fancd2* and *Blm* (the *m3* hypomorphic allele) was constructed and showed normal viability, normal reproduction, and no increase in tumors by six months of age in a mixed background, comparable to the *Fancd2*-/- mouse.

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Program Nr: 85 from the 2004 ASHG Annual Meeting

Application of restriction enzyme digest BAC fingerprints to detect chromosomal aberrations in cancer. *M. Krzywinski*[1], *S. Volik*[2], *I. Bosdet*[1], *J. Brebner*[2], *C. Mathewson*[1], *R. Chiu*[1], *D. Lee*[1], *A. Siddiqui*[1], *S. Jones*[1], *C. Collins*[2], *J. Schein*[1], *M. Marra*[1]. 1) Genome Sciences Centre, BC Cancer Agency, Vancouver, Canada; 2) Cancer Research Institute, UCSF, San Francisco, CA.

Identification of genome rearrangements is a major focus in the study of susceptibility and cause of diseases such as cancer. High-resolution, cost-effective methods are needed to generate genome-wide profiles of cancers to routinely identify causative, diagnostic and prognostic alterations. We are investigating the application of restriction enzyme digest fingerprinting of Bacterial Artificial Chromosome (BAC) clones for identification and classification of structural changes in tumor genomes. Fingerprinted BACs have certain advantages in the detection of genome rearrangements over other approaches, such as end sequence profiling (ESP) and microarray comparative genomic hybridization. Fingerprints permit the detection of rearrangements contained within a BAC, and alignment to the sequence assembly can be achieved in certain repeat-rich regions. Detected genomic alterations are also not restricted to those producing a copy number change. In silico simulations indicate that the early version of our method can be used to localize regions as small as 20kb with >95% specificity and 80% sensitivity.

We generated 5 independent restriction digest fingerprints for 700 BAC clones derived from tumor cell line and primary tumor DNA, the majority containing rearrangements identified by ESP. We compared the fingerprints to an in silico digest of the human sequence assembly to map the experimental fingerprint fragments onto the sequence. We observed fragments derived from single BACs mapping to non-contiguous regions of the genome, corresponding to potentially rearranged DNA. We corroborated ESP alignment positions to within 10 kb for 80% of the BAC ends, with a median error in end alignment position of 2.7 kb. Additionally, we detected complex and internal rearrangements of BACs, not detected by ESP. We are modifying our method to develop a high throughput application for detecting and cataloging genomic anomalies of tumor genomes.

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Program Nr: 86 from the 2004 ASHG Annual Meeting

Adaptive Chromosomal Mechanisms of Tumor Progression:- Identification of Complicons Arising from Bridge breakage Fusion Events in Genomic Regions Containing Fragile Sites. *J.A. Squire[1], G. Lim[2], J. Bayani[1], B. Vukovic[1], M. Yoshimoto[1], I. Braude[1], B. Beheshti[1], P. Marrano[1], A. Evans[1], P. Thorner[2], W. Lam[3], S. Watson[3], M. Zielenska[2].* 1) Department of Cellular & Molecular Biology, Ontario Cancer Institute, Toronto, Ontario, Canada; 2) Department of Pediatric Laboratory Medicine, Hospital for Sick Children, University of Toronto, Ontario, Canada; 3) British Columbia Cancer Agency, Vancouver, British Columbia, Canada.

We have been applying advanced molecular cytogenetics (SKY and mBAND) and tiling array comparative genomic hybridization to fine map genomic changes associated with focal gene amplification and microdeletions in tumors. The aCGH was performed using a submegabase resolution tiling set microarray that was recently developed for CGH, and consists of 32,433 BAC clones at a tiling a resolution of 80 kb, giving complete coverage of the human genome (Ishkanian, Malloff et al. 2004). This presentation will focus on fine mapping of the genomic sequences that may facilitate tumor-specific genomic rearrangement in tumors such as prostate cancer and osteosarcoma. We have shown that there is critical telomere shortening in preneoplasia associated with prostate cancer resulting in genomic instability including end to-end chromosome fusion, formation of dicentrics, ring chromosomes, and aneuploidy. In osteosarcomas we have identified a consistent pattern of gene amplification affecting cytobands at 6p21, 8q24 and 17p11.2. Molecular cytogenetics and aCGH performed on a larger series of tumors highlight the role of the bridge-breakage-fusion cycle, and telomere end-joining as critical mechanisms that fuel the adaptive chromosomal changes that accompany tumor progression n both tumor types. Moreover, our recent studies have identified patterns of inverted duplications and focal gene amplification/microdeletion taking place in regions of the genome populated by fragile sites and/or segmental duplications. Examples will be presented drawn from our recent studies using patient tumors and the prognostic implications of these observations will be discussed.

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Quantitative allelic expression differences are common in humans. *K.A. Frazer, H. Tao, K. Pant, D.R. Cox.* Perlegen Sciences, Mountain View, CA.

Differences in gene regulation have been proposed to account for a major part of the genetic basis underlying variation in heritable traits both within and between species. Expression differences between alleles of the same gene have been observed in humans by comparing the relative amounts of mRNA transcripts in normal individuals who are heterozygous for exonic SNPs. Here, we use genotyping arrays to analyze 8563 exonic SNPs located in 4102 genes for allelic expression differences in 12 individual lymphoblast cell lines. 2223 exonic SNPs located in 1500 genes are expressed in the lymphoblast cell lines and are heterozygous in at least one of the 12 individuals. 733 genes (49% of those expressed) have an indication of differential allelic expression. On average, each of the 12 individuals were heterozygous for 522 of the expressed genes, of which 106 (20%) were differentially expressed in that sample. These data indicate that alleles are commonly differentially expressed in humans. To study the transcriptional mechanisms responsible for allelic expression differences we have examined one locus, keratin 1 (KRT1), in experimental detail. For KRT1, one allele was expressed higher than the second allele in every heterozygous individual examined, suggesting that the regulatory SNPs responsible for the allelic expression differences are in linkage disequilibrium with each other as well as the assayed exonic SNP. Experimental analysis of 29 non-coding SNPs in the KRT1 interval has resulted in the identification and characterization of 5 functional SNPs which act additively and are responsible for the observed allele-specific differential expression. It is well established that KRT1 expression must be down regulated for epithelial migration to occur during skin regeneration. Here, experimental analysis suggests that epithelial cells derived from individuals homozygous for the high expressed KRT1 allele migrate slower than those derived from individuals homozygous for the low expressed KRT1 allele. Our data provide critical insights into the mechanisms underlying transcriptional regulation of allelic expression differences. Furthermore, we demonstrate that allelic expression differences can contribute to complex phenotypic traits.

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A functional genomics approach to the investigation ofhuman 6p gene function. *I. Ragoussis[1], D. Bogani[2], J. Davies[2], C. Willoughby[2], G. Duncan[3], K. Kaur[1], G. Mirza[1], R. McCeone[2], P. Denny[2], P. Sharpe[3], R. Arkell[2]*. 1) Wellcome Trust Ctr Human Gen, Univ Oxford, Oxford, UK; 2) Mammalian Genetics Unit, MRC Harwell, Oxfordshire, OX11 0RD, UK; 3) Craniofacial Development Unit, Kings College, Guys Campus, Guys Hospital, London SE1 9RT, UK.

The telomeric region of human 6p contains genes required for many aspects of embryonic development. Deletions of this region, including six new cases, have been characterised in detail and are associated with a variety of congenital abnormalities that include orofacial clefting and other craniofacial defects, CNS defects, anterior eye devlopmetal abnormalities, ear abnormalities and deafness, heart, kidney and limb defects. We have used functional genomics to generate mouse models that identify the 6p genes involved in the development of these structures. To achieve this, we have taken advantage of a large region of synteny between human 6p and the proximal portion of mouse chromosome 13. Mice monozygous for a region of chromosome 13 (corresponding to 6p25 and 6p22) are viable and fertile and replicate features of the human 6p deletion syndromes. Detailed characterisation of these mice from day 11pc to day 20 pb revealed that they lag 0.5-1 day behind in skeletal development and overall growth until birth and remain smaller than their normal siblings after that. In addition, homozygous loss of function of the Foxq1 gene that resides within the deleted material produces mice with a glossy coat. This has enabled a two-generation region specific screen for recessive, ENU induced mutations to be carried out. Screening of 1729 pedigrees has identified 11 heritable mutations that are linked to Foxq1. All of the mutations are embryonic lethal and phenotypes include clefting and other craniofacial defects, holoprosencephaly, heart, kidney and lung defects. Recombination mapping has been used to further refine the location of the mutations within the deleted area and mutation detection in candidate genes is underway for the majority of the mutant lines. The location and or identity of the mutated genes and associated phenotypes will be discussed with reference to the human 6p deletion syndromes.

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Two-stage sampling designs for gene association studies. *D. Thomas, R. Xie, M. Gebregzibher.* Preventive Medicine, Univ Southern California, Los Angeles, CA.

We consider two-stage case-control designs for testing associations between single nucleotide polymorphisms (SNPs) and disease, in which a subsample of subjects is used to select a panel of ``tagging'' SNPs that will be considered in the main study. We propose a pseudolikelihood (Pepe and Flemming, JASA 1991;86:108-113) that combines the information from both the main study and the substudy to test the association with any polymorphism in the original set. SNP-tagging (Chapman et al, Hum Hered 2003;56:18-31) and haplotype-tagging (Stram et al, Hum Hered 2003;55:27-36) approaches are compared. We show that the cost-efficiency of such a design for estimating the relative risk associated with the causal polymorphism can be considerably better than for a single-stage design, even if the causal polymorphism is not included in the tag-SNP set. We also consider the optimal selection of cases and controls in such designs and the relative efficiency for estimating the location of a causal variant in linkage disequilibrium mapping. Nevertheless, as the cost of high-volume genotyping plummets and haplotype tagging information from the International HapMap project (Gibbs et al, Nature 2003;426:789-96) rapidly accumulates in public databases, such two-stage designs may soon become unnecessary. Motivation for this approach comes from the design of a study of CHEK2 polymorphisms in breast cancer and their interactions with ionizing radiation and other genes involved in repair of double strand breaks (BRCA1/2, ATM, p53 binding protein, and others). This counter-matched study allows for the possibility of further efficiency gains for testing interaction effects by subsampling on the basis of exposure information (Bernstein et al. Breast Cancer Res 2004;6:R199-214).

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Analysis of disease-marker association studies via Bayesian partition modelling of haplotypes. *A. Morris.* Wellcome Trust Ctr Human Gen, Oxford, United Kingdom.

The most promising approach for mapping complex disease genes is generally accepted to be association studies of samples of unrelated cases and controls using high-density maps of single nucleotide polymorphisms (SNPs). The power of this approach depends, in part, on the extent of linkage disequilibrium (LD) of high-risk variants with alleles at flanking SNPs, generated as a result of their shared ancestry at the disease gene. Although each individual SNP provides relatively little information about LD, analyses of SNP haplotypes may jointly provide evidence of association for modest gene effects with realistic sample sizes.

Two major drawbacks of haplotype-based analyses are: (i) lack of parsimony and (ii) unknown phase. Here, we describe a novel method for estimating haplotype risks and testing for association in candidate genes or regions using unphased SNP genotypes directly. We begin by estimating the relative frequencies of haplotypes consistent with observed SNP genotypes. Under the Bayesian partition model, we specify cluster centres from this set of SNP haplotypes. The remaining SNP haplotypes are then assigned to the cluster with the "nearest" centre, defined in terms of marker allele matches. Within a logistic regression framework, each cluster of haplotypes is assigned the same disease risk, reducing the number of parameters required. Uncertainty in phase assignment is addressed by considering all possible haplotype configurations consistent with each unphased genotype, weighted in likelihood calculations by their relative frequencies. We develop a Markov chain Monte Carlo (MCMC) algorithm to sample over the space of haplotype clusters and corresponding risks, allowing for covariates that might include environmental risk factors or genotypes at unlinked SNPs to control for population stratification.

We illustrate the method by application to high-density unphased SNP genotype data collected across an 890kb region flanking the CYP2D6 gene for association with poor drug metaboliser (PDM) phenotype.

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Haplotype Block Analysis Using 116,000 SNPs throughout the Human Genome. *C. Rosenow*[1], *K. Hao*[2], *C. Li*[3], *W. Wong*[4]. 1) Genomics Collaboration, Affymetrix, Santa Clara, CA; 2) Department of Biostatistics, Harvard School of Public Health, Boston, MA; 3) Department of Biostatistics, Dana Farber Cancer Institute, Boston, MA; 4) Department of Statistics, Harvard University, Cambridge, MA.

There is considerable interest in using single nucleotide polymorphisms (SNPs) for mapping of complex traits. For the identification of disease associated SNPs, their non-random association with neighboring markers are analyzed in affected and unaffected individuals. Haplotype analysis has become a promising tool for this type of linkage disequilibrium (LD) -based gene searching. We have analyzed genotype data from more than one hundred thousand (116,000) SNPs throughout the genome of 54 ethnically diverse individuals using the Affymetrix Mapping 100K array. Haplotype blocks were inferred using the Wang et. al. and the Gabriel et. al. algorithm and compared to data from the HapMap project. We found a reverse correlation between haplotype block size and SNP density, indicating the identification of additional recombination sites with increasing SNP density. At the current density, more than 80 % of the typed SNPs are in haplotype blocks. On chromosome 22, most blocks contain a small number of SNPs, resulting in an average of 2.99 SNPs per block. At a haplotype block size of about 50 to 100 kb (depending on the population structure analyzed) additional SNPs did not further reduce the block size indicating the average haplotype block size in the human population is about 50 kb. Our results demonstrate the existence of haplotype block structures throughout the human chromosomes, and that the block size varies throughout the genome. Using current microarray density, the majority of the investigated SNPs are located in haplotype blocks, suggesting the untyped SNPs are likely to be in LD with the typed markers. This feature could serve as the foundation to carry out genome-wide LD mapping.

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Program Nr: 92 from the 2004 ASHG Annual Meeting

Long-range polony haplotyping in high-throughput. *K. Zhang, G.M. Church.* Department of Genetics, Harvard Medical School, Boston, MA, 02115.

Obtaining haplotypes is essential in linkage disequilibrium mapping. However, the throughput of current haplotyping technologies is several orders of magnitude lower than that of the state-of-the-art genotyping technologies. With such a huge gap, most association studies rely on haplotypes reconstructed from genotypes based on statistical methods, without taking account of the uncertainty of inferred haplotype frequencies. We have previously developed a molecular haplotyping technology based on in-gel amplification of polymerase colonies (polonies) from single DNA molecules and subsequent *in situ* single-base extensions (SBEs). Here we present a second generation of polony haplotyping technology, which has a throughput that is at least three orders of magnitude higher than the previous one. We improved haplotyping throughput by (1) reducing the feature size to 50~100 m in diameter, which translates to 3000~5000 polonies per slide and allows direct measuring haplotype frequencies in pooled DNA samples from several hundred individuals on a single slide; (2) performing multiple amplification from single molecules (MASMO) and multiple rounds of *in situ* SBEs in order to determine multi-locus haplotypes. Furthermore, we developed an experimental protocol to perform multiple amplifications from single gel-trapped intact metaphase chromosomes instead of DNA fragments, such that chromosome-wide haplotypes could be typed unambiguously without any limit of physical distance between adjacent loci. Finally, the parallel nature of polony amplification and *in situ* SBE permits haplotyping with a reagent cost as low as $0.10 per base call. This polony-based haplotyping technology can be directly applied to candidate gene based association studies.

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Ignoring Linkage Disequilibrium Between Markers Induces False Positive Evidence of Linkage for Affected Sib-Pair Analysis. *Q. Huang, S. Shete, C. Amos.* Epidemiology, Univ Texas MD Anderson Cancer , Houston, TX.

Most multipoint linkage programs assume linkage equilibrium among the markers being studied. The assumption is appropriate for the study of sparsely spaced markers with inter-marker distances exceeding a few centiMorgans, because linkage equilibrium is expected over these intervals for almost all populations. However, with recent advancements in high-throughput genotyping technology, much denser markers are available and linkage disequilibrium (LD) may exist between the markers. Applying linkage analyses that assumes linkage equilibrium to dense markers may lead to bias. Here we used simulation studies to demonstrate that LD between tightly linked markers can cause apparent over-sharing of multipoint identical by descent (IBD) between sib-pairs and false positive evidence for multipoint nonparametric linkage analysis of affected sib-pair data. LD can also mimic linkage between a disease locus and multiple tightly linked markers in certain families and thus cause false positive evidence of linkage when heterogeneity LOD score approaches are applied. This bias can be eliminated with parental data and can be reduced when additional unaffected siblings are included in the analysis.

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Linkage Analysis with Markers that Are in Linkage Disequilibrium. *G.R. Abecasis, J.E. Wigginton.* Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, MI.

Traditionally, pedigree analyses have assumed that genetic markers are in linkage equilibrium. With the advent of high-throughput SNP genotyping, closely linked SNPs are replacing microsatellites in many genome-wide scans and fine-mapping studies and this assumption can be violated. Here, we review the impact of linkage disequilibrium between markers on the results of traditional pedigree analyses, with a focus on complex trait linkage analysis. We show examples of situations where the failure to account for linkage disequilibrium between markers can result in noticeable bias in LOD scores.
We have implemented an alternative approach based on the clustering of tightly linked markers that allows for pedigree analysis in the presence of linkage disequilibrium. The approach is computationally efficient and results only in a small performance penalty, compared to traditional analyses that assume linkage equilibrium. In addition, our approach can use family samples to generate estimates of haplotype frequencies in the presence of linkage disequilibrium using a gene-counting algorithm. Our new approach is implemented in the Merlin package which can carry out many common pedigree analyses, such as parametric and non-parametric linkage analysis of discrete traits, variance components and regression-based analysis of quantitative traits and IBD estimation.

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Program Nr: 95 from the 2004 ASHG Annual Meeting

Gene Expression Profiling of Human Normal Adrenocortical Tissue and Primary Pigmented Nodular Adrenocortical Dysplasia by Serial Analysis of Gene Expression. *A.D. Horvath, Q. Vong, V. Baxendale, W. Chan, C.A. Stratakis.* NICHD, NIH, Bethesda, MD.

Serial analysis of gene expression (SAGE) is a powerful genomics tool that allows the analysis of all transcripts in a tissue or cell type, in a quantitative manner without prior knowledge of their sequence. To identify novel adrenal-specific genes putatively involved in the pathogenesis of adrenocortical tumors, we prepared SAGE libraries of human normal adrenocortical tissue (NAT) and primary pigmented nodular adrenocortical dysplasia (PPNAD) caused by a germline mutation of the PRKAR1A gene. The libraries consisted of 28,705 and 31,278 tags, representing 14,846 and 16,698 unique mRNA species from NAT and PPNAD, respectively. A total of 502 tags from the two generated libraries did not match any known expressed sequence in the GeneBank database and thus represent potentially novel transcripts. To identify tissue specifically expressed genes, we compared the relative abundance of NAT and PPNAD sequences with the corresponding expression levels in 14 other SAGE libraries from normal human tissues. A total of 128 tags, including 42 no-match tags, were found to be expressed almost exclusively in NAT and/or PPNAD and very little or not at all in other human tissues ($p<0.001$). To correctly identify the corresponding to the most adrenal-specific "no-match" tags transcripts and genes, we applied 3'RACE technique. The most 12 tissue specific "no-match" tags were converted into their corresponding longer 3' cDNA fragments using a sense primer, containing the 10-bp SAGE tag, and a single base anchored oligo(dT) antisense primer. The resulted fragments were cloned and sequenced. Differential expression between libraries was defined as a fivefold or higher tag ratio difference, combined with a $P<0.01$. In overall, 101 tags were differentially expressed in the two studied tissues. The majority of the differentially expressed tags (57%) either could not be matched to known expressed sequencies, or matched to genes with no known function, or to uncharacterized ESTs. We speculate that these genes mediate tumorigenesis in PPNAD and are potential molecular targets for this disease.

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Program Nr: 96 from the 2004 ASHG Annual Meeting

High-resolution genetic variation in classical inbred mice: implications for a haplotype map and its applications.

M.J. Daly[1,2], *C.M. Wade*[1], *A. Kirby*[1], *K. Frazer*[3], *T. Petryshen*[2], *E.J. Kulbokas*[2], *M.C. Zody*[2], *E.S. Lander*[1,2], *D. Cox*[3], *K. Lindblad-Toh*[2]. 1) Whitehead Inst Biomed Res, Cambridge, MA; 2) Broad Institute, Cambridge, MA; 3) Perlegen Sciences, Inc., Mountain View, CA.

We have previously described the mosaic haplotype structure of classical inbred lab strains of mice and suggested that a characterization of this structure would enable considerable efficiency in positional cloning efforts involving these strains. We have now obtained a detailed look at the structure of mouse variation and its applications. We describe here:

a phylogenetic analysis of the sequence of 15 strains of mice across 4.5 Mb (generated at Perlegen Sciences) which provides a detailed look at the segmental relationships and diversity in classical inbred mice

the design of and progress towards a detailed murine haplotype map of ~100,000 SNPs across an extensive panel of classical inbred strains based on these and other similar observations

the successful use of haplotype structure to guide and accelerate positional cloning efforts

the combined use of live murine resources and haplotype structure to shed light on the genetics of gene expression

The work to date suggests that a murine haplotype map, in conjunction with novel mapping resources such as crosses involving chromosome substitution strains, has the potential to dramatically increase the effectiveness of mapping complex phenotypes in mice.

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Discovery of segmental polymorphisms in the human genome using complete genome BAC arrays. *W. Cai*[1], *D. Khurana*[1], *T. Jiang*[1], *Q. Li*[1], *E. Laritsky*[1], *C.A. Chad*[1], *S.E. Scherer*[1,2], *R.A. Gibbs*[1,2], *S.W. Cheung*[1], *J.W. Belmeont*[1]. 1) Department of Molecular & Human Genetics,; 2) Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX.

Chromosomal abnormalities are common causes of many human disorders. Recent development of array based comparative genome hybridization (array-CGH) has increased the detection resolution to 50-100 fold higher than that of conventional cytogentic technique, enabling detection of subtle rearrangements in the genome as small as 100 kb. Using an innovative array construction method, we have developed BAC arrays that completely cover the entire human genome. We used a computer program to select a new set of BAC clones (over 21,500) that are optimal for array CGH. All the selected BACs have unique sequences at both ends, tightly distributed insert size and minimal repeat sequence content. The new complete human genome arrays have been validated using samples with known abnormalities in a series of blinded hybridizations. These complete genome arrays have achieved the resolution limit that is possible for the BAC cloning system. These high-resolution arrays will have huge potentials in revealing subtle cryptic rearrangements in various human syndromes. However, as the resolution of the technology improves we start to see more and more subtle abnormalities that are benign polymorphisms existing in the normal population. To distinguish the causal abnormalities from normal variants detected in patients samples we must have an extensive documentation of the population baseline variability in genomic sequence copy number (segmental polymorphisms). Toward this end, we initiated a systematic study of segmental polymorphisms in 6 different populations using our complete genome CGH arrays. Our initial results indicated that abnormalities distributed across the whole genome but the subtelomeric regions were the most variable. In each population, we will analyze samples of 100 unrelated normal individuals. This study thus will allow us to distinguish normal segmental polymorphisms from potentially causal abnormalities specific to a population with 95% confidence and over 99% confidence in the general population.

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Detection of more than 200 large-size polymorphisms in the human genome. *A.J. Iafrate[1,2], L. Feuk[3,4], L. Van Puymbroeck[1], M.N. Rivera[1,2], M.L. Listewnik[1], Y. Qi[3,4], S.W. Scherer[3,4], C. Lee[1,2].* 1) Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts, USA; 2) Harvard Medical School, Boston, Massachusetts, USA; 3) Department of Genetics and Genomic Biology and Genetics, University of Toronto, Toronto, Ontario, CANADA; 4) Department of Molecular and Medical Genetics, The Hospital for Sick Children, Toronto, Ontario, CANADA.

Using 1 megabase interval microarrays containing Bacterial Artificial Chromosome (BAC)-based DNA clones, we have identified over 200 loci in the human genome that are targets for large-size variations. Array-based comparative genomic hybridization (array-CGH) experiments were performed in pair-wise analyses using genomic DNAs from 30 unrelated and apparently healthy individuals. These experiments led to the identification of large-size polymorphisms that are scattered throughout the human genome and account for locus-specific variations, some of which involve hundreds of kilobases of DNA. An average of 11 large-size polymorphisms were detected in a given individual when a pooled DNA source containing 10 unrelated, healthy individuals is used as the control. Over 50% of these genomic regions overlap with known genes and approximately 20% of the identified loci map to regions previously thought to contain segmental duplications. Interestingly, some 10% of the loci reside within 100 kb of gaps in the current presentation of the human genome. We have established a searchable database that will provide an updated catalog of these large-size variations for accurate interpretation of whole-genome-directed array-based CGH assays in the research and clinical settings. This previously unappreciated large-scale genomic heterogeneity argues for a more dynamic impression of the structure of human genome.

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Ultra-high resolution analysis of chromosome aberrations using ROMA. *D. Warburton*[1], *V. Jobanputra*[1], *J. Sebat*[2], *W.K. Chung*[1], *K. Anyane-Yeboa*[1], *M. Wigler*[1,2]. 1) Columbia University, New York, NY; 2) Cold Spring Harbor Laboratory, NY.

Comparative genomic hybridization on genomic microarrays permits high-resolution analysis of genomic copy number variation. In traditional methods genomic DNA is hybridized to an array of BACs or cDNAs. Our new method, Representational Oligonucleotide Microarray Analysis (ROMA), (Lucito et al. 2003), substantially enhances the resolution of genomic microarray analysis. We report the first application of ROMA to patients with known cytogenetic abnormalities. After BglII digestion and adaptor-mediated PCR, patient DNA was co-hybridized with control DNA to an 85k array of oligonucleotides designed to hybridize to 200-1200 bp restriction fragments. This achieves 35 Kb resolution, >3-fold higher than a BAC array with a complete tiling path. Surprisingly, ROMA demonstrated that a 13q deletion detected by cytogenetic analysis was noncontiguous, with two distinct deletions extending from 53.08 to 61.40 Mb and from 72.88 to 74.83 Mb. The deletions occurred within a gene desert in chromosome 13, with only 6 known genes in the 10 Mb deleted region, explaining the nearly normal phenotype of the patient. FISH confirmed the noncontiguous nature of the deletion, as well as a small familial amplification involving *parkin* in 6q. In a second case with an unbalanced terminal rearrangement detected only by subtelomeric FISH, ROMA delineated a 6 Mb deletion of 22q and an 8 Mb duplication of 16q. This subtelomeric rearrangement thus involved unexpectedly large segments. In a third case, a del(4) was found by ROMA to extend from 58.8 Mb to 81.9 Mb, in agreement with the cytogenetically assigned breakpoints. The deleted genes explained some of the phenotypic features in this patient. In all cases the ROMA analysis agreed with the cytogenetic interpretation, but provided much more information. The ability to define rearrangements with great precision will enable more accurate prognosis both prenatally and postnatally for individuals with chromosomal aberrations. We are now applying ROMA to patients with congenital malformations and normal karyotypes, to identify pathogenic copy number changes below the resolution of standard cytogenetics.

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Lineage-specific gene duplication and loss in human and great ape evolution. *J. Sikela[1], A. Fortna[1], Y. Kim[2], E. MacLaren[1], M. Brenton[1], J. Pollack[2].* 1) Department of Pharmacology and Human Medical Genetics Program, University of Colorado Health Sciences Center, Denver, CO; 2) Department of Pathology, Stanford University, Stanford, CA.

We have previously used cDNA aCGH to carry out a genome-wide survey of gene copy number variation among the human and great ape lineages and identified most of the major gene copy number changes that have occurred over the past 15 MY of hominoid evolution (Fortna, et al, 2004). Over 29,000 human genes (>39,000 cDNAs) were studied in human, bonobo, chimpanzee, gorilla and orangutan lineages and 1,005 genes were identified that showed lineage-specific copy number variations. In addition, 3,100 genes were identified for which the human copy number was different than one or more great ape lineage. The chromosomal locations of these genes showed positional biases toward rearrangement-prone genomic regions such as pericentromeric and subtelomeric regions, regions of recent chromosomal inversions and fusions, and heterochromatic regions such as C-band regions on human chromosomes 1, 9, 16 and Y. In addition, there was an increase in the frequency of lineage-specific gene copy number variants in regions associated with diseases of genomic instability such as DiGeorge syndrome, Williams-Burren syndrome, Angelmann and Prader-Willi syndromes. These findings suggest a link between hominoid genome instability, recent evolutionary adaptation and human disease.
Interestingly, while such regions contained genes increased specifically in the human lineage, they also contained genes showing copy number variations specific to one or more great ape lineage. For example, the DiGeorge critical region contained several genes that were highly increased in copy number specifically in the gorilla lineage, suggesting that lineage-specific gene copy number variations that have occurred in non-human primates may be relevant to diseases of genomic instability found in humans. Finally, this aCGH dataset should provide new insights into the inter-relationship of gene duplication, genome instability, genetic disease and the genes that underlie the phenotypic differences that distinguish the human and great ape lineages.

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Evolutionary fates of gene duplications in related low copy repeats on human chromosomes 7 and 12. *A.R. Carson*[1,2], *J. Cheung*[1], *G. Occhi*[3], *M. Rocchi*[4], *S.W. Scherer*[1,2]. 1) Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, Canada; 2) Department of Medical and Molecular Genetics, University of Toronto, Toronto, Canada; 3) Department of Biology, University of Padua, Padua, Italy; 4) Sezione di Genetica-DAPEG, University of Bari, Bari, Italy.

Here we describe the genomic structure and evolution of eight related low copy repeats (LCRs) on human chromosomes 7 and 12. These LCRs range in size from 13kb to 337kb with 93% to 98% DNA sequence identity. Comparative FISH and sequence analyses indicate that the dynamic dispersal of the duplications began approximately 35 Mya during the divergence of New World monkeys. Two related genes of unknown function were identified in these LCRs and show sequence similarity to the *C. elegans* transmembrane gene *DPY-19*. These genes, *DPY19L1* and *DPY19L2*, have been copied during the LCR expansion and relaxed selection has led to pseudogenization, maintaining one functional gene. We identified two putative pseudogenes of *DPY19L1* and four putative pseudogenes of *DPY19L2*, each with a disrupted or partial open reading frame. Notably, the functional copy of *DPY19L2* is located at 12q14.2, which is not syntenic to the mouse region containing *Dpy19l2*. Instead, this mouse gene is syntenic to a putative pseudogene, *DPY19L2P1*, at 7p14.3. This depicts relocation of a functional gene through pseudogenization of the ancestral copy after gene duplication. To our knowledge, this is the first example showing a recent LCR as the causative factor of a small-scale gene rearrangement. Additionally, the two functional genes, *DPY19L1* and *DPY19L2*, share an average nucleotide sequence identity of 61% with each other, indicating that they are distantly related. Thus, the genes contained within the LCRs illustrate two fates of duplicated genes. The two related functional genes are an example of gene duplication followed by maintenance of both copies, possibly suggesting neo- or sub-functionalization. Alternately, the recent gene duplicates exemplify pseudogenization, wherein one gene copy is lost through the fixation of silencing mutations. This process can cause small-scale gene rearrangements when the ancestral copy is silenced, as is seen with *DPY19L2*.

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Program Nr: 102 from the 2004 ASHG Annual Meeting

Statistical methods for the identification of microdeletions. *Y. Li[1], C.I. Amos[2], M.S. McPeek[1]*. 1) Department of Statistics, University of Chicago, 5734 South University Ave, Chicago, IL; 2) Department of Epidemiology, U.T. M.D. Anderson Cancer Center, 1515 Holcombe Bld, Houston, TX.

Microdeletions play an important role in many genetic diseases. Identifying regions of microdeletion related to a genetic disease could lead us to the causal gene(s) located within the microdeletion. High density genotyping gives considerably more information about microdeletion. We have developed methods for the identification of microdeletions from independently sampled cases and controls using dense markers. Our methods could be easily extended to parent-offspring trios. We developed a hidden Markov model for microdeletions. This framework leads to efficient algorithms for likelihood calculation and maximization, which allow us to perform likelihood-based inference to detect and localize microdeletions associated with a trait. The model includes gamma-distributed deletion lengths and allows for heterogeneity (i.e. some proportion of the case haplotypes do not carry deletion). We have also developed permutation tests that allow us to detect microdeletions by combining information from all available markers. One test involves using the maximum z score statistic over all loci for the difference in homozygosity between cases and controls. The second test takes into account the length of the region over which there is excess homozygosity. We are testing our methods using simulation based on a dense map of markers on chromosome 18 (2300 SNPs in a 10 cM region). Supported by HG02275, HG001645 and DK55889.

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Role of Genomic Architecture in *PLP1* Duplication Causing Pelizaeus-Merzbacher Disease. *J. Lee*[1], *M. Dean*[2], *B. Gold*[2], *K. Inoue*[3], *J.R. Lupski*[1,4]. 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Human Genetics Section, Laboratory of Genomic Diversity, NCI-Frederick, Frederick, MD; 3) Department of Mental Retardation and Birth Defect Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan; 4) Department of Pediatrics, Baylor College of Medicine, Houston, TX.

Genomic architecture, a higher order structural feature of the human genome, can provide molecular substrates for recurrent submicroscopic chromosomal rearrangements, including deletion and duplication. Such rearrangements lead to gene dosage alterations within the rearranged genomic segment that may result in genomic disorders. Many disease-causing genomic rearrangements are mediated by homologous recombination between flanking low-copy repeats (LCRs), yielding common recombinant genomic segments. Pelizaeus-Merzbacher disease (PMD) is a genomic disorder that most commonly (60-70%) arises from genomic duplications of the dosage-sensitive proteolipid protein gene (*PLP1*). Interestingly, the breakpoints of the *PLP1* duplication are not common, yielding duplicated genomic segments of varying lengths. This suggests that the molecular mechanism underlying *PLP1* duplication events is likely distinct from the common mechanism. To determine whether genomic architecture also facilitates *PLP1* duplication events, we performed large-scale genome sequence analyses and identified several LCRs within the *PLP1* region. To further examine whether these LCRs may stimulate the genomic rearrangements resulting in *PLP1* duplications, we employed pulsed-field gel electrophoresis (PFGE). PFGE analyses using probes adjacent to these LCRs detected recombination-specific junction fragments on both the proximal and distal ends of *PLP1* duplications in three families. These data suggest that these LCRs may be involved in mediating the duplication events. Our study provides evidence that genomic architectural features may stimulate, although not necessarily mediate, the genomic duplications responsible for the majority of PMD cases.

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Clinical diagnosis in developmentally delayed patients; which FISH alternative to use, array-CGH or MLPA/MAPH. *J.T. Den Dunnen[1], M. Kriek[1], S.J. White[1], J. Knijnenburg[2], G.J. van Ommen[1], M.H. Breuning[1], C. Rosenberg[2], K. Szuhai[2]*. 1) Human & Clinical Genetics, Leiden Univ Medical Ctr, Leiden, Netherlands; 2) Molecular Cell Biology, Leiden, Netherlands.

Copy number changes, i.e. deletions and duplications in genomic DNA, are known to be involved in many genetic diseases. The current method of choice for their detection is karyotyping in combination with FISH. This methodology has several limitations, including a limited resolution for detecting changes and the workload and cost involved. Recent developments, such as arrayCGH and Multiplex Amplifiable Probe Hybridisation (MAPH) / Multiplex Ligation-dependent Probe Amplification (MLPA), now provide significantly improved and efficient methods facilitating the scanning for copy number changes in many genomic regions in parallel. To test and compare the performance of these techniques in a diagnostic setting we have tested 49 developmental delayed (DD) patients using both technologies. ArrayCGH analysis was performed using a 3,500 BAC array, covering the whole genome at a 1 Mb resolution. MLPA/MAPH analysis included 264 loci including the subtelomeric and acrocentric regions (n=48), regions known to be involved in microdeletion syndromes (n=25) and 191 other loci. The arrayCGH screen detected 8 independently confirmed alterations; MAPH analysis detected 5 of these, 3 alterations were not detected since these regions were not covered by the assay. MAPH analysis detected 2 alterations that were not detected by arrayCGH; in both cases the BAC array did not contain a probe for the altered region. The parallel testing shows both the reliability as well as the specific strengths of both techniques. Based on these results we suggest the following strategy as most time- and cost-effective way to screen DD patients. First, MLPA/MAPH analysis for regions known to be frequently involved (subtelomeres and microdeletion regions), subsequently arrayCGH analysis for the remaining cases.

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The use of array CGH for identifying sub-microscopic chromosomal changes in patients with intellectual disability (ID). *C. Tyson[1], C. Harvard[1], J.M. Friedman[2], S. Langlois[2], B. McGillivray[2], L. Arbour[2], S. Lewis[2], S.L. Yong[2], L. Clarke[2], M. Van Allen[2], R. Locker[4], M. Somerville[3], J. Siegel-Bartel[2], E. Rajcan-Separovic[1]*. 1) Cytogenetics, Department of Pathology, University of British Columbia, Vancouver, Canada; 2) Department of Medical Genetics, University of British Columbia, Vancouver, Canada; 3) Department of Medical Genetics, University of Alberta, Edmonton, Canada; 4) Spectral Genomics, Houston, Texas.

The recent development of whole genome microarrays offers a new approach to identifying small chromosomal imbalances at a resolution 10 times higher than routine cytogenetic analysis. We used commercial whole genome arrays with 1Mb resolution(Spectral Genomics) to look for submicroscopic gain or loss of chromosomal material in 21 patients with idiopathic ID and normal karyotypes, 2 children with ID and de-novo, apparently-balanced complex chromosomal rearrangements, and several phenotypically different but karyotypically identical members of two families with chromosomal deletions/ duplications. Our initial results show that unique changes in genomic copy number affecting one or more clones and confirmed by FISH and real time PCR were present in 2/21 children with ID. Both individuals with ID and complex chromosomal rearrangements were found by array-CGH to have submicroscopic deletions at the breakpoints not detectable by routine cytogenetic analysis. No difference in the extent of genomic loss or gain was found in phenotypically-different members of two families who carried apparently identical chromosomal rearrangements. In addition, we found evidence for frequent genomic copy number variants that do not appear to be associated with ID. Twelve clones showed apparent gain or loss in more than 1 patient, nine of which were also noted to show copy number variation in 8 normal individuals. We suspect that these recurrent sub-microscopic chromosomal abnormalities are polymorphisms, as the copy number changes were inherited from one of the parents in several cases. Our study therefore detected not only novel clinically relevant microdeletions and microduplications but also novel areas of genomic variability that may be relevant to future studies of human disease.

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Program Nr: 106 from the 2004 ASHG Annual Meeting

Charcot-Marie-Tooth disease and related hereditary polyneuropathies: management based on molecular diagnostics. *C.A. Garcia*[1], *K. Szigeti*[2], *J.R. Lupski*[2]. 1) Neurology and Pathology, Tulane Univ Health Sciences Ce, New Orleans, LA; 2) Dept of Genetics, Baylor Col Med, Houston, TX.

Thirty-seven loci and 24 genes have been identified in CMT and related peripheral neuropathies generating a complicated molecular classification, making it challenging to apply the vast amount of information in clinical practice. We have ascertained and clinically classified 153 consecutive unrelated CMT cases prior to the availability of genetic testing in commercial laboratories. We have screened 14 genes/genomic rearrangements (PMP22 dup/del, point mutations in Cx32, MPZ, PMP22, EGR2, PRX, NEFL, SOX10, SIMPLE, GDAP1, LMNA, TDP1, MTMR2) in this cohort to estimate the relative frequency of pathogenic mutations in these genes. Subsequently we have performed a combined analysis of 9 population-based studies from various ethnic backgrounds. Surprisingly similar mutation frequencies were detected in the various studies, revealing a uniform distribution of pathogenic mutations. Although it is a heterogeneous group of disorders at the molecular level, testing for a few genes/mutations yields an accurate molecular diagnosis in 65% of patients. If we apply a simple clinical classification (axonal versus demyelinating), the yield of detecting a molecular defect increases to 70% in the demyelinating group with a single test.To generate a testing scheme we have to consider that the focus and the reasons for obtaining a molecular diagnosis are different in the adult and pediatric populations. In adults with the CMT phenotype the primary aim is to establish a molecular diagnosis cost effectively to identify patients for forth-coming treatment trials and to avoid unnecessary treatment, such as immunosuppressant therapy. PMP22 duplication and Cx32 mutation analysis establishes the molecular diagnosis in 65% of patients. In the pediatric population the parents major concerns are prognosis and recurrence risk, thus a molecular diagnosis is imperative. In children, after testing for the common causes of peripheral neuropathy, PMP22 duplication and Cx32 mutations, the physician should proceed to panel testing for all the genes.

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The Poly(ADP-ribose) Polymerase 1 Modulates the Sry Function in Sex Determination. *Y.M. Li, H.J. Oh, Y.-F.C. Lau.* Dept. of Medicine, VAMC-111C5, University of California, San Francisco, CA.

Mammalian male sex determination is initiated by activation of the Sry gene on the Y chromosome. The Sry gene encodes a protein harboring a high mobility group (HMG) box that binds and bends its target DNA. Currently, the mechanism(s) by which Sry mediates this developmental process is still uncertain. Previous studies had demonstrated that the mouse Sry protein is capable of binding in vitro to specific proteins in both adult testis and embryonic gonads. We have adopted a strategy utilizing an in vitro affinity chromatography and ProteinChip technology to isolate and identify Sry interactive proteins (Sips). A 113-kDa protein was initially purified from adult mouse testis extracts with a GST-Sry fusion protein as a bait. Analysis of its tryptic peptides with SELDI-TOF mass spectrometry and database search identified the purified protein to be the mouse poly(ADP-ribose) polymerase 1 (PARP-1). Immunofluorescence staining and confocal microscopy demonstrated that PARP-1 is preferentially expressed in mouse fetal gonads at the time of sex determination and its protein co-localizes with Sry in the nuclei of Sertoli cells. GST pull-down and deletion analysis mapped the interactive domains at the HMG box of Sry and the zinc fingers of the PARP-1 protein respectively. PARP-1 binding is evolutionarily conserved among all mammalian Sry proteins. PARP-1 can be co-immunoprecipitated with an Sry antibody from protein lysate of cells co-transfected with both genes. Sry protein can be poly(ADP-ribosyl)ated by PARP-1. Electrophoretic mobility shift assays show that PARP-1 interaction decreases the binding of Sry to its target sequence and poly(ADP-ribosyl)ation of the Sry protein further abolishes its DNA binding activity. We conclude that PARP-1 modulates Sry function(s) at two levels: 1) interacting and interfering the HMG box, and 2) poly(ADP-ribosyl)ating and abolishing the Sry binding activities to its target sequence(s). Hence, PARP-1 could serve as a modulator for Sry activities in testis determination and differentiation during embryogenesis.

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Male-specific transcription of the human and mouse Spam1 genes by a conserved endogenous retroviral promoter. *C.A. Dunn*[1,2], *D.L. Mager*[1,2]. 1) Terry Fox Laboratory, BC Cancer Agency, Vancouver, Canada; 2) Dept. Medical Genetics, UBC, Vancouver.

Throughout the course of vertebrate evolution, germline retroviral infections have resulted in heritable provirus insertions into host DNA. While most of these endogenous retrovirus (ERV) insertions were lost, some became fixed in the genome. As a result, around 8% of the human and 10% of the mouse genome is derived from ERVs. ERVs contain promoter and enhancer elements that regulate transcription of retroviral genes; these signals can be adopted by nearby host genes and assume roles in their regulation. One such example is sperm adhesion molecule 1 (Spam1). Spam1 is a cell-surface hyaluronidase enzyme involved in egg cell fertilisation, and has also been implicated in metastasis by certain cancer cells. Examination of the genome sequence revealed that human, mouse and rat Spam1 transcripts initiate within the pol gene of a conserved ERV element. This is highly unusual, as all previously identified ERV transcripts initiate within the long terminal repeat viral promoter. The Spam1 locus therefore represents an example of the evolution of a promoter from protein-coding sequence. RT-PCR experiments demonstrated that mRNAs containing the human Spam1 coding region were expressed in a range of tissues. In contrast, transcripts initiating within the ERV were male-specific, expressed only in the testis and prostate. This suggests that multiple promoters are used in the regulation of human Spam1. We used 5'-RACE to determine the transcriptional start sites of Spam1 in human testis. We identified both the known ERV exon, and a nearby alternative first exon which is also derived from an ERV. Both ERV promoters were shown to be active in the testis and prostate. This represents the first known example of ERV-derived promoters acting in a gender-specific manner. We determined that only one of the two human ERV promoters is conserved in the mouse genome. RT-PCR was used to demonstrate that ERV-derived mouse Spam1 transcripts were also expressed in a male-specific manner. This ancient ERV element therefore evolved to play a role in mammalian gene regulation at least 80 million years ago.

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Meiotic synaptic patterning in the human male. *P. Brown, L. Judis, E. Chan, T. Hassold.* Genetics, Case Western Reserve Univ, Cleveland, OH.

While many studies acknowledge the importance of the synaptonemal complex (SC) to meiosis, few focus on its creation. This is especially true for humans. Most researchers have turned to model organisms and have shown that SC defects lead to abnormalities in recombination and, ultimately, nondisjunction. While these studies have been crucial to our understanding of meiotic control, most analyses have involved meiotic mutants and, therefore, their relevance to humans is unclear. Thus, we initiated a study of synapsis in the human male, analyzing testicular biopsies from men attending infertility clinics. We have studied over 1000 prophase spermatocytes from 8 control individuals, using a combination of reagents (antibodies against SCP3 to visualize the SC, CREST anti-sera to label centromeres, pan-telomeric PNA and FISH probes) to examine cells at prophase I. Initial studies suggested the importance of sub-telomeric regions in synapsis. Our more recent analyses confirm that association and provide important data on the kinetics of the synaptic process. Specifically, our results suggest that synapsis proceeds telomericly towards the centromere at a constant rate, with little evidence that specific sequences affect the process. However, in instances where one arm of a non-acrocentric chromosome is zippered up before the other, the centromere acts as a barrier to further movement from that arm. For example, in studies of chromosome 9, 43 cells had a completely synapsed p arm but a partially synapsed long arm; in 91% of these, synapsis appeared to have stopped at the centromere, and waited for the long arm to catch-up. This centromeric barrier phenotype likely extends to all males, as we were unable to detect obvious inter-individual differences in synaptic patterns. However, it is not yet clear whether all chromosomes display this behavior. To date, we have only focused on chromosomes with prominent heterochromatic blocks (9 and 16). By comparing the synaptic patterns of these chromosomes with their recombination and nondisjunction profiles, we may finally be able to determine, for the human male, factors that make individual chromosomes more or less likely to nondisjoin during meiosis.

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Dominant mutations in the human homologue of the Drosophila Nanos morphogen cause a lack of germ cells and pure sterility phenotype in men. *K. Kusz[1], A. Spik[1], A. Latos-Bielenska[2], M. Koteck[1], J. Bierla[3], P. Jedrzejczak[4], L. Pawelczyk[4], J. Jaruzelska[1].* 1) Developmental Genetics, Institute of Human Genetics, Poznan, Poland; 2) Department and Chair of Medical Genetics, University of Medical Sciences, Poznan, Poland; 3) Department of Histology, University of Medical Sciences, Poznan, Poland; 4) Clinic of Infertility and Reproductive Endocrinology, University of Medical Sciences, Poznan, Poland.

Nanos was first identified as key regulator acting in concert with a second protein, Pumilio, to repress translation of specific mRNAs in Drosophila morphogenesis and germ cell development. The highly homologous human proteins, NANOS1 and PUMILIO2, were only recently identified. These proteins form a stable complex, which is specifically expressed in the male germ line of the adult. High evolutionary conservation of this interaction indicates that both proteins may also play important roles in development of the male germ line in humans.Here, we report three types of point mutations of the NANOS1 gene resulting in a pure sterility phenotype in five patients. All three types of NANOS1 mutations were associated with a complete lack of germ cells in the seminiferous tubules with no sterility phenotype in females. Therefore, our study shows that NANOS1 may be involved in allocation and/or self-renewal of spermatogonia stem cells. Pedigree analysis suggests that inheritance of the NANOS1 gene mutations is dominant with penetration limited to males. This is the first report on autosomal point mutations causing pure sterility phenotype in men.

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Program Nr: 111 from the 2004 ASHG Annual Meeting

Mapping of PCOS susceptibility on chromosome 19p13.2 - Narrowing the candidate region around D19S884. *M. Urbanek[1], D. Stewart[2], R.S. Legro[3], J.F. Strauss[4], K.G. Ewens[2], J. D'Souza[1], W.M. Ankener[2], A. Dunaif[1], R.S. Spielman[2].* 1) Department of Medicine, Northwestern University, Chicago, IL; 2) Department of Genetics, University of Pennsylvania, Philadelphia, PA; 3) Department of Obstetrics and Gynecology, Pennsylvania State University, Hershey, PA; 4) Department of Obstetrics and Gynecology, University of Pennsylvania, Philadelphia, PA.

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among reproductive-age women. It is characterized by hyperandrogenemia and oligomenorrhea/amenorrhea, and often insulin resistance. We have identified a region near the insulin receptor gene (*INSR*) on chromosome 19p13.2 that shows evidence for both linkage and association with PCOS. Mean IBD in 124 affected sister pairs is 0.57 (P=.015). The evidence for association is strongest with allele 8 (A8) of D19S884, a dinucleotide repeat marker located 800 kb centromeric to INSR; TDT chi-square for data from 470 families is 17.9 (P = 2.3 x 10-5). For sequencing, we identified 10 PCOS probands who shared at least one D19S884 A8 allele identical by descent with an affected sister, with the goal of preferentially detecting PCOS-associated variants. For three genes (*ELAVL1*, *CCL25*, and *FBN3*) located within 100 kb of D19S884, we have sequenced more than 90% of exons, plus 1000 bp proximal promoter region, and 200 bp flanking intronic sequences. We identified 118 single nucleotide polymorphisms (SNPs). The 46 most informative SNPs, and 18 SNPs found in public sources, were genotyped in 329 families. None of the variants shows significant evidence for association with PCOS. Near D19S884, strong linkage disequilibrium (D > 0.5) extends less than 10 kb. Thus we cannot exclude the possibility that the functional variant is D19S884 itself. D19S884 is located in a 3 intron of *FBN3*, and might affect expression of this gene or the other nearby genes, or even more distant genes such as *INSR*.

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Program Nr: 112 from the 2004 ASHG Annual Meeting

Meiotic defects in infertile men. *R. Martin*[1, 2], *F. Sun*[1, 2], *E. Ko*[2], *A. Rademaker*[3], *C. Greene*[4], *P. Turek*[5]. 1) Department of Medical Genetics, University of Calgary, Calgary, Canada; 2) Department of Genetics, Alberta Childrens Hospital, Calgary, Canada; 3) Cancer Center, Northwestern University Medical School, Chicago, USA; 4) Department of Obstetrics and Gynecology, University of Calgary, Calgary, Canada; 5) Department of Urology and Reproductive Science, University of California at San Francisco, CA, USA.

Infertile men have an increased frequency of aneuploid sperm. We have determined that decreased recombination is associated with the production of aneuploid sperm in humans. The aim of this study was to determine if some cases of infertility are associated with decreased meiotic recombination. Analysis of the early stages of meiosis was performed in 7 men with nonobstructive azoospermia (NOA), 6 men with obstructive azoospermia (OA) and 11 men with normal spermatogenesis. Newly-developed immunocytogenetic techniques were used to identify the synaptonemal complex (SC) in various stages of prophase. Antibodies to meiotic proteins identified the SC (SYN1/SCP3), the centromere (CREST) and recombination sites (MLH1). Approximately 100 spermatocytes were analyzed for each man. The mean (SD) frequency of recombination was 42 (9.5) for men with NOA and 44 (6.7) for men with OA, both significantly reduced compared to controls 48.0 (4.7) ($p<.0001$, nested ANOVA). Three individual men with NOA and 4 men with OA had a significantly increased frequency of gaps in the SC compared to controls ($p<.05$, Z test). The mean frequency of cells with unsynapsed chromosome regions was significantly increased in 4 men with NOA and one with OA ($p<.05$, Z test). The mean percentage of cells containing bivalents with no recombination foci was 26.6 in men with NOA, 15.0 in the OA group and 3.9 in controls. 4 men with NOA and 3 men with OA had a significantly increased frequency of cells with nonrecombinant bivalents ($p<.05$, Z test). Thus significant numbers of men with both OA and NOA have abnormalities in chromosome pairing, a decreased frequency of recombination and an increased frequency of bivalents with no recombination foci. These meiotic abnormalities could lead to meiotic arrest or an increased frequency of aneuploid sperm.

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Program Nr: 113 from the 2004 ASHG Annual Meeting

Subfertility is associated with an increased risk of conceiving a child with an imprinting defect. *B. Horsthemke[1], S. Gross[1], A. Katalinic[2], A. Sutcliffe[3], R. Varon[4], M. Ludwig[5].* 1) Inst Humangenetik, Universitatsklinkum, Essen, Germany; 2) Inst Krebsepidemiologie, Medizinische Universitat Luebeck, Germany; 3) Dept Paediatrics, University College, London, UK; 4) Inst Humangenetik, Humboldt Universitat Berlin, Germany; 5) Endokrinologikum, Hamburg, Germany.

Recent reports on children who were conceived by intracytoplasmic sperm injection (ICSI) and have Angelman syndrome (AS) as the result of an imprinting defect (ID) have suggested that assisted reproduction may increase the risk of an ID. AS is caused by the loss of function of the maternal *UBE3A* allele on chromosome 15. In approximately 4% of AS patients, the maternal *UBE3A* allele is silenced by an ID. To investigate a possible correlation between infertility treatment and IDs, we have performed a cohort study using data from the German Angelman Syndrome Support Group. Among 79 patients we identified 16 children who were born to subfertile couples (defined as having had a time to pregnancy (TTP) >2 years and/or infertility treatment). Four of these children (25%) have a sporadic ID. Assuming that an ID accounts for 4% of AS patients, the relative risk (RR) was significantly increased in this group of patients (RR, 6.25; 95% CI, 1.68; 16.00). The RR was increased by the same factor in the untreated subgroup of couples with TTP >2 years (n=8) as well as in the subgroup of couples who underwent hormone (n=5) or ICSI (n=3) treatment (total, n=8), although the increase did not reach statistical significance. The increased RR in untreated couples with TTP >2 years suggests that IDs and subfertility may have a common, possibly genetic cause. The RR was highest in couples with TTP >2 years and infertility treatment (n=4; RR 12.5; 95 % CI 1.40; 45.13), suggesting that infertility treatment further increases the risk of conceiving a child with ID. One possible explanation is that these couples suffered from a more severe form of subfertility that necessitated treatment. Alternatively, hormonal stimulation may increase the frequency of epigenetically impaired oocytes. In summary, we suggest that genetic predisposition and superovulation, rather than ICSI, increases the risk of conceiving a child with an imprrinting defect.

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Program Nr: 114 from the 2004 ASHG Annual Meeting

Advancing age among healthy males is associated with increased frequencies of sperm with DNA fragmentation and ACH gene mutations, but not aneuploidies or diploidies. *A.J. Wyrobek[1], S. Young[2], F. Hill[1], D. Evenson[3], I. Tiegmann-Boerg[4], N. Arnheim[4], B. Eskenazi[2].* 1) Biol Biot Res Prog, Lawrence Lawrence Livermore Natl Lab, Livermore, CA; 2) Sch Public Health, UC Berkeley, CA; 3) S Dakota State Univ, Brookings, SD; 4) Univ Southern California, Los Angeles, CA.

The trend for older men to become fathers raises concerns as the probability of producing children with chromosomal or genetic defects may also increase with age. Our study contrasts the effects of male age on the relative production of several types of genetically defective sperm: DNA fragmentation (DFI) measured by SCSA; achondroplasia (ACH) gene mutations by allele-specific PCR; and numerical abnormalities by sperm FISH for chromosomes X, Y, and 21. Semen was provided by a non-clinical group of ~90 healthy non-smoking men (range 22-80y, mean 46.4) in a cross-sectional design. Linear regression models were adjusted for confounders identified by questionnaire. DFI increased with age, predicting a steady decrease in fertility potential beginning in a mans early reproductive years. DFI increased from 12.4% in 20-29 year olds to 59.9% in 70-80 year olds, ($p<0.001$ for trend). Linear regression showed a relative 3.1% increase in DFI per year of age ($p<0.001$, R2=0.55) after adjusting for duration of abstinence. The frequency of ACH mutations also increased significantly across age decades, with a 2.0% increase in ACH mutations per year ($p=0.01$) predicted from an adjusted negative binomial regression model. However, there were no detectable age effects on sperm aneuploidies (disomy 21, X, Y, XY, sex-null), or diploidies (MI and II). Aneuploidies and diploidies were highly inter-correlated but DFI, ACH, and numerical abnormalities were not. Only DFI was correlated with semen quality: DFI vs. motility, $r= -0.65$; $p<0.001$. Our findings suggest that advancing age reduces the DNA integrity of sperm and increases the frequencies of sperm with specific gene mutations, but unlike in the female, age appeared to have no effect on sperm aneuploidy or diploidy. [This work was conducted under the auspices of the US DOE by Univ of California, LLNL under contract W-7405-ENG-48 with support from NIEHS P42 ES04705 (BE and AW)].

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Meiotic outcome of translocations based on preimplantation genetic diagnosis (PGD). *Y. Verlinsky, J. Cieslak, V. Galat, A. Kuliev.* Dept Molec Genet, Reproductive Genetic Inst, Chicago, IL.

Carriers of translocations have an extremely poor reproductive outcome, with more than three quarters of their pregnancies resulting in spontaneous abortions. PGD is the only realistic option for such couples to have a normal child, showing that the proportion of abnormal oocytes and embryos may vary depending on the type of translocations and their origin. To investigate the meiotic outcome of translocations in relation to the type and origin, a segregation patterns from 112 patients carrying balanced translocations were analyzed. The meiotic outcomes were inferred either from the first and second polar body (PB1 and PB2), or blastomere analysis. Meoitic outcome detection rates by each of these methods were comparable, except for chromatid exchanges, detected only by sequencial PB1 and PB2 analysis (16.4%), and complex errors which were higher in PB analysis (17.3% vs.4.2%), 3:1 segregation found more frequently in blastomere analysis (7.7% vs.23%). Segregation patterns for paternally and maternally derived translocation showed similar tendencies, predominantly represented by alternate (35 and 34%, respectively) and adjacent I (28 and 34%, respectively), and much low adjacent II (9.1 and 11.4%, respectively). These meiotic outcomes may explain the proportion of balanced and unbalanced embryos detected, which were predicted in 76.4% embryos obtained from maternally derived reciprocal translocations, leaving only 23.6% suitable for transfer, including 11.3% balanced and 12.3% normal. On the other hand, unbalanced embryos were predicted in 68.2% embryos obtained from paternally derived reciprocal translocations, leaving 31.8% embryos suitable for transfer, including 13.1% balanced and 18.7% normal. Clinical pregnancies were obtained in 30.3% of transfer cycles, with 25% overall delivering healthy children. The data on the meiotic outcome may explain the observed 85% spontaneous abortion rate in patients prior to undertaking PGD procedure, which was reduced to 17.9% after PGD, demonstrates the tremendous positive impact PGD on the clinical outcome of pregnancies in couples carrying translocations.

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Spermatozoal RNAs contribute to the early embryo. *G.C. Ostermeier[1,2], R.J. Goodrich[1], J.S. Moldenhauer[1], D. Miller[4], J.D. Huntriss[4], M.P. Diamond[1], S.A. Krawetz[1,2,3].* 1) Department of Obstetrics & Gynecology; 2) Center for Molecular Medicine and Genetics; 3) Center for Scientific Computing, Wayne State University, Detroit, MI; 4) Reproductive and Early Development Group, University of Leeds, UK.

Until the embryonic genome is activated, zygotic maintenance is dependent on stored mRNAs and proteins. These stores alone however, are insufficient to maintain parthenogenic development and appear less than optimal for sustaining embryos derived by nuclear transfer. It has recently been demonstrated that ejaculate sperm contain a complex suite of RNAs. This has lead to the question of whether paternal RNAs are requisite for fertilization and subsequent development. To determine if unique paternal transcripts exist, cDNAs from human sperm and unfertilized human oocytes were interrogated by RT-PCR. Of the 11 candidates tested, 6 were identified as unique to sperm. A zona-free hamster egg penetration assay was employed to determine whether sperm deliver any paternal RNAs to the ooplasm at fertilization. REAL-TIME PCR was used to interrogate cDNAs produced from human sperm, hamster oocyte and hamster/human zygotic RNA. Of the 6 paternal RNAs investigated, only 2 were consistently detected in the zygote. These observations suggest that sperm deliver distinct portions of the paternal transcriptome to the ooplasm at fertilization. The presence of SI RNAs has been shown to regulate differentiation. To further explore sperm transcript functionality, a unique microarray system ideal for identifying antisense RNAs was utilized. With this approach RNA isolated from the ejaculate sperm of 6 normal fertile men was directly hybridized to olgionucleotide arrays containing 10,000 elements and the RNA-DNA hybrids were labeled with a fluorescein tagged antibody. Common to the normal fertile men, 68 candidate antisense RNAs were identified. The developmental influence of this population is being elucidated. Perhaps these and other unidentified molecules participate in processes like oocyte activation, pronuclear formation, the transition from maternal to embryonic gene control and/or the establishment of imprints in early embryos.

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The Diagnostic Yield of a Genetics Evaluation in Pervasive Developmental Disorders. *O.A. Abdul-Rahman, L.H. Hudgins.* Genetics, Stanford University, Stanford, CA.

We were able to identify 101 patients at our institution who were referred to our genetics service for PDD, autism, autistic-like features, or Asperger syndrome. In our patient population, 78 were males and 23 were females giving a male-to-female ratio of 3.4:1. The types of tests ordered were examiner-dependent and included 76 karyotypes, 66 fragile X analyses, 20 FISH studies for proximal 15q duplication, and 28 FISH studies for 22q13 deletion. Some type of metabolic testing was performed in 53 patients. MECP2 mutation analysis was carried out in six female patients. Seventeen patients did not undergo any type of testing due to noncompliance. Of the remaining 84 patients analyzed, seven (8.3%) were found to have abnormalities upon testing. Three chromosomal anomalies were found: one with 5p duplication, one with low-level mosaicism for trisomy 21, and one with an unbalanced translocation involving chromosomes 10 and 22. The translocation resulted in monosomy for 22q13.3 and was only identified after a high-resolution study was performed. FISH demonstrated one hybridization signal for the ARSA probe. None of the patients with chromosomal anomalies had any major or significant minor anomalies upon examination by a clinical geneticist. Three females were diagnosed with Rett syndrome after MECP2 analysis identified a disease-causing mutation. One case was initially evaluated at 25 months of age and was not suspected of having Rett syndrome until regression was noted at the age of 4. The remaining patient was found to have an elevated urine orotic acid, with a normal ammonia level, of unknown significance. Based on our series, the yield of a genetics evaluation in patients with some form of PDD or autism who do not necessarily meet DSM-IV criteria is 8.3%. These findings suggest that a high-resolution karyotype provides the greatest diagnostic yield for patients with autistic-like features. FISH analysis for 22q13 deletion may also be indicated, even in the absence of major and/or minor anomalies. MECP2 analysis should be considered for younger females who present with autistic behaviors. Routine metabolic evaluations are of low diagnostic yield.

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Program Nr: 118 from the 2004 ASHG Annual Meeting

Diagnostic yield of the routine assessment of patients with developmental delay/mental retardation and autism in a genetics clinic: A retrospective study. *T. Pesaran[1], M. Fox[2], V. Vandergon[1], S. Cederbaum[2]*. 1) Genetic Counseling, California State Univ, Northridge, CA; 2) Pediatric Genetics, CaliforniaUCLA, Los Angeles, CA.

Developmental delay (DD), mental retardation (MR) and autism are some of the most common reasons for a referral to a pediatric genetics clinic. Guidelines have been established regarding the evaluation of these patients, emphasizing the high diagnostic utility of cytogenetic studies and neuroimaging. The purpose of this study is to assess the utility of diagnostic studies undertaken in children with MR/DD and /or autism without significant genetic diagnosis when they presented to the UCLA Genetics Clinic. We report a retrospective analysis of 82 patients who were referred to our clinic for MR, DD and/or autism over a 3-year period. Diagnostic studies (history, physical examination, high-resolution chromosome analysis, fragile X testing, molecular studies, metabolic screening tests, and neuroimaging studies) yielded a causal diagnosis in 11 (13.4%) of the 82 patients. Causal categories included chromosome abnormalities (5) and known genetic syndromes (6). In individuals who were classified as autistic, with or without developmental delay, an etiologic diagnosis was made 3/21 (14.3%) of the time. Of these 1/21 (4.8%) was a clinical diagnosis, while 2/21 (9.5%) were based on laboratory studies. The diagnostic study with the highest yield was high-resolution chromosome analysis while fragile X testing was the most commonly ordered test with the lowest yield (0%). The majority of the patients (78%) were seen only once while the rest were seen twice. These findings suggest that in a general genetics clinic the diagnostic yield of these patients is not as high as previously suggested and that continued follow-up care is necessary in order to identify the underlying cause of these patients MR/DD and autism.

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Program Nr: 119 from the 2004 ASHG Annual Meeting

Genetic Testing for Autism:Current State of Practice in the Southeastern United States. *C.M. Wolpert[1], M.L. Cuccaro[1], H. Cope[1], S.L. Donnelly[1], H.H. Wright[2], R.K. Abramson[2], A. Ashley-Koch[1], G.R. DeLong[1], M.A. Pericak-Vance[1]*. 1) Duke Center for Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) W.S. Hall Psychiatric Institute, Columbia, SC.

Genetic testing is available for several disorders that show clinical overlap with autism. An estimated 10 to 15% of individuals with autism have an identifiable genetic disorder. Yet it is not known what percentage of individuals with autism will yield positive results for the currently available genetic tests. Clinical recommendations for genetic testing are limited to individuals with autism who also have mental retardation, dysmorphic features, or both. This study examined the frequency of genetic testing in a sample of 625 families with one or more children with a confirmed diagnosis of autism enrolled in a genetic study. Some participants had genetic testing as part of private clinical evaluations prior to entering the study. In this data set, less than 20% of individuals with autism have had genetic testing. The most common genetic tests ordered in this sample were: Fragile X testing (18%), chromosome analysis (14%), FISH (5.2%), amino acids (9%), and organic acids (8%). Some individuals had more than one genetic test. Two comparisons were conducted to examine potential differences related to genetic testing. First, adaptive functioning scores were compared for participants with autism who had genetic testing vs. those who did not have genetic testing. The group with genetic testing had significantly lower mean VABS composite scores($p = 0.0001$). Lower scores reflect poorer level of functioning. Secondly, the data set was grouped on the basis of family history for autism. The family history positive group had a significantly higher number of individuals who had genetic tests ($p=0.004$). These data suggest that genetic testing is more likely to be ordered for individuals with lower adaptive functioning or a positive family history. Less than 20% of this data set have had genetic testing, but 72% of the participants have mental retardation. Therefore, more than 50% of eligible individuals did not have genetic testing suggesting that in this data set genetic testing is underutilized.

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Autism symptoms are less severe in girls with Essential Autism. *J.H. Miles, T.N. Takahashi, R.E. Hillman, K.L. Martin.* Med Gen Div, Univ Missouri Hosp, Columbia, MO.

Historical dogma maintains girls with autism are more retarded and have poorer outcomes than boys. We reported earlier that essential autism, for which there is no evidence of altered morphogenesis, should be studied separately from complex autism (diagnosed by dysmorphology & microcephaly). We report a comparison study of girls and boys with essential autism. Boys were matched to the girls for age, SES and evaluation date. IQ scores (Leiter, Wechsler), Vineland Adaptive Behavior scores, autism diagnostic scores (CARS, ADI-R, DSM IV), age & type of onset were compared. There were no significant differences in IQ mean scores (girls=89 vs boys=77), ranges (g=51-124 vs b=32-105) or distribution. Vineland scores were similar for communication (g=66 vs b=57), daily living (g=70 vs b=55), social (g=67 vs b=62), motor (g=75 vs b=73) and composite (g=66 vs b=57). Yet, for each task girls' scores surpassed the boys. There were no significant differences in age & type of onset though girls tended to develop symptoms earlier (60% in year 1 vs. 11% of boys, p=0.057); boys developed symptoms more frequently in the 2nd year with language regression (b=60% vs g=30%). Autism symptom scores were similar; CARS (g=34.9 vs b=34.0), # of DSM IV criteria met (g=8 vs b=9) and ADI-R composite scores (Social g=15.4 vs b=19.5), (Verbal g=10.5 vs b=15), (Nonverbal g=9 vs b=11.3), (Repetitive behaviors g=4.1 vs b=5.1). For all categories the girls scores were slightly better than the boys. Moreover, boys were more severely affected in 26/36 items vs 8 for girls (p=0.00004). Girls excelled most in spontaneous imitation (p=0.005), range of facial expression (p=0.06), interest in children (p=0.07), directing attention (p=0.06) and imaginative play (p=0.07). We conclude that girls within the more homogeneous essential autism subgroup are less severely autistic than boys and probably out perform boys in cognitive & adaptive measures. This is consistent with our previous data showing lower recurrence risks for girls regardless of the sex of the proband and the higher male to female ratio (6.5:1). This supports the hypothesis that girls are somehow protected from developing autism.

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High Density, High Resolution Haplotype Structure of 750Kb on Chromsome 17 based on 1300 Founder Chromosomes. *J.L. Stone[1], B. Merriman[1], D.H. Geschwind[2], S. Nelson[1].* 1) Dept Human Gen, Gonda, #5554, Univ California, Los Angeles, Los Angeles, CA; 2) Dept Neurology, Univ California, Los Angeles, Los Angeles, CA.

Studies have suggested that the human genome is organized in a block structure and that a high fraction of diversity can be captured using a small proportion of available polymorphisms in an interval. Studies of complex genetic disorders look to this phenomenon to conserve resources by assaying only those SNPs that capture and define haplotype blocks. The value added from knowing the haplotype block structure *a priori* when designing an association study to determine if common variants underlie disease susceptibility is a main impetus for the HapMap project. Most information on the block-like nature of the genome has been gathered from assaying tightly spaced markers in modest numbers of individuals of different ethnicity. However, many disease studies in the US are conducted on primarily Caucasian families and the impact of the HapMap project remains to be determined. To more completely assess the structural nature of the genome and the ability of htSNPs to capture sufficient information for a family based association study, we assayed 214 SNPs over a region of ~753Kb on Chr17 in 1000 individuals from 325 families. The study included trio families from autism and ADHD disease studies. The region is located at a previously reported region of autism linkage on Chr17 at ~50cM (Yonan et al. 2003). We detected 24 blocks across the region. The size of the haplotype blocks marked by more than 1 SNP ranged from 0.1-168 Kb. 80% coverage of the different haplotypes in any one block of greater than 1 SNP ranged from 2 to 6 distinct haplotypes. 52 SNPs (30% of SNPs with MAF>1%) were flagged as htSNPs to capture 80% of genomic diversity in the region. Our results, based on a large number of chromosomes and focused on a population relevant to many complex genetic disorders studied in the US, fit the general trend of a block-like genomic structure of haplotypes found by other groups. Our greater depth of sampling shows, however, greater diversity within a single ethnic population than previously thought with greater numbers of SNPs needed to capture even 80% of that diversity.

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Genome scan replicates linkage of autism to 17q11. *R.M. Cantor-Chiu[1], N. Kono[1], M. Alarcón[2], D.H. Geschwind[2].* 1) Human Genetics; 2) Neurology, Center for Autism Research, David Geffen School of Medicine at UCLA, Los Angeles, CA.

Autism, characterized by deficits in language and reciprocal social interactions combined with repetitive and stereotypic behaviors, exhibits the hallmarks of a genetically complex disorder. A non-Mendelian mode of inheritance, coupled with a 90% monozygotic vs. a 6% dizygotic twin concordance rate implies it is likely to develop from the interactions of multiple susceptibility genes. Given the genetic complexity, confirmation of linkage signals with independent well-matched study samples is critical, however, such samples are rare. We report the replication of autism linkage to 17q11 first identified by Yonan et al. (AJHG 73:886) with a genome scan of 345 sibpairs from the Autism Genetic Resource Exchange (AGRE). In an independent sample of 110 AGRE sibpairs diagnosed using the same ADI-R criteria and using the same statistical methodology, multipoint allele sharing statistics were estimated and lod scores calculated by the Genehunter software using 401 multi-allelic markers genotyped by the Center for Inherited Disease Research. Two loci exhibited lod scores exceeding the replication threshold of 1.4 ($p<.01$). A 25 cM region with all lod scores > 1.4 on 17q11-23 had a peak at 61 cM with a lod of 1.9 near D17S1299, and a 12 cM region with all lod scores > 1.4 on 3p14-12 had a peak at 99 cM near D3S2406 with lod of 1.8. Furthermore, when the analysis of the linked region on Chromosome 17 was limited to the 58 same-sex male pairs, a lod score of 4.2 was observed, replicating the results of Stone and colleagues found in an independent AGRE sample (IMFAR, 2004), and implying that the linkage of autism to this region derives primarily from genes expressed in males. The single point lod score of the peak marker in this region for the combined AGRE sample of 455 sibpairs was 3.0. Fine mapping of this combined sample will better localize the putative risk gene. This study confirms linkage of autism to 17q11 and provides critical information for effective gene identification in this complex disorder.

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Program Nr: 123 from the 2004 ASHG Annual Meeting

Replication of association of *ENGRAILED 2* in 485 autism spectrum disorder families. *N. Gharani[1], R. Benayed[2], V. Mancuso[2], G. Lazar[2], S. Kamdar[2], L.M. Brzustowicz[1,4], J.H. Millonig[1,2,3].* 1) Dept Genetics, Rutgers Univ; 2) CABM; 3) Dept of Neuroscience and Cell Biol UMDNJ-RWJMS, Piscataway, NJ; 4) Psychiatry, UMDNJ-NJMS, Newark, NJ.

Autism Spectrum Disorder (ASD) is a complex behavioral disorder with a genetic and neurodevelopmental basis. Previously, using 167 families from the Autism Genetic Resource Exchange (AGRE), and the transmission-disequilibrium test (TDT) we demonstrated that 2 intronic SNPs (*rs1861972* and *rs1861973*) from the homeobox transcription factor *ENGRAILED 2* (*EN2*) were significantly associated with ASD both individually and as a haplotype under both narrow and broad diagnostic criteria. In the present study, association of *rs1861972* and *rs1861973* was tested in 2 additional datasets, 239 further AGRE families and 141 families from the NIMH autism dataset, using the family-based association analysis program PDTPHASE (2.404). Each new dataset analyzed alone either displayed minimal or no association with ASD. However, when the data was analyzed from the entire AGRE population, greater significance of association was observed than originally reported (*rs1861972-rs1861973* haplotype: narrow: P= 0.000041; broad: P=0.000016). Likewise, when TDT analysis is performed on data from all three populations, yet further significance is achieved (*rs1861972-rs1861973* haplotype: narrow: P=0.0000098; broad: P=0.0000039), indicating that *rs1861972* and *rs1861973* are associated with ASD in three different datasets. Further LD mapping and TDT analysis was then performed in the original 167 AGRE families using 3 additional polymorphisms, 2 in the promoter (*rs6150410*, *rs1345514*) and 1 intronic (*rs3824068*). None were found to be associated with ASD individually and only *rs3824068* was in strong LD with *rs1861972* and *rs1861973*. Multi-SNP haplotype analysis failed to identify a single predisposing ancestral haplotype and provided further evidence that *rs1861972* and *rs1861973* are unlikely to be functional. Together these data are consistent with an as yet unidentified functional variant in strong LD with *rs1861972* and *rs1861973* existing in all three ASD datasets providing further evidence that *EN2* may act as an ASD susceptibility locus.

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Program Nr: 124 from the 2004 ASHG Annual Meeting

Mutations of STK9 cause early onset seizures, mental retardation (MR), an autistic disorder and a Rett Syndrome (RTT)-like phenotype. *J. Christodoulou[1], L.S. Weaving[1], S.L. Williamson[1], K.L. Friend[2], O.L.D. McKenzie[2], H. Archer[3], J. Evans[3], A. Clarke[3], G.J. Pelka[1, 4], P.P.L. Tam[4], C.M. Watson[4], H. Lahooti[1], C.J. Ellaway[1], B. Bennetts[1], H. Leonard[5], J. Gécz[2].* 1) Western Sydney Genetics Program, Childrens Hospital at Westmead, Sydney, Australia; 2) Dept of Genetic Medicine, Womens & Childrens Hospital, Adelaide, Australia; 3) Dept of Medical Genetics, University of Wales College of Medicine, Heath Park, Cardiff, Wales; 4) Embryology Unit, Childrens Medical Research Institute, Sydney, Australia; 5) Telethon Institute for Child Health Research, University of Western Australia, Australia.

RTT is a severe neurodevelopmental disorder primarily affecting females, caused by *MECP2* mutations in about 75% of classic cases. We report two families where probands had RTT-like features, which had pathogenic mutations in the putative serine/threonine kinase, *STK9*. One family had a proband with a RTT-like phenotype with early onset seizures, her identical twin sister with autism and mild-moderate MR, and a brother with profound MR and seizures. No pathogenic *MECP2* mutations were found. Microsatellite mapping identified a candidate region at Xp22.31-p22.2 between DXS8051 and DXS1683. Sequencing of the *STK9* gene revealed a deletion (c.183delT) in the coding sequence in the affected family members. In a screen of 44 RTT cases, a single splice site mutation, IVS13-1G>A, was identified in an atypical RTT-like female. Whole mount in situ hybridization *Stk9* analysis revealed widespread expression of the *Stk9* gene in the week-8 mouse brain, strongest in regions overlapping with but not identical to those of *Mecp2*. Interestingly, *Stk9* expression was similar to wild-type in the brains of mice that lack *Mecp2* function, suggesting that Stk9 expression is independent of *Stk9* activity, indicating that STK9 may act either in parallel to or upstream of MeCP2. Thus, mutations in *STK9* can lead to a clinical phenotype overlapping with RTT. It remains to be determined whether STK9 interacts directly or indirectly with MeCP2, and whether *STK9* mutations are more prevalent in specific clinical subgroups of RTT or other clinical presentations.

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Program Nr: 125 from the 2004 ASHG Annual Meeting

Low copy repeat (LCR) associated 10q rearrangements affecting developmental delay and autism. *L. Schimmenti[1], B. Hirsch[1], K. Iyadurai[1], L. Oseth[1], T. Paton[2], B. Bill[1], W. Roberts[2], D. Czapansky-Beilman[1], L. Charnas[1], S. Scherer[2], S. Selleck[1].* 1) Univ of Minnesota, Minneapolis, MN; 2) The Hospital for Sick Children, Toronto, Ontario, Canada & Univ of Toronto.

LCR mediated genomic rearrangements represent a significant cause of developmental disability. We describe a family identified through a male autistic proband with an LCR associated deletion of 10q. High resolution G-band analysis (850 band level) revealed a deletion from 10q23.2 to 10q23.32. Two maternal male first cousins, presented with developmental, speech and language delays, have the same 10q deletion but do not meet autism criteria. The mother, maternal grandfather and 5 of 6 evaluated maternal siblings bear the deletion and display a range of developmental, speech, and language delays, with males more severely affected. A maternal sibling without the deletion is unaffected. Characterization of the deletion by FISH using probes from BACPAC Resources and Affymetrix 10K GeneChip SNP analysis defined a deletion spanning 7Mb. Complex LCRs of 0.7Mb proximally and 0.5Mb distally, appeared as duplicated FISH signals at the breakpoints. We pursued and identified by FISH an additional LCR at 10q11.2. BLAST analysis showed 98-99% identity between LCRs at the breakpoints and confirmed the proximal LCR at 10q11.2. Deleted genes within the segment include NRG3, SNGC, and GLUD1 while PTEN, a gene that has been associated with autism, is located approximately 0.36 Mb from the distal breakpoint. To determine the frequency of deletions involving 10q11.2 and 10q23 in autistic probands, we screened short tandem repeat polymorphic markers from 10q in 167 families collected through the Toronto Hospital for Sick Children Autism Clinic. We have evidence for non-Mendelian segregation of 10q markers in 22 probands. We replicated the genotyping data and are confirming the presence of putative deletions using FISH and quantitative PCR. The presence of a deletion at 10q23.3-23.32 identified in a family with an autistic proband and the identification of putative LOH independently ascertained for autism suggests a potential role for this LCR flanked genome segment in the pathogenesis of autism and related disorders.

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Phenotype of the Fragile X Premutation in Childhood: A Preliminary Study. *F. Farzin*[1], *H. Perry*[1], *D. Hessl*[1,2], *D.Z. Loesch*[4], *J. Cohen*[5], *L.W. Gane*[1], *M. Kradin*[1], *R.J. Hagerman*[1,3]. 1) Univ California MIND Inst, Sacramento, CA; 2) Department of Psychiatry, University of California at Davis Medical Center, Sacramento, California,; 3) Department of Pediatrics, University of California at Davis Medical Center, Sacramento, California,; 4) School of Psychological Science, La Trobe University, Melbourne, Australia; 5) Fragile X Alliance, Victoria, Australia.

This study reports on phenotypic features in 25 males (ages 5-22) with the fragile X mental retardation 1 (FMR1) premutation (55 to 200 CGG repeats). Fifteen are probands and 10 are nonprobands, identified through pedigree analysis. Sixteen male siblings without the FMR1 mutation (ages 5-22) were included as controls. Probands ranged in full scale IQ (FSIQ) from 46-135 (M=92), nonprobands from 80-112 (M=96, and controls from 82-130 (M=101). 20% of probands, 10% of nonprobands, and 6% of controls had a borderline or mentally retarded FSIQ (FSIQ<85). Mean SCQ and CGI scores of probands and nonprobands combined were significantly different ($p<0.05$) compared to controls. Results show 94% of probands, 30% of nonprobands, and 13% of controls met criteria for ADHD on the CGI and DSM-IV criteria. Symptoms of ASD were confirmed on the SCQ in 60% of probands, 10% of nonprobands, and none of controls. By DSM-IV criteria, 73% of probands, 20% of nonprobands, and none of controls are on the autism spectrum. 80% of probands, 10% of nonprobands, and 6% of controls were being treated with medications, including stimulants, SSRIs, and/or atypical antidepressants. Our results show that the majority of young boys who present to clinic with the premutation have autism spectrum disorders and ADHD. In addition, cognitive deficits and other behavior problems, including anxiety and poor eye contact, are common. Even in premutation boys who do not present to clinic, there is a significant rate of autism spectrum disorders and ADHD. Therefore, it is important for clinicians to carry out DNA testing on all siblings of individuals identified with fragile X syndrome so children with the premutation can be identified, evaluated, and treated for these problems.

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Integrated transcriptional profiling and linkage analysis for disease gene identification in cardiovascular and metabolic disorders. *E. Petretto[1], N. Hübner[2], C.A. Wallace[1], H. Zimdahl[2], H. Schulz[2], F. Maciver[1], M. Müller[1], O. Hummel[2], J. Monti[2], V. Zidek[3], V. Kren[4], T.W. Kurtz[5], S. Patel[1], J. Whittaker[1], M. Pravenec[3], T.J. Aitman[1].* 1) Faculty of Medicine, Imperial College, London, UK; 2) Max-Delbrück-Center for Molecular Medicine, Berlin, Germany; 3) Czech Academy of Sciences, Prague, Czech Republic; 4) Charles University, Prague, Czech Republic; 5) University of California, San Francisco, USA.

The BXH/HXB panel of rat recombinant inbred (RI) strains, derived from Spontaneously Hypertensive Rats (SHR) and Brown Norway parental strains, is the largest RI panel developed for genetic analysis of cardiovascular and metabolic phenotypes. Over 1000 genetic markers and more than 70 phenotypes have been characterized in this panel. We have used this RI panel to identify genes and regulatory pathways underlying complex SHR phenotypes. Gene expression profiles across 30 RI strains were generated in adipose and kidney tissues. mRNA transcript abundance for each of the 15,923 genes on the array was treated as a quantitative trait and used to map cis- and trans-acting modulators of gene expression (eQTLs) to the rat genome. Following removal of redundant linkages (transcripts linked to multiple adjacent markers), around 2700 and 2300 distinct eQTLs (genomewide significance $p<0.05$) were identified in kidney and fat tissues respectively. Around 300 eQTLs were in common in both datasets suggestive of co-regulated genes in different tissues. In the fat dataset we observed that among linkages where marker and probeset can be mapped around 70% are trans-acting. Sequencing of promoter and coding regions is ongoing to identify allelic variants that may underlie phenotypic differences in the parental strains. The cis-acting eQTLs represent a valuable data set of functional positional candidates for the cardiovascular and metabolic traits mapped in the SHR strain. The genes and pathways controlling these phenotypes, including hypertension, insulin resistance and dyslipidaemia may also merit testing as candidate genes for similar phenotypes in human populations.

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Characterising phenotype in genetic studies of blood pressure. *M.D. Tobin[1], N.A. Sheehan[1], S.M. Raleigh[2], N.J. Samani[2], P.R. Burton[1].* 1) Department of Health Sciences, University of Leicester, Leicester, UK; 2) Department of Cardiovascular Sciences, University of Leicester, Leicester, UK.

Maximising the genetic signal in association and linkage studies of blood pressure (BP) is of crucial importance if one is to detect the modest genetic effects that underlie this complex trait. Choices in the design of such studies include: (1) the method of BP measurement; (2) whether to study systolic or diastolic BP, the difference between them (pulse pressure), or the presence or absence of hypertension and; (3) the method of adjustment for antihypertensive treatment that may otherwise distort estimates of genetic effects. Concern about the latter has often resulted in the study of hypertension rather than BP as a continuous trait, and has more recently led to the study of pulse pressure, which may be less affected by treatment than systolic or diastolic BP. We investigated the impact of these choices in simulated data and in an analysis of 547 subjects from an ongoing study of representative UK Caucasian nuclear families, in which ambulatory and conventional BP was measured in parents aged 40-60 and offspring >18 years (the GRAPHIC Study). Statistical analysis was undertaken for these different traits using generalised linear mixed models in WinBUGS 1.4, incorporating different adjustments for treatment, and adjusting for age, sex and smoking. We show results consistent with a greater power to detect the effects of covariates (including polymorphisms in the *PRKWNK1* gene) using mean 24-hour ambulatory systolic and diastolic BP rather than conventional measures of BP. Furthermore, when we adjusted mean 24-hour systolic BP for treatment effects using a semi-parametric algorithm, the impact of these polymorphisms appeared to be greater and the narrow sense heritability estimates (h^2_N) increased from 50.1% to 60.3%, indicative of a greater power to detect genetic determinants. Using mean 24-hour pulse pressure, adjusted h^2_N fell to 49.2%. These findings highlight the importance of ambulatory BP measurement and of appropriate adjustment for antihypertensive treatment effects, but do not support the use of pulse pressure to maximise the genetic signal in studies of BP.

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Multiple essential hypertension susceptibility genes on chromosome 1q. *Y. Chang[1], X. Liu[2], M. Ikeda[1], J. Kim[1], M. Layton[1], R. Cooper[3], A. Weder[4], A. Chakravarti[1].* 1) Inst. of Genetic Medicine, Johns Hopkins Univ., Baltimore, MD; 2) Dept. of Epidemiology and Biostatistics, Univ. of California, San Francisco, CA; 3) Dept. of Preventive Medicine and Epidemiology, Loyola Univ. Chicago, IL; 4) Division of Hypertension, Univ. of Michigan School of Medicine, MI.

Essential hypertension (HTN), believe to be caused by renal dysfunction in sodium balance, is a major predisposing factor to renal failure and cardiovascular diseases. To uncover genes involved in blood pressure (BP) regulation, we performed genome-wide linkage analysis on 1,875 individuals (585 families) collected through the GenNet network of the NHLBI Family Blood Pressure Program. We employed variance components linkage analysis, used systolic BP, diastolic BP, pulse pressure and mean arterial pressure as phenotypes, and analyzed African American and Caucasians separately and combined. We found evidence of linkage in 8 genomic regions with one or more phenotypes. The most significant linkage result (LOD=3.2) is between diastolic BP and the marker D1S1589 (192 cM, 1q25.1) in Caucasians. This region has been implicated by 2 other genome-wide efforts to identify HTN loci: the NHLBI Family Heart Study and the Finnish Twin Cohort Study. Furthermore, 1q21-1q32 is syntenic to a region on rat chromosome 13 and a region on mouse chromosome 1 that contain HTN QTLs. The linkage region spans 43 cM and contains > 200 known genes. Based on expression patterns and known or putative function, we identified 29 HTN candidate genes. We genotyped 16 additional STR markers in the linkage region (intermarker distance < 2Mb) and prioritized the 29 candidate genes based on evidences of linkage and association. We have examined 58 SNPs in and flanking 9 candidate genes and found significant and consistent association in 3 genes: RGS5, ATP1B1 and SELE. Since these genes are not in linkage disequilibrium, it is likely that 1q21-1q32 contains multiple genes that determine BP. Only one of the 3 genes is known to play a role in regulating sodium balance. Therefore, other processes, such as endothelial cell activation and vascular morphogenesis, might also contribute to the pathophysiology of HTN.

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Two-locus non-parametric linkage analysis of essential hypertension: a multidimensional genome scan. *J. Tzenova*[1], *C. Wallace*[2], *R. Dobson*[2], *J. Pembroke*[2], *M. Brown*[3], *D. Clayton*[3], *N. Samani*[4], *A. Dominiczak*[5], *J. Webster*[6], *M. Lathrop*[7], *J. Connell*[5], *P. Munroe*[2], *M. Caulfield*[2], *M. Farrall*[1]. 1) Univ Oxford, UK; 2) Queen Mary's School of Medicine; 3) Univ Cambridge; 4) Univ Leicester; 5) Univ Glasgow; 6) Aberdeen Royal Infirmary; 7) Centre National de Genotypage, France.

Genes that affect a trait through epistatic interactions may have no significant main effects and might not be detected in a single-locus genome scan. We perform the first two-dimensional genome scan in humans to investigate the disease etiology of hypertension. We extend the work of Farrall (1997) to obtain a two-locus non-parametric linkage test for two linked or unlinked disease loci. The method can be applied to affected sib-pairs and half-sib-pairs on genome-scan data and is used to test different genetic models: a general two-locus model, additive and epistatic models, and single-locus tests. The computational challenge of calculating joint two-locus IBD probabilities for genome scan data is solved by using Merlin. We examine the distribution of the two-locus test statistic under the null hypothesis through simulation. The 0.05 alpha threshold for a 2D scan in 100 ASPs falls just below a lodscore of 6. We perform a 2D genome scan using 424 microsatellite markers in a sample of 2000 hypertensive sib-pairs and 73 hypertensive maternal half-sib-pairs from the British Genetics of Hypertension Study (www.brightstudy.ac.uk). A test of the general two-locus model, which includes epistasis, versus a model in which neither locus contributes to hypertension identifies 8 pairs of linked regions. The pairs are composed of 10 independent regions, 6 of which do not show single locus suggestive evidence for linkage (MLS < 1). We perform a detailed investigation of the 8 pairs of regions using a denser grid search and fitting different two-locus genetic models. The peak two-locus lodscore occurs between two linked loci on chromosome 8p23 under an epistatic model. This analysis demonstrates the advantage of performing a multidimensional scan in complex traits to identify novel susceptibility loci, which would not be detected in a single-locus scan.

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Association between the *KLOTHO* KL-VS allele and HDL-C, blood pressure, stroke, and longevity. *H.C. Dietz*[1,2], *G. Atzmon*[3], *A. Arking*[4], *N. Barzilai*[3], *D.E. Arking*[1]. 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Howard Hughes Medical Institute; 3) Institute for Aging Research, Albert Einstein College of Medicine, Bronx, NY; 4) Johns Hopkins University, Baltimore, MD.

We have previously identified a functional variant of *KLOTHO*, termed KL-VS, that is associated with human aging and early-onset occult coronary artery disease. Here we determine whether the KL-VS allele influences cardiovascular risk factors, cardiovascular events, and ultimately, mortality. 525 Ashkenazi Jews (ages 51-107) were genotyped for the KL-VS allele. In concordance with our previous data in Czech individuals (age 79, P0.01), a heterozygous advantage for longevity was observed for individuals 79 years (P0.004). Combined analysis indicates a 1.57-fold (95% CI 1.23-1.98) increased odds ratio (OR) for 5-year survival in two independent populations (P0.0002). Cardiovascular risk factors were assessed through multivariate regression analysis, revealing that KL-VS allele status is an independent determinant of both HDL-C (P0.05) and systolic blood pressure (SBP) (P0.008). History of vascular events was analyzed using logistic regression, indicating that, after adjustment for traditional risk factors, heterozygous individuals were at significantly lower risk for stroke than wild-type individuals (OR=5.88, 95% CI 1.18-29.41), while homozygous KL-VS individuals had the highest risk (OR=30.65 95% CI 2.55-368.00). Mortality data was available for a subset of the cohort (age 95 years), and prospective analyses using Cox Regression indicates that wild-type individuals have a 2.15-fold (95% 1.18-3.91) and homozygous KL-VS individuals a 4.49-fold (95% CI 1.35-14.97) increase in relative risk for mortality, as compared to KL-VS heterozygotes, after adjusting for potential confounders. We conclude that the KL-VS allele of KLOTHO is a significant predictor of longevity in both cross-sectional and prospective studies, and is associated with HDL-C levels, SBP, and stroke.

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A novel Apolipoprotein CIII gene polymorphism is associated with Exceptional Longevity. *G. Atzmon[1], M. Rincon[2], R.B. Lipton[3], N. Barzilai[4], A.R. Shuldiner[5].* 1) Institute for Aging Research,AECOM, New York; 2) Department of Pediatrics, AECOM, New York; 3) Department of Epidemiology and Population Health,AECOM, New York; 4) Head of Institute for Aging Research,AECOM, New York; 5) University of Maryland School of Medicine, Baltimore.

The ability to achieve an extended and healthy life is strongly clustered in families of centenarians, suggesting genetic determinants for longevity. We have previously demonstrated that large particle size of HDL and LDL lipoproteins, and increased HDL levels were features of exceptional longevity. Here we focused on apolipoprotein C-III (ApoC-III), a major component of lipoproteins, as a candidate for the unique lipoprotein profile of longevity. We recruited over 300 Ashkenazi Jews (98.2 (0.36) years [mean (SE)]) their offspring (68.3 (0.46) years), and an control group age-matched to the offspring, and genotyped them for 6 selected ApoC-III SNPs in the promoter region, previously shown to be associated with cardiovascular disease. Strikingly, the frequency of subjects homozygous for the C allele at position -641 was ~2.5 fold higher in centenarians and their offspring compared to age-matched controls (0.25, 0.21 and 0.11, respectively; p=0.001). Plasma levels of ApoC-III were significantly lower in centenarians and their offspring than controls [mean(SE): 9.8(0.6), 9.3(0.5) vs. 11.7(0.7) ug/ml, respectively; p=0.01]. Subjects carrying the 641 CC genotype compare to those carrying the AA genotype had lower plasma levels of ApoC-III [10(1.1) vs. 13 (1.1) ug/ml; p=0.05]. This ApoC-III genotype was associated with the unique lipoprotein profile of longevity i.e. high HDL levels, large HDL and LDL particle sizes, and low triglyceride levels. Analysis of an independently recruited Ashkenazi cohort (117 subjects with AA or AC genotype and 31 with CC genotype) showed that subjects who carried the CC genotype had a significant 3.9(1.1) year survival advantage after age 80 compared with subjects carrying the CA or AA genotype (p=0.02). We suggest that sequence variation in the ApoC-III gene may be implicated in human exceptional longevity through decreased ApoC-III levels resulting in a favorable lipoprotein profile.

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Program Nr: 133 from the 2004 ASHG Annual Meeting

GATA2 and six other transcription factors specific for cis-regulatory elements significantly over-abundant in genes differentially expressed in arthrosclerotic aortas map to linkage regions in the GeneCard study. *J.E. Stenger[1,2], R. Karra[2], D. Seo[2], A. Dorba[3], S.T. Burks[4], H. Xu[1,2], E.R. Hauser[1,2], E. Iverson[3], M. West[3], J.M. Vance[1,2], P.J. Goldschmidt-Clermont[1,2], for the GENECARD and AGENDA Investigators.* 1) Duke Center for Human Genetics,; 2) Department of Medicine, DUMC,; 3) Institute of Statistics and Decision Sciences, Duke University, Durham, NC, USA; 4) Department of Biological Sciences, NC Agricultural and Technical College, Greensboro, NC, USA.

Integrating gene expression and linkage data (genomic convergence) is a useful approach for identifying and prioritizing genes that may contribute to complex diseases. However, mRNA levels do not necessarily correlate with protein levels, metabolite levels or phenotype. Also, inappropriate gene expression is more likely attributable to changes in *cis*-regulatory elements, *trans*-activators, or both, than reflective of mutations directly altering gene function.

To identify TAs that may be causative of aberrant gene expression observed in artheroschlerotic aortas that we hypothesize may contribute to coronary artery disease, we used bioinformatics to push our genomic convergence approach to the next level and take pathway and network data into consideration.

To this end, we identified TAs encoded by genes mapping to linkage regions from the Genetics of Early Onset Cardiovascular Disease (GENECARD) study that were known to bind specifically to regulatory motifs that were statistically over-represented in the 3,000 bp upstream of the coding sequence of genes significantly differentially expressed in artheroschlerotic arteries versus controls. We identified seven genes encoding TAs mapping to regions within linkage peaks with extrapolated lod scores ranging from 1.0 to 4.0 in decreasing order: *GATA2, EGR1, IRF1, AHR, NKX25, HOX13* and *IRF2. GATA2* mapped to a region within a linkage peak with a lod score greater than 3.9 in a multipoint analysis using GENECARD data. We conclude that this is worthwhile strategy that allows us to extend and to re-prioritize our candidate gene list in a logical way.

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Genotype at common variants in C-Reactive Protein (CRP) significantly alters plasma CRP levels in young adults: Results from the CARDIA Inflammation Genomics and Atherosclerosis Prevention (IGAP) Study. *C. Carlson[1], A. Reiner[1], S. Force-Aldred[2], P. Lee[1], M. Rieder[1], K. Liu[3], O.D. Williams[4], C.E. Lewis[4], M. Fornage[5], E. Boerwinkle[5], R. Tracy[6], M. Gross[7], C. Iribarren[8], R.M. Myers[2], D.A. Nickerson[1], D. Siscovick[1].* 1) University of Washington; 2) Stanford University; 3) Northwestern University; 4) University of Alabama at Birmingham; 5) University of Texas, Houston; 6) University of Vermont; 7) University of Minnesota; 8) Kaiser Permanente.

A number of prospective epidemiologic studies have demonstrated that mildly elevated levels of CRP predict future cardiovascular events independently of traditional cardiovascular risk factors. CRP levels were measured at examinations in 1992-3 and 2000-1 for the CARDIA young adult cohort. Single nucleotide polymorphisms were identified by completely resequencing the CRP gene in a variation discovery panel of 24 blacks and 23 whites. No amino-acid changing SNPs were identified in either population. Patterns of linkage disequilibrium between common variants were used to select a subset that describe all common patterns of variation in either population. These tagSNPs were genotyped in the CARDIA cohort (1814 blacks and 1952 whites) and then assessed for associations with plasma CRP at each exam, using regression to adjust for other known environmental and genetic risk factors. Eight common haplotypes were observed. Relative to the population mean plasma CRP level (3.2 mg/L in 2000-1), a haplotype in blacks showed significantly higher CRP (+1.5 mg/L per copy), a haplotype in whites showed significantly lower CRP (-1.5 mg/L per copy) and a clade of haplotypes shared by both populations showed consistently lower CRP (-1.1 mg/L per copy). The changes in CRP levels associated with these haplotypes are consistent at both time points. Each of these haplotypes was tagged specifically by at least one SNP private to the haplotype. In vitro expression analysis confirms that several promoter region polymorphisms associated with these common haplotypes alter promoter function. The common haplotypes described here could prove to be important risk factors for cardiovascular disease.

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Program Nr: 135 from the 2004 ASHG Annual Meeting

Allelic variants of Upstream Stimulatory Factor 1 (USF1)-gene represent a risk factor for cardiovascular events in females in a prospective study. *K. Komulainen[1], M. Perola[1], R. Kilpikari[1], P. Pajukanta[2], J. Saarela[1], K. Silander[1], M. Alanne[1], P. Ellonen[1], S. Kulathinal[3], K. Kuulasmaa[3], V. Salomaa[3], L. Peltonen[1]*. 1) Dept. of Molecular Medicine, NPHI, Biomedicum, 00290 Helsinki, Finland; 2) Dept. of Human Genetics, David Geffen School of Medicine at UCLA, University of California, Los Angeles, California 900957088, USA; 3) Dept. of Epidemiology and Health Promotion, NPHI, Mannerheimintie 166, 00300, Helsinki, Finland.

Upstream stimulatory factor I (USF1) is a ubiquitously expressed transcription factor controlling genes involved in lipid and glucose metabolism. USF1 has been associated with Familial Combined Hyperlipidemia (FCHL), one of the most common dyslipidemias predisposing to a premature coronary heart disease (Pajukanta et al, Nat Genet 2004) and has also been shown to influence features of glucose and lipid homeostasis in an European study (Putt et al, Hum Mol Genet 2004). However, cross-sectional studies do not estimate correctly the population risk for a variant and its public health impact. Here we report the results of a first population-based prospective study on the effect of the variants of the USF1-gene to the timing of cardiovascular events. We explored a possible association between USF1 allelic variants and cardiovascular survival times in a prospective case-cohort study drawn from a population cohort of Finns. Cox proportional hazard model was used to examine timing of cardiovascular events among different USF1 genotype or haplotype carriers. In the analyses female carriers of a risk genotype of the USF1 were found to have 4.3 (95% CI 1.26-14.66, P 0.0196) times increased risk of cardiovascular event. Interestingly, when diabetes and baseline CVD persons were not included in the model, another USF1 SNP genotype showed a 2.5 (95% CI 1.16-5.23, P 0.0196) times elevated risk of a cardiovascular event in females. The results imply that USF1 gene confers to the risk of cardiovascular events at the population level. The finding in females is underlined by the generally known difficulty to clinically identify women with high risk of cardiovascular events, and suggests that genetic profiling of females for the USF1 variants might have clinical relevance.

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Program Nr: 136 from the 2004 ASHG Annual Meeting

Identification of genetic risk factors for atherosclerosis and drug response markers using clinical trials of the cholesterol-lowering drug Pravastatin. *K. Ranade[1], O.A. Iakoubova[2], J.J. Devlin[2], T.G. Kirchgessner[1], Z. Tsuchihashi[1], L.M. Ploughman[1], K.E. Zerba[1], C.J. Packard[3], J. Shepherd[3], F.M. Sacks[4], M.S. Sabatine[4], E. Braunwald[4], T.J. White[2], R.E. Gregg[1], P.M. Shaw[1], N.C. Dracopoli[1].* 1) Bristol-Myers Squibb, Princeton, NJ; 2) Celera Diagnostics, Alameda, CA; 3) University of Glasgow and Royal Infirmary, Glasgow, UK; 4) BWH, Harvard Medical School, Boston, MA.

Morbidity and mortality trials-with their rigorous diagnostic criteria, significant follow-up and large sample sizes-of drugs used to lower cholesterol levels and treat cardiovascular disease present a unique opportunity to identify genetic risk factors for atherosclerosis and genetic determinants of drug response.For this reason, we have begun large-scale genetic studies of patients enrolled in trials of the lipid-lowering drug Pravastatin.As a first step, we evaluated almost 3 million genotypes from 3000 patients enrolled in the Cholesterol and Recurrent Events trial genotyped with 1000 missense single nucleotide polymorphisms in over 500 candidate genes.A selected set of 100 of these thousand SNPs was also evaluated in the West of Scotland Coronary Prevention trial.Using this rich dataset, we replicated several published associations and identified novel genetic risk factors.One example follows.Paraoxonase 1(PON1), an enzyme that resides on circulating lipoprotein particles has been implicated in atherosclerosis in previous studies.A glutamine (Gln)/arginine(Arg) SNP at residue 192 that affects the ability of PON1-bearing HDL particles to protect LDL from oxidation in vitro was significantly associated with stroke (P = 0.002).For Gln/Arg heterozygotes and Arg/Arg homozygotes the odds ratios were 2.7(95% CI 1.28-5.79) and 2.4(1.45-4.05) respectively. When combined with two other published studies, the association between this SNP and stroke was highly significant (P = 0.000001).The attributable fraction was ~40% in this cohort of high-risk Caucasians. Sequence analysis of the PON1 gene from stroke cases revealed two new polymorphisms, an Arg/Stop and Ala/Val at residues 32 and 201 respectively. SNPs in the PON2 gene were not associated with risk of stroke.

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Program Nr: 137 from the 2004 ASHG Annual Meeting

COMPREHENSIVE ANALYSIS OF SNPS IN ENPP1: ASSOCIATION WITH DIABESITY. *CP. Jenkinson*[1,2], *DK. Richardson*[1], *L.. Rodriguez*[1], *J. Schneider*[2], *P. Streng*[1], *R. Arya*[1], *R. Duggirala*[2], *MP. Stern*[1], *RA. DeFronzo*[1], *J. Blangero*[2]. 1) UTHSCSA; 2) SFBR, TX.

The ENPP1 gene encodes an enzyme that interacts with the insulin receptor and attenuates insulin signaling. Previous studies have shown genetic association of the K121Q variant with diabesity and related conditions. ENPP1 is the strongest positional candidate gene at the chromosome 6q23 locus where we previously detected linkage to diabesity in Mexican Americans, from the San Antonio Family Diabetes Study (SAFADS). Therefore, we genotyped all known SNPs within ENPP1 in 429 subjects in whom we detected the initial linkage signal. We chose a comprehensive approach to clearly define what contribution ENPP1 makes to diabesity in this population. Of 133 known SNPs, 27 failed design criteria. Of 106 SNPs genotyped, 80 were polymorphic with minor allele frequency (MAF) > 0.01. Association analyses were performed with a variety of diabesity phenotypes. We used a variance components approach with a fixed effects measured genotype model, to detect association of genotypes with these variables. Of the 80 SNPs examined, 28 had nominal p values less than 0.05 with at least one trait, 9 had p <0.01, and 1 had a p < 0.001 with single traits. Only 3 SNPs were located within coding sequence, two of which (S650S, A835A) were synonymous and one, (K121Q) was non-synonymous and non conservative. The K121Q variant was significantly associated with fasting plasma glucose (FPG) (P=0.043) and the A835A variant was significantly associated with FPG and HDL-cholesterol (P=0.015 and 0.0047, respectively). The most strongly associated trait, with multiple SNPs, was HDL-C, followed by FPG and leptin. The observed distribution of SNP variants was highly non random. We also noted a non linear distribution of MAF with a sharp discontinuity at MAF=0.25. The SNP data were analyzed for haplotype structure and for contribution to the QTL using a novel Bayesian Quantitative Trait Nucleotide approach (BQTN). We detected association of several SNPs in a separate population sample of 680 subjects from the VA Genetic Epidemiology Study.

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Program Nr: 138 from the 2004 ASHG Annual Meeting

Isolation of a gene located on chromosome 5q34-q35.2 conferring risk of type 2 diabetes mellitus. *S.F.A. Grant[1], I. Reynisdottir[1], G. Thorleifsson[1], I. Schmitt[1], M. Martin[2], R. Benediktsson[3,4], J. Vrang[1], J-B. Cazier[1], M.J. Linn[2], S. Dutta[2], A.D. Christ[5], K.E. Amrein[5], V. Emilsson[1], G. Sigurdsson[3,4], U. Thorsteinsdottir[1], V. Gudnason[3], A. Kong[1], M.E. Gurney[1], J.R. Gulcher[1], K. Stefansson[1]*. 1) deCODE Genetics, Reykjavik; 2) Hoffmann-La Roche, Nutley, NJ; 3) Icelandic Heart Association, Reykjavik; 4) Landspitali-University Hospital, Reykjavik; 5) F. Hoffmann-La Roche, Basel.

We previously reported the localization of a susceptibility gene for type 2 diabetes mellitus (T2D) to 5q34-q35.2. The genome-wide scan, using non-obese (BMI<30) T2D patients in Iceland, resulted in linkage with a LOD score of 3.64 ($p=2.12x10^{-5}$) (Reynisdottir *et al*,AJHG 2003;73:323-35). For the subsequent association analysis and LD mapping of the 3.7Mb region corresponding to a drop of 1 in lod score at this locus, a dense set of microsatellite markers were genotyped in an expanded patient cohort together with controls derived from the general population. Across one gene we found haplotype association to the linkage phenotype of non-obese T2D. In a follow-up analysis of the gene, using a set of 66 SNPs, the strongest single point association to unrelated non-obese T2D was found to the common allele of a SNP located within intron 1 ($p\text{-val}=1.3x10^{-3}$;aff allelic freq=0.725;RR=1.39). Functional characterisation of the gene product showed that it has a limited tissue distribution, with highest expression in brain and lower levels in pancreas and kidney. Specific analysis of the pancreas revealed that expression was localised to -cells. Subsequently, knockdown of gene expression with siRNA in a pancreatic -cell line, INS1, resulted in an increase in basal and glucose stimulated insulin secretion. Furthermore, the intron 1 SNP yielded a significant correlation to serum insulin levels ($p\text{-val}=5.8x10^{-3}$) in 274 unrelated non-medicated GAD negative T2D patients. Together, these data demonstrate that this gene plays a role in insulin secretion. In conclusion, we find that a region on 5q34-q35.2 contains at least one gene conferring T2D risk, and its dysregulation suggests an effect on insulin secretion. The identity of the gene and the corresponding data will be presented.

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Inherited variation in mitochondrial DNA and risk of type 2 diabetes. *R. Saxena[1,2], P.IW. de Bakker[1,2], V.K. Mootha[2], K. Singer[1], J.N. Hirschhorn[2,3], M. Daly[2], L. Groop[4], D. Altshuler[1,2].* 1) Department of Molecular Biology, Massachusetts General Hospital, Boston, MA; 2) Broad Institute of Harvard and MIT, Cambridge, MA; 3) Childrens Hospital, Harvard Medical School, Boston, MA; 4) Department of Endocrinology, Lund University, Malmo, Sweden.

Mitochondria play a central role in many disease processes, with rare mutations in mitochondrial proteins causing many metabolic, neurological and muscular disorders. Whether common variation in mitochondrial DNA (mtDNA) plays a primary, causal role in common multifactorial diseases is less clear. We present an analytical framework to comprehensively assess the influence of common mtDNA variants on risk of disease, taking advantage of 1255 publicly available complete sequences. We catalogued all common variation in mtDNA sequences from European, Asian and African samples, and selected minimal sets of tagSNPs that efficiently capture all common variants (>1%) with a minimal coefficient of determination of $r^2 > 0.8$. For comparison, we show that haplotype tagging SNPs used in previous papers about mtDNA (to Parkinsons disease and longevity) capture only 30% and 20% of common variants with $r^2 > 0.8$, respectively. As an initial application of this approach we genotyped 42 mtDNA tags in a panel of 1109 diabetic individuals and 1109 matched controls. We analyzed all common single sites and haplotypes of putative functional SNPs for association to type 2 diabetes, and evaluated the statistical significance using permutation testing. Our best result from a preliminary test of all single sites are two sites conferring greater risk of type 2 diabetes (odds ratio 1.9, 95% CI 1.3-2.8, $P_{nominal} = 6x10^{-4}$, $P_{study-wide} = 0.023$). Our results raise the intriguing possibility that mtDNA variants play a causal role in common forms of type 2 diabetes. More generally, our methodology is valuable for assessing the influence of mtDNA variants on other diseases or quantitative traits, and should increase the power and efficiency of association studies in mtDNA.

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Association of Protein-Tyrosine Phosphatase N1 Gene Polymorphisms with Type 2 Diabetes and Insulin Resistance. *D.W. Bowden*[1], *J.L. Bento*[1], *N.D. Palmer*[1], *J.C. Mychaleckyj*[1], *C.D. Langefeld*[1], *B.I. Freedman*[1], *S.S. Rich*[1], *S.M. Haffner*[2], *J.M. Norris*[3], *R.N. Bergman*[4]. 1) Wake Forest Univ Sch Med, Winston-Salem, NC; 2) Texas Health Sciences Cntr at San Antonio, San Antonio, TX; 3) Colorado Health Sciences Center, Denver, CO; 4) Keck School of Medicine, Los Angeles, CA.

Protein-tyrosine phospatase-1B (PTP-1B), encoded by the PTPN1 gene, negatively regulates insulin signaling by dephosphorylating the phosphotyrosine residues of the insulin receptor kinase activation segment. PTPN1 is located in 20q13; a type 2 diabetes (T2DM) linked region. We have carried out an association analysis of 35 noncoding SNPs spanning the 161 kb genomic region including the PTPN1 gene. Initially these SNPs were assessed for association with T2DM in two independent collections of Caucasians with T2DM and two matching control groups. Consistent and significant evidence for association is observed, especially SNPs spanning the 3-end of intron 1 of PTPN1 through intron 8 (P-values 0.043-0.004 in one case-control set and 0.038-0.002 in a second case-control set). Analysis of combined case-control data increased the evidence of SNP association with T2DM (P=0.005-0.0016). All associated SNPs lie in a single 100 kb haplotype block that encompasses the PTPN1 gene. Haplotype analyses identified a significant difference between cases and controls (P=0.0035-0.0056) with one common haplotype contributing strongly to the evidence for association with T2DM. Odds ratios are approximately 1.3 for risk haplotypes. In addition, PTPN1 was evaluated for association with measures of glucose homeostasis in 811 Hispanic subjects in the IRAS Family Study. Consistent with the function of PTP-1B, all SNPs with minor allele frequencies > 0.1 are significantly associated with insulin sensitivity index (SI; P-values=0.044-0.003) and fasting glucose (P-values=0.029-<0.001); there was no evidence of association with AIR (a measure of -cell function). In haplotype analysis, the diabetes risk haplotype is associated with lower SI and higher fasting glucose (P=0.005 and P=0.00003, respectively). These results suggest that PTPN1 is a significant contributor to T2DM susceptibility and insulin resistance.

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A functional polymorphism in the promoter of phosphatidylinositide 3-kinase p110 gene protects obese juvenile from insulin resistance. *C. Le Stunff[1], D. Fallin[2], PH. Roméo[3], P. Bougnères[1].* 1) Dpt of Pediatric Endocrinology, INSERM 561, Paris, France; 2) Dpt of Epidemiology, Johns Hopkins University, Baltimore, MD; 3) Dpt of Hematology, Institut Cochin, U567, Paris, France.

Through insulin resistance, obesity has become the leading cause of Type 2 Diabetes (T2D) in millions of humans. The degree of insulin resistance varies among obese patients according to environmental and genetic factors that remain almost entirely unknown. Phosphatidyl 3-kinase (PI3-K) is a key effector of the metabolic pathways that determine the insulin sensitivity of peripheral tissues. We studied a common -359 C/T polymorphism located in the promoter of the gene encoding PI3-K p110 subunit. We genotyped two independent cohorts of 358 and 397 obese children. None of these children had T2D and only 11/755 had impaired glucose tolerance. Insulin resistance was quantified using the HOMA index derived from oral glucose tolerance tests (OGTT). We investigated the effect of the -359C/T polymorphism on the relationship between insulin resistance and body mass index (BMI). We observed a strong relationship between BMI and HOMA ($p< 0.001$) as well as between the -359C/T genotype and HOMA ($p< 0.001$). The effect of BMI on HOMA did appear to be different by genotype after adjustment for pubertal stage, age, and sex. The C variant was associated with a near complete protection from insulin resistance. In vitro, we have shown a 40% increase of transcriptional activity of the C variant in transiently transfected Jurkat cells with the two promoter alleles. Our observation is consistent with PI3K being an important determinant of insulin resistance variability in humans.

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A more powerful transmission/disequilibrium test based on haplotype similarity, with application in the family based association study between the Leptin gene and obesity from the NHLBI Family Heart Study. *K. Yu[1], A. Kraja[1], I. Borecki[1], R.H. Myers[2], M.A. Province[1]*. 1) Division of biostatistics, Washington University, St. Louis, MO; 2) Department of Neurology, Boston University, Boston, MA.

Taking advantage of increasingly available high-density single nucleotide polymorphism (SNP) markers within genes and across genome, various types of transmission/disequilibrium tests (TDT) using haplotype information have been developed. A practical challenge arising in such studies is the possibility that transmitted haplotypes have inherited disease-causing mutations from different ancestral chromosomes, or do not bear any disease-causing mutations (founder heterogeneity). To reduce the loss of signal strength due to founder heterogeneity, Yu et al. (Genetic Epidemiol, in press) proposed a sequential peeling procedure in the context of population based case-control studies. Here we extend that approach to family based studies. The new method is applicable to nuclear families with multiple affected sibs, and with ambiguous phase information. Through preliminary simulation studies, we find that the method has the correct type I error rate in a structured population, and higher power than some existing haplotype based TDT. For example, compared to HS-TDT (Zhang et al., Am J Hum Genet, 2003, 73: 566-579), we observe an over 20% power increase in considered simulation scenarios.
As an application, we apply the new method to the association study between the Leptin gene and obesity from the National Heart, Lung, and Blood Institute Family Heart Study. The analysis is based on 20 SNPs within the Leptin gene. The result shows that there is a strong association with the p-value of 0.027 (contrast to an estimated p-value of 0.9 by HS-TDT).

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Physiologic analysis of GDF-3 knockout mice. *C.W. Brown, J.J. Shen.* Dept Molec/Human Gen/Pediatric, Baylor Col Medicine, Houston, TX.

The transforming growth factor beta superfamily is a large collection of critically important signaling molecules. It consists of more than 35 growth factors involved in diverse processes such as cell cycle control, embryonic development, and organogenesis. Growth differentiation factor 3 (GDF-3, also known as Vgr-2) was originally identified through screens utilizing conserved regions present in other superfamily members. Several lines of evidence indicate a possible role for GDF-3 in adipogenesis, including detectable transcript in murine adipose tissue, changes in expression pattern during differentiation of preadipocytes, and upregulation of expression in mice homozygously null for aP2 (or FABP4), a fatty acid binding protein predominantly present in adipocytes. GDF-3 knockout mice were created in our laboratory. These mice were born without overt congenital malformations, fertility remained intact, and life span was normal. The weight curves of GDF-3 knockout mice maintained on a normal diet were indistinguishable from wild type mice. However, protection from obesity was observed when the knockout mice were fed a high fat diet.

Physiologic studies have been undertaken to determine the mechanism(s) by which GDF-3 deficiency results in the diet-induced obesity phenotype. Data was collected from 6 and 12 week-old mice, spanning the transition point of body weight divergence. Preliminary data indicate that there are no significant differences in food intake or the basal metabolic rates of GDF-3 knockout mice relative to wild type mice. However, detailed tissue dissections reveal specific fat depot mass differences, pointing to possible perturbation of adipogenesis or adipocyte function. Obesity is a rapidly growing worldwide problem with an increasingly negative impact on global health. These studies will provide valuable insight into adipogenesis and obesity, leading to a greater understanding of the underlying pathophysiology.

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A GAD2 variant confers obesity resistance. *W.D. Li, C. Dong, D. Li, C. Garrigan, R.A. Price.* Department of Psychiatry, Behavioral Genetics, University of Pennsylvania Medical Center, Philadelphia, PA.

Several research groups have found linkage of an obesity phenotype (body mass index 27 kg/m^2) to a narrowly defined interval in chromosome region 10p21 (e.g., Hager et al., 1998; Price et al., 2001), with the strongest linkage found for a marker (D10S197) in an intron of the GAD2 (Glutamic acid decarboxylase) gene. More recently, GAD2 polymorphisms have been associated with obesity (Boutin et al., 2003), but the association did not account for the linkage signal. To test whether a particular allele might account for the 10p21 linkage in our samples, we carried out the recently developed Genotype-IBD Sharing Test (GIST, Li et al., 2004) in 260 European American nuclear families segregating extreme obesity and normal weight. We found that allele 2 of marker D10S197 (allele frequency = 0.2, located in the fourth intron of GAD2 gene) showed strong associations in both a recessive (p = 0.000004, weighted NPL score = 5.17) and additive model (p = 0.000021, weighted NPL score = 5.37), while another allele, 4, had p = 0.021 and weighted NPL = 3.14 in a recessive model. To verify the GIST result, we made allele 2 of D10S197 unknown, and the single point linkage npl score (GENEHUNTER) for D10S197 dropped from 2.81 to 2.18. Furthermore, we selected the fifteen families yielding the highest npl scores on D10S197 and genotyped 10 SNPs and marker D10S197 all of which are located within the 1.28Mb GAD2 flanking region (from 25.87-27.15 Mb). The D10S197 allele 2 was unlikely to be transmitted to obese offspring (transmitted vs. untransmitted = 1/27, p = 0.000001). A four marker haplotype (rs1443775 -243 D10S197 rs1443798) showed transmitted/untranmitted = 0/15, p = 0.0001. A preliminary analysis of 106 extremely discordant sisters (average within pair weight difference 72.4 kg, 25.7 kg/m^2) found normal weight and thin sisters more likely to be homozygous for allele 2. Since D10S197 allele 2 is associated with normal weight, it could be a protective allele contributing to but not fully accounting for the 10p21 linkage. More studies are needed of GAD2 and its flanking regions.

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Haplotype block structure and haplotype tagging SNP selection are dependent on analytical approach: lessons for complex disease gene mapping. *E. Zeggini[1], W. Rayner[1], R. Hanson[2], B. Mitchell[3], J. O'Connell[3], P. Deloukas[4], L. Cardon[1], M. McCarthy[1], for the Type 2 Diabetes 1q Consortium.* 1) University of Oxford, UK; 2) DAES/NIDDK/NIH, Phoenix, AZ; 3) University of Maryland, MD; 4) Wellcome Trust Sanger Institute, UK.

The characterisation of haplotype blocks and haplotype tagging (ht)SNPs within these blocks is proposed as a shortcut to aid disease gene discovery in complex traits such as type 2 diabetes. The potential utility of blocks is, however, challenged by concerns about their consistency and, hence, applicability across different populations, and by the arbitrary choice of block definition. To fine-map diabetes-susceptibility genes, the Type 2 Diabetes 1q Consortium has densely SNP-typed (median interval: 7kb), seven populations (Amish, Pima, French, Hong Kong, Shanghai, UK, Utah) for a 13Mb region using the Illumina platform. Using data from a ~3Mb subregion (300 SNPs), we have compared block structure as derived using two different block definitions (Gabriel et al, Wang et al) in controls from these seven populations. In addition, htSNPs have been defined to generate the total number of SNPs (within and between blocks) required to capture common variation. Similar patterns of pairwise LD were observed across the populations, with isolates demonstrating higher LD (eg average D in Amish:0.4 vs UK:0.2). Application of different block definitions gave rise to variability in the apparent genomic architecture of the region, both within and across populations. The Gabriel et al method consistently gave rise to fewer blocks and categorised fewer SNPs within blocks [ranging from 33 blocks (159 SNPs in blocks) in the Shanghai population to 46 (219) in the Amish] compared with the Wang et al method [ranging from 52 (242) in the Amish to 60 (236) in the UK populations]. Overlap in the haplotype block method-driven selection of htSNPs ranged from 54% to 95% between the two definitions and varied across populations. In conclusion, differences in analytical approach influence block structure and htSNP definition, with ramifications for the success of LD mapping of complex disease genes.

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Program Nr: 146 from the 2004 ASHG Annual Meeting

Case-control differences in linkage disequilibrium as a tool for gene mapping in complex diseases. *M.G. Hayes*[1], *C.A. Roe*[1], *M. Ng*[2], *L. del Bosque-Plata*[2], *T. Tsuchiya*[5], *X. Wu*[3], *N.G. Ambrose*[6], *E. Yairi*[6], *E.H. Cook*[3,4], *N.J. Cox*[1,3]. 1) Human Genetics; 2) Biochemistry & Molecular Biology; 3) PAAR/PGRN; 4) Psychiatry, Univ Chicago, Chicago, IL; 5) Medicine and Molec Sci, Gunma Univ, Japan; 6) Speech and Hearing Sci, Univ Illinois, Urbana-Champaign, IL.

Powerful multipoint linkage disequilibrium (LD) methods are commonly used to fine-scale map complex phenotypes, but they are too computationally intensive to apply to genome-wide association studies. We present a novel, less computationally intensive method for LD mapping at a genome-wide level. In follow-up studies of fine mapping *NIDDM1* (2q37.3) and positionally cloning *CAPN10* as a type 2 diabetes susceptibility gene in Mexican Americans, we observed more extensive LD in 110 diabetics than in 112 random samples. This difference was readily observed using LD Unit (LDU) plots, simple pairwise measures of LD (e.g. r^2 and D), and estimates of the population genetic recombination parameter (), assessing significance with likelihood ratio ^{2} and permutation tests. The latter two analyses point to the large intron between exons 13 and 14 as the center of this LD difference. These results are consistent with the hypothesis that increased frequency of common, historically-related susceptibility allele(s) in the region reduces the haplotype diversity in cases and increases the degree and extent of LD. To pilot this approach at a genome-wide level we examined the LD patterns in Affymetrix 10K SNP chip data from a linkage study of persistent stutterers of European descent. Case and control sets were constructed by selecting one affected individual and one unrelated unaffected individual from each of 90 families. Genomic regions with case-control LDU and LDU/kb differences correspond to areas showing evidence for linkage, as measured by Kong and Cox LOD and HLOD scores, and/or association, as measured by family based association tests. We are currently investigating these regions at a finer-scale using the methods described above. The development of such a computationally feasible genome-wide multipoint LD mapping approach will greatly aid the identification of susceptibility loci in complex diseases.

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Genetic Structure, Self-Identified Race/Ethnicity and Confounding in the US Population. *H. Tang[1], T. Quertermous[2], B. Rodriguez[3], S.L.R. Kardia[4], X. Zhu[5], A. Brown[6], J. Pankow[7], M.A. Province[8], S.C. Hunt[9], E. Boerwinkle[9], N.J. Schork[9], N. Risch[2]*. 1) Dept Public Health Sci, Fred Hutchinson Cancer Res Ctr, Seattle, WA; 2) Stanford U Medical School, Stanford, CA; 3) U of Hawaii, Honolulu, HI; 4) School of Public Health, U of Michigan, Ann Arbor, MI; 5) Loyola U Medical Center, Maywood, IL; 6) U of Mississippi Medical Center, Jackson, MS; 7) U of Minnesota, Minneapolis, MN; 8) Washington U, St. Louis, MO; 9) Other U.

There has been considerable debate regarding the correspondence between genetically defined population structure and categorizations based on self-identified race/ethnicity in the US population. To address this question, we have analyzed genetic data for 366 microsatellite markers typed uniformly in a large multi-ethnic population sample of individuals as part of a study of the genetics of hypertension (NHLBI Family Blood Pressure Program). Subjects self-identified into four major racial/ethnic groups (Caucasian, African American, East Asian, Hispanic) and were recruited from 15 different geographic locales within the United States and Taiwan. Genetic cluster analysis on the microsatellite markers produced four major clusters, which showed near perfect correspondence with the four self-reported race/ethnicity categories. Among 3,636 subjects of varying race/ethnicity, for only 5, or 0.14%, was their genetic cluster membership different from their self-identified race/ethnicity. On the other hand, we detected only modest genetic differentiation within race/ethnicity group between current geographic locales. Thus, ancient geographic ancestry, as represented by self-identified race/ethnicity, as opposed to current residence, is the major determinant of genetic structure in the US population. Our results also indicate near perfect confounding between population genetic and socio-cultural categorizations.

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Program Nr: 148 from the 2004 ASHG Annual Meeting

Analysis of human population genetic structure and diversity using polymorphic LINE-1 insertions.
D.J. Witherspoon[1], *C.T. Ostler*[1], *E.E. Marchani*[1], *W.S. Watkins*[1], *D.A. Ray*[2], *M.A. Batzer*[2], *L.B. Jorde*[1]. 1) Dept. of Human Genetics, University of Utah, Salt Lake City, Utah; 2) Dept. of Biological Sciences, Louisiana State University, Louisiana.

L1 (*LINE-1*) elements are a large family of mammalian retrotransposons that have been replicating in mammals for more than 100 Myr. We have genotyped 51 unlinked polymorphic *L1* insertions of the active human *Ta L1* class in 317 individuals from 21 populations from sub-Saharan Africa (108 individuals), Asia (54), Europe (85) and the Indian subcontinent (40). Since these genetic markers are essentially free of homoplasy, they allow robust inference of population relationships and demographic history. Results obtained using *L1* markers are compared with those obtained using 100 polymorphic *Alu* insertions genotyped previously in these same population samples. The average heterozygosity for *L1* elements is greater in African populations (0.402) than in the three non-African population groups (range: 0.345 - 0.365). The F_{ST} value for continental population groups is 12%, which is very similar to the value of 14% obtained for 100 *Alu* insertion polymorphisms. We used genetic distance trees to study the relationships between populations. Inferred relationships between the populations are similar for the two data sets, in that populations from the same continent tend to cluster together. The largest distance in the tree is seen between African and non-African populations, with a larger Asian-African distance than the European-African distance. A series of south Indian populations is intermediate between the European and East Asian populations, and the upper-caste Brahmin population has the smallest distance to the European cluster (as seen in previous analyses of *Alu* and other polymorphisms). The root of the </>L1 tree is located within the African population cluster, supporting an African origin of modern humans.
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Program Nr: 149 from the 2004 ASHG Annual Meeting

Genome-wide screening of genomic variation in the human population using array CGH. *A. Sharp[1], D. Locke[1], R. Vallente[2], E. Eichler[1]*. 1) Dept. of Genome Sciences, University of Washington, Seattle, WA; 2) Dept. of Genetics, Case Western Reserve University, Cleveland, OH.

The availability of the human genome sequence has allowed the identification of genomic regions rich in low copy repeats (LCRs) which may represent unstable regions susceptible to chromosomal rearrangement. To determine whether large-scale genomic variation and polymorphisms occur in the human population, we utilized array CGH using ~2400 unique BAC clones with a mean resolution of ~1.4Mb to screen a human diversity panel for submicroscopic genomic variation. This panel comprised 20 individuals of sub-Saharan African, Asian and Caucasian descent. We identified a total of 53 putative loci that showed copy-number variation between human populations. Five of these variant sites were shown by FISH to represent bona fide differences between sub-Saharan African and Caucasian individuals. These verified sites all localized to either pericentromeric or subtelomeric chromosomal regions flanked by intrachromosomal segmental duplications, suggesting these act as mediators of genomic variation. Comparison of sensitivity of array CGH to detect duplications and deletions with FISH results revealed excellent concordance. However, some cases of copy number alteration involved translocation of genomic material to new locations, which could only be defined by FISH. Array CGH technology provides improved resolution as well as high-throughput detection of genomic DNA copy number alterations that may be involved in genome rearrangements. The linked variation of certain genomic loci with specific human populations suggests that array CGH will be increasingly important in understanding the architecture of the human genome with respect to chromosome rearrangements. We are currently conducting further studies to investigate the role of LCRs in genomic variation using a custom array of ~2500 BAC clones targeted specifically to these regions.

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Program Nr: 150 from the 2004 ASHG Annual Meeting

Population differentiation among SNPs associated with common disease. *K.E. Lohmueller*[1,2], *M.M. Mauney*[2], *D.E. Reich*[3], *J.M. Braverman*[2]. 1) Institute for Molecular & Human Genetics, Georgetown University Medical Ctr, Washington, DC; 2) Georgetown University, Washington, DC; 3) Department of Genetics, Harvard Medical School, Boston, MA.

Association studies have consistently identified a handful of common single nucleotide polymorphisms (SNPs) associated with common disease. However, the extent of allele frequency differences among major populations (Africans, Asians, and Caucasians) for these disease SNPs is unclear. Only with the recent advent of large-scale sequencing and genotyping projects describing genetic variation in different populations (e.g. the SNP Consortium, Seattle SNPs, and the HapMap) has it become possible to empirically examine patterns of population differentiation. We gathered allele frequency data from the literature as well as from the SNP Consortium and Seattle SNPs databases for SNPs consistently associated with disease (at least 16 loci), SNPs reportedly associated with disease (at least 8 loci), and SNPs in genes that were reportedly associated with disease (at least 49 loci). To measure population differentiation, we calculated Wright's fixation index (F_{ST}) and compared F_{ST} values from the disease datasets to genome-wide values obtained from 23,083 SNPs throughout the genome. Preliminary results suggest that certain disease SNPs may have elevated levels of population differentiation relative to other loci in the genome. However, additional analyses are being performed to confirm this finding. To our knowledge, this is the largest study of its kind to examine population differentiation of putative disease SNPs. Our results have implications for understanding the evolution of disease loci as well as for the design and interpretation of association studies in different populations.

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Program Nr: 151 from the 2004 ASHG Annual Meeting

The introduction of the lactase persistence mutation into global population. *N.S. Enattah*[1], *A. Trudeau*[1], *V. Pimenoff*[2], *J.K. Seo*[3], *S. Rahgozar*[4], *D. Comas*[5], *S.Q. Mehdi*[6], *T. Sahi*[7], *E. Savilahti*[8], *M. Perola*[1], *A. Sajantila*[2], *I. Jarvela*[9], *L. Peltonen*[1]. 1) Depts of Molecular Medicine, National Public Health Institute & Medical Genetics, University of Helsinki, Finland; 2) Dept of Forensic Medicine, University of Helsinki; 3) Seoul National University Hospital,South Korea; 4) Blood Transfusion Center, Esfahan, Iran; 5) Universitat Pompeu Fabra, Barcelona, Spain; 6) Biomedical and Genetic Engineering Division, Dr A. Q. Khan Research Laboratories, Islamabad, Pakistan; 7) Dept of Public Health,University of Helsinki; 8) Hospital for Children and Adolescents, Helsinki; 9) HUSLAB, Laboratory of Molecular Genetics,Helsinki.

To trace the history of the T-13910 allele specific for the lactase persistence (LP) variant of the lactase gene (LCT) in global populations, we have carried out an analysis of the allelic background of the C/T-13910 variant associated with lactase persistence/nonpersistence in 1567 samples representing 36 populations using 8 SNPs and one deletion-insertion polymorphism . An excellent agreement with the reported prevalence of LP was observed in various global populations. Haplotype analysis revealed that two major haplotypes could be identified to carry the lactase non persistent variant whereas only one major background haplotype was observed in lactase persistent alleles in all global populations. Based on estimated age of the most common recent ancestor for the LP allele (4800-6600 years ago) and the regional prevalence of the haplotypes, a plausible scenario for the history of LP seems to emerge. We propose that the geographic region between Ural mountains, Volga river, and North of Caucasus represents the most likely origin of the LP mutation. From this location this allele was distributed westbound towards Europe, Western Asia and Middle East. This would imply that LP was introduced to Europe by migrations of Indo-European tribes from Asian Steppes, not from Middle East, the region where the farming and dairy practice are supposed to originate. This data provides some basis for understanding the evolutionary history of LP and the role of different forces shaping the present day prevalence of LP in global populations.

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Shared Polymorphism in Chimps and Humans: A Case for Balancing Selection? *J.C. Stephens, M.S. Pungliya, B.A. Salisbury.* Population Genomics, Genaissance Pharmaceuticals, New Haven, CT.

Humans and chimpanzees diverged approximately 4-6 million years ago, making shared retention of ancestral polymorphism highly improbable. An exception to this argument is polymorphism that is maintained by balancing selection, such as the case of shared alleles at human and chimpanzee MHC loci. We have an opportunity to survey the human and chimpanzee genomes for sites of balanced polymorphism, through resequencing efforts conducted at Genaissance Pharmaceuticals. We sequenced 5,275 autosomal genes in order to discover single nucleotide polymorphisms in 79 unrelated subjects from diverse ethnic backgrounds: African-American (20), East Asian (19), European-American (20), Hispanic-Latino (17), and Native American (3). In addition, we also sequenced a single chimpanzee Pan troglodytes and a gorilla Gorilla gorilla. From the human samples, we identified 96,834 SNPs. Of these, 79% also had genotype data for the chimpanzee and 27% had data for the gorilla. We found 812 SNPs (1.1% of those that have data for the chimpanzee) that were heterozygous, and hence polymorphic, in the chimpanzee. Not all such SNPs are likely to be under the influence of balancing selection, since recurrent mutation is almost certainly operating as well. Hence, characterization of several attributes of these SNPs is required to discriminate between balancing selection and recurrent mutation as the source of such SNPs. Among those attributes investigated are their physical clustering, their frequency spectra, their distribution among functional regions, and their haplotypic context. Analysis of the transition to transversions ratios implicates recurrent mutation as one major explanation of the shared polymorphisms. Analysis of the allele frequencies implicates low functional constraint as another factor.

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Program Nr: 153 from the 2004 ASHG Annual Meeting

Signature of selection at evolutionarily conserved non-coding sequences in the human genome. *M. Hawrylycz, J. Wallace, J. Stamatoyannopoulos.* Dept. of Computational Biology, Regulome, Seattle, WA.

Evolutionarily conserved non-coding sequences (CNSs) have been proposed to mark functional elements in the human genome. However, it is unknown to what degree the selective pressures that shaped such sequences remain active in human populations. We analyzed 117 diverse gene domains and identified 289 highly conserved elements. Analysis of data from deep re-sequencing (90 individuals) of these elements revealed a striking reduction in genetic diversity compared with other non-coding sequences, and approximating that of exons. Surprisingly, diversity was not correlated with the degree of overall conservation of individual elements. Moreover, we found the majority of segregating sites to coincide with bases predicted to be under purifying selection, implying the existence of a substantial, mildly deleterious non-coding genetic load of recent origin.

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Program Nr: 154 from the 2004 ASHG Annual Meeting

Comparison of recombination patterns in humans and chimpanzees. *W. Winckler[1,2], S. Myers[3], R. Bontrop[4], D. Reich[1,2], P. Donnelly[3], D. Altshuler[1,2].* 1) Dept. of Genetics, Harvard Medical School, Boston, USA; 2) Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, USA; 3) Dept. of Statistics, University of Oxford, Oxford, UK; 4) BPRC, Rijswijk, Netherlands.

Recent data have shown that much of human recombination is clustered in hotspots (Jeffreys et al, 2001; McVean et al, 2004; Crawford et al, 2004). We utilized the independent recombination histories of chimpanzees and humans to assess the effect of local sequence features on recombination patterns. We hypothesized that since the genome sequences of humans and chimpanzees are 98.6% identical, if recombination patterns were determined by primary sequence alone we would expect to observe hotspots of recombination in chimpanzees at the same sites as we do in humans. To test our hypothesis, pilot studies were performed on six known human hotspots (-globin, DNA3, DNA2, DMB1, DMB2, and TAP2). To further investigate variation in location of hotspots, 20 additional regions spanning 1.7 Mb were evaluated. Regions were sequenced in 3 or 22 Western chimpanzees and 24 humans to obtain dense coverage of SNPs in each species. We designed genotyping assays for the SNPs, and genotyped them in 38 Western chimps or 94 humans. The genotyping data for both species were aligned to the human reference genome and LD patterns were compared for each population. The hotspots were analyzed using coalescent-based methods as described in McVean et al. The significance of the statistics is evaluated by simulation, as is its power and sensitivity to demographic assumptions and SNP ascertainment. The known hotspots are clearly evident in the human samples. In the cross-species comparison, there is no instance where an imputed hotspot in chimps aligns precisely with the hotspot in humans. This suggests that 98.6% sequence identity is insufficient to create a strong correlation in hotspot localization. However, preliminary results suggest that there may be a recombination rate correlation over larger windows. This could address whether factors other than local sequence identity (such as chromatin structure or position effects) might be involved.

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Identifying genotype-phenotype associations by comparing the distribution of genotypes along a phenotypic gradient. *B.A. Payseur[1], A.G. Clark[1], E. Boerwinkle[2], J. Hixson[2], C.F. Sing[3].* 1) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY; 2) Human Genetics Center and Institute of Molecular Medicine, University of Texas Health Science Center, Houston, TX; 3) Department of Human Genetics, University of Michigan, Ann Arbor, MI.

Association testing in the context of a cohort study generally requires explicitly identifying specific genotypic classes and then testing the null hypothesis that the distributions of phenotypes across those classes are homogeneous. Alternatively, in agricultural settings one often sees application of bulked segregant analysis, a method that tests for differences in allele frequency between the upper and lower tails of the phenotype distribution. We generalize this approach to ask whether the distribution of multi-site genotypes is homogeneous along a ranked gradient of phenotypes. Several metrics for quantifying the degree of association are explored, and statistical significance of observed association is assessed by randomly permuting genotypes along the phenotypic array. We evaluate the performance of this method using computer simulations that account for variation in linkage disequilibrium and allele frequencies generated by population history. If a single SNP is responsible for phenotypic effects, the method cannot do better than the test of this single SNP. However, when two or more SNPs influence a trait, the method can show greater power than single SNP tests. In particular, the approach identifies SNPs that influence a trait through epistatic interactions, even if they have no marginal effect on the phenotype.

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Program Nr: 156 from the 2004 ASHG Annual Meeting

Fine mapping of major histocompatibility complex region for susceptibility genes that cause carbamazepine-induced Stevens-Johnson syndrome. *S.I. Hung[1], W.H. Chung[2], H.S. Hong[2], M.S. Hsih[3], L.C. Yang[2], J.Y. Wu[1], Y.T. Chen[1]*. 1) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) Department of Dermatology,Chang Gung Memorial Hospital, Taipei, Taiwan; 3) Department of Neurology, Chang Gung Memorial Hospital, Taipei, Taiwan.

Stevens-Johnson syndrome (SJS) is a serious, acute life-threatening adverse reaction, most often caused by medication. It is characterized by rapidly developing blistering exanthema of macules and target-like lesions accompanied by mucosal involvement and skin detachment. Carbamazepine (CBZ) is a frequent cause of SJS worldwide and is the most common offending drug in the Han Chinese population. We have recently identified HLA-B*1502 allele as a genetic marker for carbamazepine-induced Stevens-Johnson syndrome (CBZ-SJS) (Nature. 2004, 428: 486). To further investigate the genetic susceptibility region, we conducted fine mapping in the major histocompatibility complex (MHC) region in an extended patient population consisted of 56 CBZ-SJS patients and 194 control individuals (93 healthy subjects and 101 carbamazepine tolerant patients). In the 4-Mb MHC region, 211 single-nucleotide polymorphisms (SNPs) and 20 microsatellite markers were selected for genotyping. Forty-six SNPs located in the MHC class I, II and III regions showed significant association ($p< 0.01$), among them six SNPs located between HLA-DRA and HLA-B had strong association (Chi-square=40~50, $p <0.000001$) and one SNP 35kb telemetric to the HLA-B locus demonstrated even greater significance (Chi-square=134; $p <0.000001$). HLA-B*1502 was present in 100% of the CBZ-SJS cases and only 3% of the controls (odds ratio, 3,180; $p<0.000001$). Furthermore, the recombinant map of HLA-C/HLA-B/MICA loci defined the susceptibility locus within 80kb flanking the HLA-B*1502 gene. These data indicate that genes in the vicinity of the HLA-B locus, if not B*1502 itself, participate in the pathogenesis of CBZ-SJS. The high predictive values and sensitivity (100%) of this marker provide a plausible basis for the development of such a test to identify individuals at risk for this life-threatening condition.

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Trimethylation of Histone 3 Lysine 4 is an Epigenetic Mark for Regions that Escape X Inactivation. *A.M. Khalil, D.J. Driscoll.* Depts of Pediatrics and Molecular Genetics & Micro., Univ. of Florida College of Medicine, Gainesville.

It is now estimated that 150-200 genes escape X inactivation (Xi) in somatic cells of human females. These genes are clustered in several discrete regions on the X chromosome. However, it is not currently known how these genes escape Xi. Here, we show that although the human female inactive X chromosome is largely devoid of histone 3 lysine 4 (H3-K4) trimethylation, regions that escape Xi are enriched with this modification. Also, H3-K4 trimethylation, unlike histone acetylation, is restricted to discrete regions on metaphase chromosomes, and is completely absent from constitutive heterochromatin. In contrast to humans, there are only few genes that are known to escape Xi in the mouse. Therefore, we examined mouse female somatic cells with H3-K4 trimethylation to identify candidate regions with genes that escape Xi. We found the mouse female inactive X to have 7 discrete regions, including the pseudoautosomal region, that are enriched with H3-K4 trimethylation. Xi also occurs in males during spermatogenesis. We found the inactive X in mouse male meiosis to become devoid of H3-K4 trimethylation except for 7 discrete regions. Those regions are at the same chromosomal position as the ones we observed on the inactive X in somatic cells of the mouse female, suggesting that regions that escape Xi in females are either 1) marked during meiosis and/or 2) genes that escape Xi in females also escape Xi in male meiosis. We also show here that unlike Xi in females, histone 4 deactylation of the inactive X in male meiosis precedes H3-K9 dimethylation. Also, the inactive X in male meiosis is hyper(di)methylated at H3-K4; however, the female inactive X is devoid of this modification. In summary, we have 1) identified H3-K4 trimethylation as a cytogenetic mark for regions that escape Xi in females and possibly in male meiosis, and 2) shown that the sequence of changes in histone modifications associated with male meiotic sex chromosome inactivation has key differences from those associated with Xi in females, suggesting that the male germ line has developed a different strategy of Xi than the female somatic lineage.

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The majority of chromosome inversions arise independently and act as powerful suppressors of recombination.
N.S. Thomas, B. van Zyl, J.A. Crolla, S. Youings, P.A. Jacobs. Wessex Regional Genetics Lab, Salisbury District Hospital, Salisbury, United Kingdom.

Chromosome inversions are relatively common in humans and a few are so frequent they are considered to be polymorphic variants. The remainder are less frequent and are assumed to have arisen from unique events. We are investigating the origin of chromosome inversions and their effect on recombination during meiosis. Since 1967 we have identified 167 independently ascertained non-variant inversions. The majority had unique cytogenetic breakpoints but there 20 different inversions represented in our data more than once with apparently the same breakpoints; 6 appear unique to our laboratory while 14 have been reported in published seies of inversions. We have used microsatellite analysis to define haplotypes on the inverted chromosome to differentiate between apparently identical rearrangements that originate from a common ancestor, ie identical by descent (IBD), and those that are unrelated and potentially recurring. To date we have tested two inversions that were unique to our region, inv(10)(q21.3q24.1) and inv(13)(q33.2q34) [both in 2 families] and three that were more widely distributed, inv(3)(q21q25), inv(7)(q22.1q34.1) and inv(14)(q24q32)[in 4, 4 and 3 families respectively]. We found four of the five inversions to be IBD; for the inv(14), two families were IBD while the third was unrelated. However, FISH mapping using tiling path clones showed the breakpoints in the third family to be different from the two IBD families, so the inversions are independent but not truly recurring.Using our microsatrellite data to look at the effect of each inversion on recombination, we found no recombinant in any of the five inversions tested. Furthermore, recombination supression included the entire inverted region and also extended for variable distances beyond the inversion, eg for inv(10) over 50Mb/60cM. As these inversions must have passed through a large number of meioses, the extent of haplotype sharing suggests that many inversions have a profound effect on recombination, suppressing it not only within the inversions but forconsiderable distances beyond the breakpoints.

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Breakpoint mapping in apparently balanced de novo chromosome rearrangements: no evidence for consistent loss of chromosome material at the breakpoints. *J. Fantes[1], E. Boland[2], J. Ramsay[1], D. Donnai[2], V. van Heyningen[1], J. Clayton-Smith[2], D. FitzPatrick[1], G. Black[2].* 1) MRC Human Genetics Unit, Edinburgh, United Kingdom; 2) Dept. of Medical Genetics and Regional Genetic Service, St Mary's Hospital, Manchester, United Kingdom.

The birth incidence of de novo apparently balanced rearrangements (DN-ABCRs) is 1in 3700: ~8% are born with serious congenital abnormalities. It has been assumed that these adverse phenotypic effects are due to deletion of chromosome material at the breakpoint, and/or breakpoint disruption of the function of one or more genes. To test this hypothesis we plan to FISH map 100 breakpoints from DN-ABCRs ascertained with various abnormal phenotypes (ophthalmic, craniofacial malformation or learning disability), and a similar number from phenotypically normal DN-ABCRs. The breakpoints are initially localised using the Sanger Centre 1 Mb clone set, and their position is then refined using a contig of BAC/PAC clones from the Ensembl and UCSC Genome Browsers. Overlapping clones or 10-20 kb genomic PCR fragments define the breakpoints further.

To date we have identified 38 breakpoint-spanning clones in 23 DN-ABCRs with an abnormal phenotype: a known gene was disrupted at 10 breakpoints; no genes were disrupted at 16 breakpoints and at a further 12 breakpoints the BP-spanning clone contains one or more genes that may be disrupted. We only found three deletions, one some distance from the breakpoint.

When compared to the pattern of results for 15 DN-ABCRs without a phenotype it suggests that breakpoint disruption of the function of one or more genes is more frequent than significant loss of chromosome material at the breakpoint.

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Program Nr: 160 from the 2004 ASHG Annual Meeting

Analysis of the centromeric regions of the human genome. *K. Rudd, H.F. Willard.* Duke Univ, Durham, NC.

The centromere plays a critical role in chromosome segregation at mitosis and meiosis. Human centromeres are comprised of megabases of satellite DNA, which has been shown to be involved in centromere function by genetic, biochemical, and artificial chromosome assays. Despite the functional importance of satellite, the human genome assembly has largely excluded centromeres. Thus, for each chromosome assembly there exists a centromere gap. We have analyzed the proximal 1 Mb on either side of all centromere gaps (except for the acrocentric short arms) to define the sequence content of these complex regions of the genome. Of the 43 Mb so analyzed, only 14% is comprised of satellite and 4.8% is other satellites; the remaining DNA is consistent with euchromatin typical of chromosome arms, with an average gene density of 2.5 genes/Mb. satellite is based on ~171 bp monomers that are organized in a highly homogeneous higher-order structure or that lack any periodicity and exist in a more divergent monomeric form. Previous experimental data suggest that higher-order satellite is the predominant type in the genome, typically flanked by monomeric. In contrast, > 95% of the ~7 Mb of satellite in the Build 34 genome sequence is of the monomeric type, reflecting the incomplete nature of the current assembly. Phylogenetic analyses of higher-order and monomeric satellite from multiple chromosomes demonstrate that higher-order satellite is evolutionarily distinct from monomeric satellite, likely reflecting the recent emergence of higher-order satellite in the primate lineage. Monomeric satellite is well conserved between chimpanzees and humans (98.2 +/- 1% identical) whereas higher-order satellite is less conserved (93.0 +/- 2% identical), suggesting that the two types of satellite are evolving at different rates. As monomeric satellite is organized in a chromosome-specific manner, centromere-proximal BACs containing these regions of the genome can be used to determine the identity of marker chromosomes. Further analysis of the centromeric regions of the genome will not only elucidate the functional requirements and evolutionary history of satellite, but should also allow true completion of the human genome sequence.

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Packaging of meiotic chromosomes correlated to GC-content, loop size, and recombination rates. *H. Heng*[1,2,3], *J.B. Stevens*[1], *F. Yang*[4], *G. Liu*[1], *S.W. Bremer*[1], *X. Chen*[5], *J. Korenberg*[5], *B. Spyropoulos*[6], *P. Moens*[6], *C. Ye*[7]. 1) CMMG, Wayne State University, Detroit, MI; 2) Department of Pathology, Wayne State University, Detroit, MI; 3) Karmanos Cancer Institute, Detroit, MI; 4) Center for Veterinary Science, University of Cambridge, Cambridge, UK; 5) Cedars-Sinai Medical Center, Department of Human Genetics and Pediatrics, University of California, Los Angeles, CA; 6) Biology Department, York University, Toronto, ON; 7) SeeDNA Biotech Inc, Windsor, ON.

When the cell undergoes a meiotic division, the chromatin forms a series of loops around a proteinaceous structure called the synaptonemal complex(SC). We report a strong correlation between SC representation, GC-content and different recombination rates among chromosomes; and between human, mouse and rat. This led to the discovery that GC-rich areas of meiotic chromosomes formed shorter loops than AT-rich areas and that telomeric loops were smaller than interstitial loops regardless of GC-content. Thus the chromosome architecture is responsible for the different recombination rates between different species and chromosomes, as well as among AT/GC-rich, telomeric and interstitial chromosomal regions. These novel features of meiotic chromosome packaging also provide a structural explanation for the inconsistence between the physical map (the size of mitotic chromosomes determined by the total number of nucleotides) and the genetic map (the length of synaptonemal complex defined by the number of nucleotides and GC-content).

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Development of yeast model system for palindrome-mediated chromosomal translocation. *T. Ohye[1], H. Inagaki[1], H. Kogo[1], B.S. Emanuel[2], H. Kurahashi[1]*. 1) Div Molecular Genetics, Fujita Health Univ, Toyoake, Aichi, Japan; 2) Div Human Genetics, Childrens Hosp Philadelphia, Philadelphia, PA.

The constitutional t(11;22)(q23;q11) is the most frequently occurring non-Robertsonian translocation in human. The breakpoints of the t(11;22) were identified within palindromic AT-rich repeats (PATRRs) on chromosomes 11 (PATRR11) and 22 (PATRR22), suggesting that cruciform structures mediate double-strand-breaks (DSBs) leading to this recurrent translocation. Although PATRR11 was cloned in its entirety, PATRR22 was located within one of the unclonable gaps of the human genome, which makes detailed mechanism far from fully elucidated. We have developed yeast model for t(11;22) using artificial PATRR22 generated from the der(11) and the der(22) of a balanced t(11;22) carrier.We constructed two transgenes; one has a PATRR11 with yeast Trp1 gene, and the other has a PATRR22 with Leu2 gene. For PATRR22, we have liberated the chromosome 22 part from both the der(11) and der(22) PCR products using restriction enzyme, and then fused them to generate a putative original PATRR22. These transgenes were introduced into haploid yeast strains of different mating types separately. The PATRRs were integrated into the yeast chromosome by homologous recombination; PATRR11 into Trp1 locus of chromosome IV and PATRR22 into Leu2 locus of chromosome III, respectively. By mating of these yeast strains, we obtained diploid yeast having each copy of the PATRRs in the chromosomal context. After 36 hours of growth in liquid medium, genomic DNA was isolated. We performed translocation-specific PCR using primer pairs flanking the PATRRs each on chromosome IV and on chromosome III. As a result, we successfully detected translocation-specific PCR products of *de novo* origin. The sequence of the junction fragments indicated that the translocations always occur at the region with microhomology between PATRR11 and PATRR22. It is demonstrated that DSB within the PATRRs followed by single-strand-annealing is the mechanism of the palindrome-mediated chromosomal translocation. This yeast system will give us a lot of clues to the mechanism involved in the translocation.

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The DSCR is not critical for Down syndrome. *R.H. Reeves[1], L.E. Olson[1,2], J. Leszl[3], J.T. Richtsmeier[3].* 1) Dept Physiology, P202, Johns Hopkins Univ Sch Med, Baltimore, MD; 2) University of Redlands, CA; 3) Penn State University, State College, PA.

Trisomy 21 is among the most complex genetic disorders compatible with human survival. Several major features of Down syndrome (DS) are posited to be caused by trisomy of a small subset of chromosome 21 genes located in a Down syndrome critical region (DSCR). The DSCR is defined as the smallest region of overlap between rare individuals who have segmental trisomy 21 and share a given feature of Down syndrome. Although the DSCR concept has dominated the field of DS research for four decades, to date there has been no method for definitive testing of this idea. Among the features mapped to the DSCR are short stature, protruding tongue, facial dysmorphology related to the craniofacial skeleton, and mental retardation of the DS type. Ts65Dn mice have segmental trisomy for the region corresponding to the DSCR plus an additional ~70 genes. These mice have small stature. Quantitative morphological assessment demonstrates anomalies of the craniofacial skeleton, skull and mandible with direct parallels to DS. We used chromosome engineering to create a duplication and deletion of the mouse chromosome 16 segment orthologous to the DSCR. Mice with three copies of the DSCR do not display the craniofacial anomalies seen in Ts65Dn and in DS as predicted, and do have a mild but completely distinct dysmorphology. In contrast, mice trisomic for all of the Ts65Dn segment except the DSCR exhibit a craniofacial phenotype similar to Ts65Dn. These results are contrary to the simplistic predictions of both the DSCR hypothesis and the amplified genetic instability hypothesis, indicating that a new and more sophisticated basis is needed to understand the global effects of trisomy on development.

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Program Nr: 164 from the 2004 ASHG Annual Meeting

The splitting of chromosome bands into sub-bands analyzed by multicolor-banding (MCB) and chromosome stretching. *U. Claussen*[1], *H. Lehrer*[1], *R. Hliscs*[1], *A. Kuechler*[1,2], *A. Weise*[1], *T. Liehr*[1]. 1) Inst Human Gen & Anthropology, Jena, Germany; 2) Department of Radiotherapy,Jena, Germany.

The splitting of chromosome bands into their sub-bands has its implications for the precise mapping of DNA probes at the sub-band level and for the understanding of the chromosome architecture. Surprisingly, there have been nearly no scientific investigations dealing with that process. Here we investigated the hierarchically organized splitting of bands in detail using the multocolor banding (MCB) probe set of different human chromosomes (#5, 6, 18 and 19) hybridized to normal human metaphase and prometaphase chromosomes at the ~850-, ~550-, ~400-, and ~300-band level. The analysis were performed by comparing the disappearance and appearance of pseudo-color bands of the four different band levels. The regions to split first are telomere- and centromere-near. The directions of band splitting towards the centromere or the telomere could be assigned to each band separately. In contrast to the GTG-band ideograms published in ISCN 95 at the 850-, 550-, and 400-band level pseudo-colors assigned to GTG-light bands are resistant to band splitting. GTG-dark bands split into their dark and light sub-bands because inside dark bands light sub-bands appear which are rather resistant to further elongation. This confirms the results obtained by stretching of GTG-banded chromosome 6 published by Hliscs et al. (Cytogenet Cell Genet 79:162-166, 1997). In this respect the nomenclature of the ideograms of GTG-banding patterns for normal human chromosomes should be reassessed. Furthermore, the results indicate to fundamental doubts on the well established concept of chromosome condensation during mitosis which should be replaced by the recently proposed concept of chromosome region-specific protein swelling. Supported in part by the Deutsche Krebshilfe/Mildred Scheel Stiftung fr Krebsforschung (70-3125-Li1) and the IZKF together with the TMWFK (TP 3.7 and B307-04004).

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Program Nr: 165 from the 2004 ASHG Annual Meeting

Chromosome Damage and Changes of Gene Expression in Leukemia Cells Surviving Ultra-short Pulsed Electric Fields. *P. Fox[1], K. Rohrer[1], K. Schoenbach[2], M. Stacey[1]*. 1) Center for Pediatric Research, Norfolk, VA; 2) Center for Bioelectrics, Old Dominion University, Norfolk, VA.

Ultra-short, high intensity, pulsed electric field (nsPEF) applications have emerged from recent advances in electronics. nsPEF exposures are of high electrical intensity with pulse duration in the order of nanoseconds. In different cell lines exposed to nsPEF we found that those derived from leukemia and lymphoblastoid B-cells (LCLs) are sensitive to nsPEF. We hypothesize that cells exposed to nsPEF show evidence of nuclear damage. Our objective is to determine changes in chromatin structure and function in various cell lines exposed to nsPEF. Telomere localization in nsPEF exposed vs unexposed cells was determined by FISH, and quantification of telomere damage by FLOW-FISH. Differences in chromosomal structure in nsPEF exposed vs unexposed cells were estimated by FISH using chromosome 18 and 19 paint probes. Changes in DNA/nuclear matrix interaction were measured by DNA halo formation, and reduction of gene expression. Observation of telomeres by FISH revealed that as the number of pulses increased, the fluorescence signal intensity of telomeres decreased. Individual telomere signals migrated away from the nucleus, which otherwise appeared intact. FLOW-FISH revealed a significant difference in signal intensity between nuclei of exposed vs unexposed Jurkat cells, suggesting telomere damage. Cell lines of non-leukemia origin did not show nuclear or telomere damage. Contrary to FLOW-FISH, chromosomal painting did not reveal changes in morphology as measured by the relative area occupied by each chromosome within each nucleus. DNA halo formation in nsPEF exposed vs unexposed cells suggested that nsPEF exposure disrupted the nuclear architecture. Transcribed genes are associated with the nuclear matrix, and their expression could be disrupted following exposure to nsPEF. We performed microarray analysis on leukemia and LCL cell lines exposed vs unexposed to nsPEF and found a down regulation of 51-53% of 1921 cancer related genes. We plan to investigate the sensitivity of leukemia cell lines to nsPEF, which may be a result of gene interaction with the nuclear matrix.

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A genome-wide survey of segmental duplications that mediate common human genetic variation and their involvement in syntenic rearrangements. *R.A. Ophoff*[1,2], *M.R. Mehan*[1], *M. Almonte*[1], *P.N. Rao*[3], *N.B. Freimer*[1,2]. 1) UCLA Center for Neurobehavioral Genetics, Los Angeles, CA; 2) Dept Human Genetics, UCLA, Los Angeles, CA; 3) UCLA Dept of Pathology and Lab Medicine, Los Angeles, CA.

Recent studies have identified several genomic rearrangements that occur frequently in the general population. Genome-wide surveys of higher order structures predisposing to such common variations in genomic architecture indicate that segmental duplications (SDs) -constituting up to 5% of the genome- may play an important role in generating additional rearrangements and in disease etiology. We previously conducted such a survey and identified >230 loci in the human genome that could mediate common human genetic variation (Mehan et al (2004) *Human Genomics*). Some of these sites harbor common inversions or are associated with diseases characterized by duplications, deletions, or inversions. We have further observed that some of the large blocks of duplicated segments present at these loci are members of families with multiple, almost identical segments occurring on different chromosomes. Comparative analysis between human and mouse of one such family consisting of at least 16 members revealed a specific pattern of syntenic rearrangements. Often, SDs mark the breakpoints of synteny between human and mouse. However, for this family of duplicated segments, in 14/16 cases the presence of an SD did not simply mark a breakpoint of human-mouse synteny but also revealed a continuation of synteny with a region elsewhere in the human genome, which is flanked by an SD from the same family. We are now investigating whether this is a widespread phenomenon, indicating a specific role of individual families of SDs in shaping the human genome.

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Program Nr: 167 from the 2004 ASHG Annual Meeting

Effects of the inversion on chromosome 8p23: Extensive linkage disequilibrium and association to panic disorder and gene expression. *T.E. Thorgeirsson[1], S. Bjornsdottir[1], C. Lopez-Correa[1], N. Desnica[1], H. Oskarsson[2], J.G. Stefansson[3], H. Kolbeinsson[3], E. Lindal[3], M. Steinarsdottir[3], J. Pop Kostic[1], S. Matthiasdottir[1], A. Baker[1], J. Sainz[1], G. Thorleifsson[1], H. Petursson[1], M.F. Frigge[1], H. Stefansson[1], J.G. Gulcher[1], K. Stefansson[1], A. Kong[1]*. 1) DeCode Genetics, Reykjavik, Iceland; 2) Therapeia, Reykjavik, Iceland; 3) Landspitalinn-University Hospital, Reykjavik, Iceland.

There is increasing evidence for a role of genomic rearrangements in complex disorders. We describe studies on a common inversion polymorphism on chromosome 8p23 originally reported by Giglio et al (Am J Hum Genet, 68(4):874-83). FISH measurements indicated that the less frequent form of the inversion was in association to Panic Disorder (PD) in Iceland. Joint analysis of FISH and genotype data identified excellent surrogate markers for the inversion status. Using surrogate markers the association to PD replicated in a larger cohort of Icelandic patients and controls (relative risk ratio of 1.5, p = 5 x 10-4). Genotype analysis also revealed extensive linkage disequilibrium (LD) within the region, most likely arising from the admixture of the different orientations of this ancient genomic rearrangement as recombination is suppressed in heterozygotes. This is also evident from our analysis of recently released SNP data from the Hapmap Project, which also shows that, although there has been some mixing of genetic material, each orientation has over time developed its own distribution of alleles. Furthermore, quantitation of RNA from blood and lymphoblast cell lines shows that the expression of several genes within the inversion region is strongly associated with the orientation of the segment. Thus, our results indicate a role of the inversion polymorphism on 8p23 in the etiology of complex phenotypes. They also clearly demonstrate how genomic rearrangements can influence LD structure in the genome with implications for the interpretation of case-control association data and the origin of LD in general, as inversion polymorphisms elsewhere in the genome could similarly influence the LD-block structure of the human genome.

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A common haplotype in the 5 region of the SCN5A gene is strongly associated with ventricular conduction impairment. *S. Kaab[1], A. Pfeufer[2], C. Bezzina[3], S. Jalilzadeh[2], T. Koopmann[3], S. Perz[4], J. Müller[2], M. Hinterseer[1], C. Huth[5], G. Steinbeck[1], H-E. Wichmann[5], P. Yang[6], D. Roden[6], A. Wilde[3], T. Meitinger[2].* 1) Dept. of Cardiology, LMU University Clinics Grohadern, Munich, Germany; 2) Institute of Human Genetics, TUM Munich and GSF National Research Center for Environment and Health, Neuherberg, D; 3) Experimental and Molecular Cardiology Group, Academic Medical Center, Amsterdam University, NL; 4) Institute of Medical Informatics, GSF National Research Center for Environment and Health, Neuherberg, D; 5) Institute of Epidemiology, GSF National Research Center for Environment and Health, Neuherberg, D; 6) Vanderbilt University School of Medicine, Nashville, TN, USA.

Background: The SCN5A gene encodes the -subunit of the cardiac voltage dependent sodium channel. Coding region mutations cause Brugada Syndrome and other familial conduction disturbances. Recent studies have suggested SCN5A promoter mutations may also contribute to arrythmias.Aim: We investigated the influence of common SCN5A promoter and gene variants on ECG parameters in a central European general population sample. Methods: We genotyped 702 individuals from the population based KORA survey 2000 for 96 SNP markers. Haplotypes were inferred by the Haploview software package.Results: We identified a block of high linkage disequilibrium extending from 10 kb upstream of noncoding exon 1 to 10 kb into intron 1. Within the block the third most frequent haplotype (hap3, AF= 16.8%) was significantly associated with the width of the QRS complex (p=0.0075; QRS 93.4 ms in wt/wt (n=474), 96.3 ms in wt/hap3 (n=201) and 100.8 ms in hap3/hap3 (n=18)). The effect was more attributable to left bundle branch conduction delay (complete LBBB: 3/479 (0.6%) in wt/wt, 7/203 (3.4%) in wt/hap3 and 2/18 (11.1%) in hap3/hap3, OR (hap3 dominant model) = 6.74 (1.81-25.1), p=0.0011). The association was confined to males both with respect to QRS width and LBBB. These data support the concept that variability in channel expression by polymorphisms in the regulatory region of the gene influences cardiac conduction even in unselected individuals from the general population.

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Common Gene Variants in Myocardial Potassium Channel Genes Act Additively to Modify the QT-Interval in the general population. *A. Pfeufer[1], S. Perz[4], J. Müller[1], S. Jalilzadeh[1], J. Hinterseer[2], G. Goppel[2], T. Illig[3], H. Löwel[3], G. Steinbeck[2], M. Näbauer[2], R. Holle[3], H-E. Wichmann[3], S. Kääb[2], T. Meitinger[1]*. 1) Institute of Human Genetics, TU Munich & GSF National Research Center, Munich, Germany; 2) Department of Internal Medicine, Cardiology, LMU University Clinics Grosshadern, Munich, Germany; 3) Institute of Epidemiology, GSF National Research Center, Munich, Germany; 4) Institute of Medical Bioinformatics, GSF National Research Center, Munich, Germany.

AIM: Cardiac arrythmias are frequently caused by delayed repolarization under control of voltage gated potassium channels. Extreme phenotypes are the monogenic long QT Syndromes (LQT1-LQT6). We undertook LD mapping of the genes KCNQ1 (LQT1, 11p15.5), KCNH2 (LQT2, 7q36, 35kb), KCNE1 and KCNE2 (LQT5 and 6, 21q22) and performed association analysis of frequent gene variants with QT-interval in the general population. METHODS: In n=702 population based probands we recorded ECGs and calculated a QT-time corrected for age, sex and heart rate (QTc). Probands were genotyped for 82 SNPs in and around KCNQ1, 107 SNPs in and around KCNH2 and 40 SNPs in the neighboring KCNE1 and KCNE2 genes. LD-structure was characterized by determining D' and r2 and haplotypes were determined statistically. Probands with ventricular pacemakers, complete bundle branch blocks or brady- or tachyarrhythmia were excluded from association analysis. In the remaining probands (n=657) the corrected QTCN-interval was associated with genetic variants by linear regression. RESULTS: The strongest association to the QTc-interval was between two SNP markers in a block in KCNQ1 Intron 1 (AFmin = 0.37, dQTc= +7,0ms, p=0,0072) and in a block in KCNH2 extending from Intron 2 to Exon 15 (AFmin = 0.23, dQTc=+7,2ms, p=0,0049) explaining 1.3% resp. 1.1% of the entire variance of QTc. Both SNPs were in HWE and in complete linkage equilibrium with each other. They were independent with respect to their effects on QTc (p=0.004), their combined effect explaining 2,4% of its variance. CONCLUSION: Common gene variants in the genomic region of the KCNQ1 and KCNH2 genes independently and additively influence myocardial repolarization.

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Linkage to an HLA-DR2 Independent Locus on Chromosome 1q43 in Multiple Sclerosis. *S.J. Kenealy[1], L.A. Herrel[1], Y. Bradford[1], N. Schnetz-Boutaud[1], J.R. Oksenberg[2], S.L. Hauser[2], L.F. Barcellos[4], S. Schmidt[3], M.A. Pericak-Vance[3], J.L. Haines[1].* 1) Vanderbilt University, Nashville, TN; 2) University of California, San Francisco, CA; 3) Duke University, Durham, NC; 4) University of California, Berkeley, CA.

Multiple sclerosis (MS) is a debilitating neuroimmunological and neurodegenerative disease that affects an estimated 400,000 individuals in the United States. Genomic screens have identified numerous chromosomal regions of interest for MS loci. However, with the exception of the HLA locus, studies have failed to consistently identify genes that modulate MS disease expression.

An MS dataset of 186 multiplex families was used to further localize seven non-HLA regions (1q43, 2q32, 9q34, 13q12, 16q23, 18p11, and 19q13) identified in a genomic screen performed by the U.S. and French MS Genetics Groups. SNPs located at ~1 Mb intervals extending >10 Mb to each side of peak genomic screen markers were genotyped. Parametric two-point analyses identified peak LOD scores > 2.0 for regions 1q43 (LOD=2.52) and 19q13 (LOD=2.26). Non-parametric multipoint analyses identified a peak LOD score > 3.0 only for the 1q43 region.

Because 1q43 provided the most promising evidence for linkage after follow-up, data from this region was prioritized for examination using HLA-DR2 as a covariate. Ordered subset analyses (OSA) using the inverse of HLA-DR2 LOD scores generated a LOD score of 4.37 on 1q43 in the subset of families with no linkage to HLA-DR2. Similar results were obtained when negatively weighting families by the number of HLA-DR2 alleles in affected individuals. OSA also substantially narrowed the linkage peak in the 1q43 region to ~6Mb (corresponding to a -2.0 LOD score confidence interval) that contain 42 positional candidate genes.

These results suggest the presence of an independently acting MS locus in the 1q43 region. They also suggest that covariate analysis is a useful approach for narrowing intervals of interest in complex disease gene mapping studies. Analyses of the remaining linkage regions and candidate genes in the 1q43 region are ongoing.

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Migraine Susceptibility: Role for Vascular and Hormonal Gene Variants. *L. Griffiths*[1], *N.J. Colson*[1], *M.P. Johnson*[1], *R.P. Curtain*[1], *S. Quinlan*[1], *J. MacMillian*[2]. 1) Genomics Res Ctr, Sch Hlth Sci, Griffith Univ, Southport, Aus; 2) QLD Clinical Genetics Service,Brisbane, Aus.

Migraine is a multifactorial disorder characterised by headpain, nausea, vomiting, photophobia and often severe, neurological disturbances. Neurotransmitter-related pathways have been the main focus of studies investigating the molecular mechanisms of the disorder. However vascular and hormonal disturbances also occur in migraineurs, as highlighted by alterations in cerebral blood flow and hormonal triggers of migraine, particularly in women. We have recently investigated a number of genetic factors involved in these functions. These studies have provided evidence implicating variants in the methylenetetrahydrofolate reductase (MTHFR) and angiotensin I-converting enzyme (ACE) genes, as interacting determinants of migraine. Specifically, our results indicated that MTHFR acts synergistically with ACE to increase total migraine risk by ~2-fold and migraine with aura-specific risk by ~3 fold.We have also investigated a number of hormonal gene variants. Steroid hormones mediate their activity via hormone receptors, which have a wide tissue distribution. A recent study in our laboratory examined estrogen receptor and progesterone receptor genes for a potential role in migraine. Results of the ESR1 analysis showed a significant difference between 224 unrelated migraineurs compared to 224 controls in both allele frequencies (P=0.003) and genotype frequencies (P=0.008). An independent follow-up case/control study of 520 individuals also resulted in a significant association with regard to allele frequencies ($P=8x10^{-6}$) and genotype frequencies ($P=4x10^{-5}$). Results of the progesterone receptor analysis also showed significant results, both in the first study group, (allele frequencies P=0.02, and genotype frequencies P=0.04), and in the independent follow-up group (allele frequencies P=0.003, and genotype frequencies P=0.02). Furthermore, interaction analysis revealed that individuals who carried at least one copy of both susceptibility genotypes were 3.2 times more likely to suffer from migraine, indicating that these genes appear to act synergistically to increase the risk of migraine.

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Malic Enzyme May be a Causative Locus in Idiopathic Generalized Epilepsy. *D.A. Greenberg[1,2,3], E. Tzilianos[3], S. Marathe[1], M. Durner[1], G. Alvin[2], L. Strug[1,2], D.K. Pal[1,2], I. Klotz[1], E. Dicker[1], E.B. Bromfield[9], S. Shinnar[5], S. Resor[4], J. Cohen[7], S.L. Moshe[6], C. Harden[8], H. Kang[5].* 1) Biostat/Div Statistical Genetics, Columbia, New York, NY; 2) Psychiatry Dept, Columbia Univ, NY, NY; 3) Genome Center, Columbia Univ, NY, NY; 4) Neurology, Columbia Univ, NY, NY; 5) Neurology & Pediatrics, Montefiore Med Cntr, NY, NY; 6) Neurology, Albert Einstein, NY, NY; 7) Beth Israel Hospital, NY, NY; 8) Neurology, NY Hospital-Cornell, NY, NY; 9) Neurology, Brigham and Women's, Boston, MA.

We previously reported linkage of adolescent-onset Idiopathic Generalized Epilepsy (IGE) to D18S474 on chr. 18 (maxlod=5.2) [Durner et al 2001]. We report identification of the likely susceptibility locus. We typed 35 SNPs in 147 IGE patients and 126 controls near D18S474 for case-control (CC) as well as family members for family-based association studies. SNPs centromeric to D18S474 showed significant association with CC and family-based tests ($p<.0001$). The strongest allelic association appeared in the gene ME2 (Malic Enzyme). The original linkage analysis supported recessive inheritance, so we tested for association of the disease on the basis of genotype. SNP homozygotes were highly associated with the disease compared to heterozygotes. We identified a 9 SNP haplotype, located in the ME2 and promoter regions, associated with disease. The frequency of haplotype homozygotes is 35% in cases vs 8% in controls. The OR for epilepsy for this homozygote haplotype is 6.1 (CI: 2.9-12.7). This report represents the second suggested IGE locus based on analysis of families collected specifically for common forms of IGE, and in a genomic region previously identified through linkage analysis. The association evidence suggests that ME2 is a major susceptibility locus for IGE. There are no known channel genes in this region. ME2 is a genome-coded enzyme which localizes in the mitochondria. It may be related to epilepsy through its reported role in GABA synthesis, an inhibitory neurotransmitter; or, localization is mitochondria might suggest a connection to energy metabolism or apoptosis during brain development. (This work supported by grants NS27941, DK31775, MH48858, and NS37466 from NIH).

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Does mutant glucocerebrosidase trigger protein aggregation? *O. Goker-Alpan, K.S. Hruska, B.K. Stubblefield, E. Sidransky.* NSB, NIMH/NHGRI, NIH, Bethesda, MD.

Protein misfolding results in many disease states, including adult-onset neurodegenerative disorders, characterized by the accumulation of intra- or extra-cellular aggregates and cellular damage. A prime example is Parkinson disease (PD), where -synuclein accumulation in Lewy bodies (LB) causes degeneration of the dopaminergic neurons. In Gaucher disease (GD), the inherited deficiency of the lysosomal enzyme glucocerebrosidase (GC), there are rare patients who develop early-onset, L-Dopa-refractory parkinsonian manifestations. Since the neuropathology observed in these cases shares similarities with other parkinsonian syndromes, we hypothesized that protein misfolding might also play a role in Gaucher disease. Immunofluorescence and confocal microscopy performed on fibroblasts from subjects with neuronopathic Gaucher disease showed that GC accumulated in the early endosomes instead of the lysosomes. In order to determine whether this abnormal intracellular processing of GC is due to misfolding, the fibroblasts were then grown at a reduced temperature, which may allow misfolded proteins to be processed and targeted correctly. Culturing the cells at 30C appeared to correct the observed processing defect, and also to increase the residual enzyme activity and steady-state protein levels on Western blots.

To investigate whether mutant GC contributes to protein aggregation in the subset of patients with GD and parkinsonism, brain samples from these subjects and from subjects with PD alone were stained with antibodies against GC and -synuclein. Glucocerebrosidase localized to Lewy bodies only in parkinsonian patients with GC mutations. Additionally, intraneuronal inclusions in which GC and -synuclein co-localized were identified in these subjects. These findings suggest that mutant GC may have a synergistic effect on aggregate formation by interacting with -synuclein and /or by altering degradation through lysosomal dysfunction. Exploring these interactions in vivo will further our understanding of the mechanisms that trigger protein misfolding and cellular damage in adult-onset neurodegenerative disorders.

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Mutations in the Glucocerebrosidase gene contribute to Parkinson's disease in Ashkenazi Jews. *R. Gershoni-Baruch[1,4], H. Rosenbaum[2], J. Aharon-Peretz[3,4].* 1) Institute of Human Genetics, Rambam Medical Center, Haifa, Israel; 2) Department of Hematology and Bone Merrow Transplantation, Rambam Medical Center, Haifa, Israel; 3) Cognitive Neurology and Movement Disorder Unit, Rambam Medical Center, Haifa, Israel; 4) Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel.

Background: An association between Parkinsonism and type 1 Gaucher disease (GD) was previously described. The objective of the present study was to evaluate the relevance of glucocerebrosidase (GBA) mutations to idiopathic Parkinson disease (PD). Methods: A clinic-based case series of 99 Ashkenazi patients diagnosed with idiopathic PD was screened for six GBA mutations (N370S, L444P, 84GG, IVS+1, V394L, R496H). These mutations account for the vast majority of GD causing alleles in Ashkenazi Jews. Seventy four Ashkenazi patients diagnosed with Alzheimers disease (AD) and 1543 Ashkenazi healthy individuals who presented for heterozygous detection of recessive diseases were equally evaluated.Results: Thirty one PD patients [31.3%, 95 confidence interval (C.I.) 22.2-40.4%] carried either one or two GBA mutated alleles. Twenty-three N370S heterozygotes, four 84GG heterozygotes, three N370S homozygotes and one R496H heterozygote were identified. Among 74 AD patient three GD carriers (two N370S heterozygotes and one 84GG heterozygote; 4.1%; 95% CI: 0.0-8.5%) were identified. Among 1543 healthy controls 95 GD carriers (92 N370S heterozygotes and three 84GG heterozygotes) were identified; 6.2%, 95% CI: 5-7.4%) were identified. The number of GD carriers, among PD patients, significantly exceeds that observed among AD patients (OR = 10.8; CI 3.0-46.6; p<0.0001) and normal controls) OR = 7.0; CI 4.2-11.4; p<0.0001). PD-GD carriers tended to younger compared to non-carriers (mean age at onset 60.0+14.2. years vs 64.2+11.7 years p=0.04). Conclusions: Our results suggest that heterozygosity for a GBA mutation predisposes to PD.

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Program Nr: 175 from the 2004 ASHG Annual Meeting

Dopamine system genes and characteristics of mental activity. *M.M. Vanyukov*[1,2,3,5], *B.S. Maher*[1,2,4,5], *B. Devlin*[1,2,3,5], *G.P. Kirillova*[1,5], *R.E. Tarter*[1,3,5], *R.E. Ferrell*[1,2,5]. 1) Center for Education and Drug Abuse Research (CEDAR), Dept. of Pharmaceutical Sciences; 2) Dept. of Human Genetics; 3) Dept. of Psychiatry; 4) School of Dental Medicine; 5) University of Pittsburgh, Pittsburgh, PA.

The risk for substance use disorders (SUD) has been shown to be significantly heritable. The dopaminergic system (DS) has been implicated in the etiology of SUD and its common precursor, attention deficit hyperactivity disorder (ADHD). The genes encoding components of the DS may contribute to the heritability of SUD and ADHD liabilities and the association between them. Using the family-based association test (FBAT), we investigated the association between SNPs in six DS genes and a battery of neuropsychological tests in a sample of 267 males and their parents. The battery was designed to measure mental processes related to SUD and ADHD liabilities. It included the Stroop Color Word Task measuring attentional control of a response (e.g. color naming) while suppressing a more dominant response (word rendering), the Vigilance task (a continuous performance test of attention and impulsiveness), the Porteus Maze test measuring planning ability and impulse control, and the Actigraph measuring motor activity. A total of 19 SNPs in the dopamine transporter (DAT1) and the five dopamine receptors (DRD1-DRD5) genes were genotyped. Robust associations were found between the -521 C/T SNP of the DRD4 gene and each of the measures: a modest association for mean total motor activity (p=0.02) and a strong association for the Porteus Maze impulsive errors score (p=0.0019), Stroop Interference (p=0.00016), and Vigilance Target Hit Rate, THR (p=0.00006). The latter also correlated with the genotype for the -521 DRD4 SNP (Spearmans rho=0.17, p=.04), with the T allele related to higher THR values. THR was also highly predictive of SUD (odds ratio, OR=7.4 [95% CI, 1.8-30.6] p=.006), as well as demonstrated a trend to predict the rate of SUD development (hazard ratio, HR= 3.2 [95% CI, 0.94-11.02], p=.06). No strong associations were observed for the SNPs in the other genes.

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Program Nr: 176 from the 2004 ASHG Annual Meeting

Association of GABRG2 gene polymorphisms with schizophrenia and visual working memory. *J.A. Turunen[1,2], T. Paunio[1], A. Tuulio-Henriksson[3], J. Ekelund[1,3], J. Suhonen[1], T. Varilo[1], T. Partonen[3], J.S. Sinsheimer[2], J.D. Terwilliger[4], A. Jokiaho[3], A. Loukola[1], T. Ylisaukko-oja[1], W. Hennah[1], J. Lnnqvist[3], L. Peltonen[1].* 1) Deparment of Molecular Medicine, National Public Health Institute, Helsinki, Finland; 2) Department of Human Genetics,David Geffen School of Medicine,University of California,Los Angeles, USA; 3) Department of Mental Health and Alcohol Research,National Public Health Institute, Helsinki, Finland; 4) Department of Psychiatry and Columbia Genome Center,Columbia University, New York, USA.

We have identified a region on chromosome 5q as a potential susceptibility locus for schizophrenia in a Finnish family sample. Furthermore, several other studies have also displayed evidence for linkage within the vicinity of this locus. In order to further track putative schizophrenia predisposing genes at 5q31-34, we performed a SNP-based analysis of positional candidate genes. The first stage analysis was performed using 61 nuclear families from the late settlement region of Finland. Two SNPs within the gamma-aminobutyric acid receptor A subunit gamma 2 gene (GABRG2) provided suggestive evidence of association using the family based association test. The analysis was expanded to cover the complete schizophrenia sample containing 449 nuclear families with 967 affected individuals. Multiple SNPs within the GABRG2 gene provided evidence for association, which was further strengthened by a three-SNP haplotype at the 3end of the gene (p = 0.000079).To further study the role of the GABRG2 gene we tested endophenotypes derived from neuropsychological test data of cognitive functioning for which individuals with schizophrenia perform worse than controls. 12 SNPs in the GABRG2 gene were analyzed using a sample of 168 Finnish families originally ascertained for schizophrenia. Of these, 5 SNPs in the 3 end of the gene were associated with visual working memory (p = 0.0298 - 0.0031). The GABA-signaling pathway has been widely speculated to be involved in the etiology of schizophrenia. This study now suggests that the GABRG2 gene affects this etiology putatively through a role in neurocognitive functioning.

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***Maspin* as a gender- and polymorphism-independent fetal DNA marker for noninvasive prenatal diagnosis by maternal plasma analysis.** *Y.K. Tong[1], S.S.C. Chim[1], L.Y.S. Chan[1], R.W.K. Chiu[1], T.K. Lau[2], T.N. Leung[2], C. Ding[3], Y.M.D. Lo[1]*. 1) Departments of Chemical Pathology, and; 2) Obstetrics and Gynaecology, The Chinese University of Hong Kong, Hong Kong SAR, China; 3) Bioinformatics Program and Center for Advanced Biotechnology, Boston University, Boston, MA.

Circulating fetal DNA analysis is a promising approach for noninvasive prenatal diagnosis. Many workers have used Y-chromosomal sequences as fetal-specific markers, which are only applicable to pregnancies with male fetuses. Thus, the development of a gender- and polymorphism-independent fetal DNA marker is long-awaited. We explored the use of epigenetic markers with differential methylation profiles between fetal and maternal tissues for achieving this goal. *Maspin* (*SERPINB5*) has been noted to be expressed in the placenta, but not in adult peripheral blood cells. *Maspin* promoter methylation has been postulated to be involved in determining its tissue-specific expression (Futscher et al *Nat Genet* 2002). Hence, we investigated the methylation patterns of *maspin* in placental and maternal blood cells by bisulfite sequencing. The placental cells were predominantly unmethylated while the maternal blood cells were heavily methylated. Placental demethylation increases with gestational age. We further developed a real-time quantitative methylation-specific PCR assay for detecting placenta-derived unmethylated *maspin* sequences (U-*maspin*) from maternal plasma. Using this assay, U-*maspin* sequences were detected in all 10 and all 6 maternal plasma samples collected during the first and third trimesters, respectively. In women carrying male fetuses, maternal plasma U-*maspin* and *SRY* concentrations showed a similar trend. U-*maspin* sequences were cleared from maternal plasma 24 hours after delivery. Our data therefore demonstrate the feasibility of developing generic fetal epigenetic markers for maternal plasma detection. The first of such markers, *maspin*, may allow the application of plasma DNA-based noninvasive prenatal monitoring, such as for preeclampsia and preterm labor, to all pregnancies. (Supported by the Innovation and Technology Fund and Hong Kong Research Grants Council.).

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Program Nr: 178 from the 2004 ASHG Annual Meeting

Ready detection of paternally inherited fetal point mutations in maternal plasma by the use of size-fractionated circulatory DNA. *Y. Li[1], E. Di Naro[2], A. Vittuci[3], B. Zimmermann[1], W. Holzgreve[1], S. Hahn[1].* 1) University Women's Hospital, University of Basel, Basel, Switzerland; 2) Department of Obstetrics and Gynecology, University of Bari, Italy; 3) Division of Hematology II, University of Bari, Italy.

Introduction: Currently fetal DNA in maternal plasma cannot be used for the reliable analysis of fetal alleles involving point mutations. We have recently shown that a selective enrichment of fetal DNA sequences can be achieved on the basis that fetal DNA molecules are smaller than maternal sequences. We have now examined whether this approach can be used for the detection of three common beta thalassemia point mutations: IVSI-110, IVSI-1, IVSI-6 and codon 39.
Methods: We examined 32 maternal blood samples (12 wks) in a blinded manner. Circulatory DNA was extracted, size-fractionated by gel electrophoresis and PCR amplified using a PNA clamp. The paternal mutant allele was detected by allele-specific real-time PCR.
Results: Our blinded analysis indicated that the presence or absence of the paternal mutant allele had been correctly determined in 28 of the 32 cases examined. One false positive result was scored, and 2 were flagged as uncertain due to the amount of template DNA being too low. 1 result could not be confirmed. A parallel assessment of un-fractionated total circulatory DNA samples resulted in the incorrect evaluation in almost 50 percent of cases.
Conclusions: Our study clearly demonstrates that fetal DNA sequences can be enriched for by size-fractionation, thereby permitting the reliable determination of otherwise masked fetal genetic traits. This method is simple and is easily modified to permit non-invasive prenatal assessment of other disorders involving compound heterozygous mutations.

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Program Nr: 179 from the 2004 ASHG Annual Meeting

Global Gene Expression Analysis of the Living Human Fetus using Amniotic Fluid Supernatant as a Source of Cell-Free mRNA. *P.B. Larrabee[1], K.L. Johnson[1], C. Lai[2], J.M. Cowan[1], U. Tantravahi[3], D.W. Bianchi[1].* 1) Div Genetics, Dept Pediatrics, Tufts-New England Med Ctr, Boston, MA; 2) Nutrition and Genomics Lab, JM-USDA HNRC, Tufts Univ, Boston, MA; 3) Dept Pathology, Women and Infants' Hospital, Providence, RI.

Background: Human fetal development in vivo is difficult to study on a molecular level. Following our discovery of cell-free fetal (cff) DNA in amniotic fluid (AF), we hypothesized that cff RNA in AF could also be used for fetal gene expression analysis. Profiling of human fetuses with microarrays to analyze thousands of genes could provide important information about fetal well-being, disease progression, and normal vs. abnormal development. We aimed to determine if fetal mRNA in the acellular fraction of AF could be used to study large-scale gene expression in living human fetuses, with gestational age (GA) and pathology as variables. **Methods:** We utilized Affymetrix U133A microarrays to determine expression levels of over 14,500 human genes in 5 samples: 4 from pregnant women between 20-32 weeks undergoing amnioreduction for polyhydramnios (2 with twin-twin transfusion (TTT) syndrome and 2 with hydrops fetalis). For a control, we pooled 6 AF samples from 17-week genetic amniocenteses. Data analysis was performed using Microarray Suite 5.0 software. Gene expression in each of the samples from fetuses with polyhydramnios was compared to the pooled 17-week sample. **Results:** On average, 21% of the genes present differed in their expression. For many developmental genes, such as surfactant proteins, keratins and mucins, we observed a clear change in expression level (up to 32-fold difference) with increasing GA. Expression of Aquaporin 1, a water transporter gene, was significantly increased (up to 16-fold) in both TTT samples. Placental gene transcripts were not present in any samples. **Conclusions:** Microarray profiling of cff mRNA in AF is feasible and can detect significant differences in gene expression in fetuses of different GA, with and without disease. Cff mRNA in AF originates from the fetus, not placenta. Microarray analysis of AF has the potential to become an important tool for evaluation of the living fetus.

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Expression of Keratin 8 and TRAIL in Down Syndrome placentae. *S. Klugman, D. Khabele, J. Liang, K. Livne, M. Lopez-Jones, S. Reznik, S. Gross.* Dept OB/GYN, Montefiore Medical Ctr, Bronx, NY.

OBJECTIVE: Previous data from our group has shown that keratin 8 (KRT8) is overexpressed in Down Syndrome placentae in comparison to normal controls. There is evidence that KRT8 may modulate the external apoptotic pathway that is mediated by tumor necrosis factor (TNF) genes. Our goal was to evaluate expression of KRT8 and the TNF apoptotic gene tumor necrosis factor related ligand (TRAIL) in tissue from Down syndrome (DS) placentae in comparison to normal controls. **STUDY DESIGN:** Tissue samples were obtained from euploid placentae (n=8) and Trisomy 21 placentae (n=11) from women who underwent indicated pregnancy terminations between 17 and 24 weeks of gestation through an approved IRB protocol. Western blotting and immunohistochemistry (IHC) were used to analyze the specimens. Quantitative data was analyzed by Student t tests. IHC results were reviewed with a placental pathologist. **RESULTS:** KRT8 was significantly overexpressed (p= 0.008) in the DS placenta specimens, in comparison to euploid placentae. However, a prominent inverse relationship between KRT8 and TRAIL was observed in eight out of the eleven DS samples, a phenomenon that was not observed in the euploid placentae. This result was observed on a cellular level using IHC where both KRT8 and TRAIL were expressed at the outermost portions of the apical membranes of syncytiotrophoblast cells. **CONCLUSIONS:** Aneuploid pregnancies often result in placental dysfunction leading to poor pregnancy outcomes. While histological changes have been previously observed in DS placentae, the present study identifies changes in molecular pathways associated with apoptosis, which is a critical process in normal placental development. Perturbations in TRAIL-related apoptosis in DS placentae may provide additional information as to the underlying cellular regulation in aneuploid placentae.

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Program Nr: 181 from the 2004 ASHG Annual Meeting

Should maternal age remain an indication for invasive prenatal testing? *A. Summers, T. Huang, C. Meier.* Genetics Program, North York General Hosp, Toronto, ON, Canada.

Advanced maternal age (AMA) has been an indication for amniocentesis since the 1970s when ~5% of pregnant women were 35 years. Over recent decades, this proportion has increased and currently, in Ontario, 15% of pregnant women are AMA. If all of these women had amniocentesis, we would detect ~50% of cases of Down syndrome (DS). The introduction of maternal serum screening in the late 1980s has enabled us to use amniocentesis more effectively. With second trimester triple marker screening, which involves alpha fetoprotein (AFP), unconjugated estriol (uE3), human chorionic gonadotrophin (hCG) and maternal age, 65% of DS can be detected for a 5% positive rate. In the Ontario Maternal Serum Screening Program between 10/1993 and 9/2000, 85% of cases of DS from AMA women were detected with a positive rate of 20%. Integrated Prenatal Screening (IPS) combines first trimester nuchal translucency and pregnancy associated plasma protein-A, with second trimester AFP, uE3 and hCG with or without inhibin A into a single risk estimate for DS. Between 11/1999 and 4/2004, 7,574 AMA women were screened using IPS in our program. With a risk cut-off of 1:200, 94% (30/32) of DS cases were detected for a 7% positive rate. The 6% loss in detection with IPS compared to doing amniocentesis on all women in this group, is compensated by the 93% reduction in amniocentesis. Although IPS has missed 2 cases of DS, 6,927 amniocentesis procedures and 346 subsequent fetal losses have been avoided as a direct benefit of the screen. Assuming a cost of C$1,000 for an amniocentesis and counselling, and C$60 for an IPS, IPS has resulted in C$6 million cost saving. Recent studies show that prenatal screening also provides useful information for the early diagnosis of congenital heart defects and adverse obstetric outcomes. Nevertheless, despite the effectiveness of prenatal screening, maternal age remains a major indication for invasive prenatal testing. We believe that it is time to revise the policy of offering amniocentesis based on maternal age in favour of basing invasive testing only on the results of prenatal marker screening.

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Program Nr: 182 from the 2004 ASHG Annual Meeting

The First 100,000 Pregnancies Screened in the First Trimester. *G. Tsukerman[1, 2], I. Kirillova[1, 2], O. Pribushenya[2], N. Gusina[2], G. Krapiva[2], L. Lishtvan[2], N. Venchikova[2], S. Shreder[2], S. Kovalev[2], I. Solovyeva[2], L. Savenko[2], H. Golovataya[2], I. Novikova[2]*. 1) Joint Research Program, Reproductive Genetics Institute, Chicago, IL, USA; 2) Institute for Hereditary Diseases, Minsk, Belarus.

Objective: To assess the effectiveness of first trimester screening by ultrasonography and maternal serum testing (MST) in detecting structural anomalies and aneuploidies of the fetus in the general obstetric population.

Design: In 8 years period 100,000 unselected pregnancies were screened between 10.5 and 13 weeks of gestation. 1.34% of pregnant women were under 18, and 6.1% were aged 35 or older.

Early Pregnancy Failure and Multiple Pregnancy: 2,294 empty sacs and non-viable fetuses and 1012 multiple pregnancies were detected.

Fetal Malformations: 346 cases of fetal anomalies were discovered, resulting in an overall incidence of 1 in 274 pregnancies. Together with routine findings (NTD), the ability to detect a range of rare monogenic malformations in the first trimester was demonstrated. The first trimester detection rate for fetal malformations increased from 21% to 47% during the study period.

Aneuploidies by NT: Learning curve effect explained the increase of the detection rate for trisomy 21 from 40% to 70%.

Aneuploidies by Combined Screening (NT+MST): 94% of trisomy 21 cases were detected at 3.4% of false-positive rate. An overall detection rate for chromosomal abnormalities was 1 in 360 pregnancies in the first trimester.

Pathological Verification of Prenatal Diagnosis: Pathological verification of prenatal diagnosis in the beginning of the study period was performed in the 2nd trimester in 60% of cases while by the end of this period 60% of post abortion autopsies were done in the first trimester.

Conclusions: Today, at least 50% of fetal structural anomalies and 90%of chromosomal abnormalities can be detected in the first trimester in a general obstetric population.

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Evaluation of rapid prenatal diagnosis by QF-PCR in 18.000 consecutive prenatal samples. *V. Cirigliano[1,2], G. Voglino[3,4], A. Marongiu[3], P. Canadas[1], M. Ejarque[1], E. Ordonez[1], M. Massobrio[4], T. Todros[4], C. Fuster[2], M. Campogrande[4], J. Egozcue[2], M. Adinolfi[5].* 1) Molecular Genetics, General Lab Barcelona; 2) Cellular Biology, Universitat Autonoma de Barcelona; 3) Molecular Genetics and Cytogenetics, Promea Turin; 4) Obstetrics and Gynaecology University of Turin; 5) The Galton Laboratory University College London.

The Quantitative Fluorescent PCR (QF-PCR) assay, introduced during the last few years, allows to perform prenatal diagnoses of common chromosome aneuploidies in a few hours after sampling. We report the first assessment of QF-PCR performed on a large cohort of 18.000 consecutive clinical specimens analyzed in two different centers. All samples were investigated using several selected STR markers together with the Amelogenin gene for fetal sexing. Results were compared with conventional cytogenetic analysis.In 17.179 cases, normal fetuses were detected by QF-PCR. No false positives were observed. A total of 800 abnormal karyotypes were detected by cytogenetic analysis, 732 cases were correctly diagnosed by QF-PCR as due to trisomy 21, 18, 13, triploidies, double trisomies and aneuploidies of the XY chromosomes. Two partial trisomies 18q were also identified together with 12 out of 25 cases of chromosome mosaicism. In 14 cases of cell culture failures QF-PCR was the only evidence of fetal X, Y, 21, 18 and 13 chromosome complement. The molecular assay demonstrated overall 93,3% sensitivity detecting 95% of clinically significant chromosome abnormalities. QF-PCR showed 100% specificity with PPV of 100% and NPV of 99.7%. QF-PCR proved efficient and reliable in detecting common chromosome aneuploidies. The assay reaches the purposes of relieving anxiety of most parents within 24 hours from sampling or to accelerate therapeutical interventions in case of abnormal result.Main advantages of QF-PCR are its very low cost, speed and automation, enabling to analyze up to 100 samples per day. In countries where large scale cytogenetic analysis is hampered by its high cost and lack of technical expertise QF-PCR may be used as the only prenatal diagnostic test.

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Program Nr: 184 from the 2004 ASHG Annual Meeting

Maternal cell contamination analysis of 959 prenatal specimens received for molecular diagnostic testing. *E.M. Rohlfs, N. Faulkner, L. Rosenblum-Vos, B.A. Allitto, E.A. Sugarman.* Genzyme Genetics,Westborough, MA.

Contamination of prenatal specimens by either maternal cells or cells from a twin or triplet fetus may result in misinterpretation of molecular analyses. In November 2003, the American College of Medical Genetics (ACMG) published standards and guidelines stating all prenatal specimen types having molecular analyses should be assessed for maternal cell contamination (MCC). From Nov. 1, 2003 to May 31, 2004 we analyzed MCC in 959 prenatal and cordblood specimens received for prenatal diagnoses. Cystic fibrosis, sickle cell anemia and RhD accounted for 43%, 19% and 11% respectively of the tests ordered. The remainder were tested for at least one of 14 other diseases. Maternal and fetal samples were analyzed using ProFiler Plus (ABI) followed by capillary electrophoresis. ProFiler Plus analyzes 9 tetranucleotide repeats on multiple chromosomes and has been validated to detect MCC levels of at least 10%. If less than 2 markers were informative, the samples were also tested using the COfiler system to analyze 4 additional markers. Maternal alleles one repeat smaller than the fetal allele were not considered informative due to the presence of the PCR stutter peak. Contamination levels ranging from 2% to 66% were detected in 5% (16/320) of the amniotic fluid specimens. Reanalysis of cultures derived from 12 of the MCC positive amniotic fluids did not identify MCC. In one case, both MCC and fetal cell contamination (FCC) from a twin was detected. Five of 505 (1%) amniotic fluid cultures were positive for MCC at levels ranging from 4% to 39%. Significant MCC persisted in 3 of the 5 cases when additional flasks were analyzed. Of 62 CVS samples, MCC was detected in one case (1.6%) and FCC from 2 of 3 triplets in another case (1.6%). We detected a single case of MCC in both CVS culture (n=46) and cordblood specimens (n=26). In this sample set, we have identified levels of MCC that would have interfered with appropriate prenatal result interpretation. Our findings provide data quantifying the frequency of MCC in prenatal samples referred for molecular analysis (2.6% overall) and support the ACMG standards and guidelines regarding assessment of prenatal specimens.

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Program Nr: 185 from the 2004 ASHG Annual Meeting

Prenatal and preconceptional screening for fragile X syndrome Experience with 37,000 tests. *M. Berkenstadt*[1], *L. Ries-Levavi*[1], *B. Goldman*[1], *M. Spiegel*[1], *L. Gerard*[1], *H. Cuckle*[2], *G. Barkai*[1]. 1) The Danek-Gertner Institute of Human Genetics, Sheba Medical Center, Tel-Hashomer, Israel 52621; 2) Reproductive Epidemiology, Centre for Reproduction, Growth and Development, School of Medicine, University of Leeds, 26 Clarendon Road, Leeds, LS2 9NZ, UK.

Fragile X syndrome is the most common cause of inherited mental retardation and the discovery in 1991 of the molecular basis for the disorder made routine population screening feasible. Since early 1994 the Danek Gertner Institute of Human Genetics has offered both pre-conceptional and prenatal screening to women with no known family history of fragile X syndrome. Women applied for testing on their own initiative or on the advice of their physician. Prenatal diagnosis is offered to those with a full or pre-mutation (FM or PM). By December 2003 a total of 37,446 women had been tested. 34,014 women had no family history of mental retardation and/or learning disorder and we will focus only on this sub-group. Using the cut-off of 55 CGG repeats for PM, the overall carrier frequency In Israel was found to be 1 in 154 (1 FM and 220 PM). Prenatal diagnosis was carried out in 284 pregnancies of carriers. 47% of the fetuses received the normal allele and 53% the mutated one. Sixteen fetuses had an FM and all of these pregnancies were terminated. Expansion to FM occurred in 10% of fetuses receiving the mutated allele. Owing to the high PM rate in our population, we conclude that screening for fragile X syndrome among women of reproductive age should be more widely offered.

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Program Nr: 186 from the 2004 ASHG Annual Meeting

Perceptions of the risks of herbal, over-the-counter, and prescription medications in pregnancy. *N.D. Fernandes, J.M. Friedman.* Medical Genetics, University of British Columbia, Vancouver, BC, Canada.

Although herbal medicines are widely used and generally believed to be safe, there have been few studies of possible teratogenic effects related to herbal treatments during pregnancy. Given the popularity of herbal medicines, the fact that risk perception may govern behaviour and the fact that many herbs are pharmacologically active and could pose a risk to the developing fetus, we undertook this study to evaluate the perceptions of pregnant women and their health care providers on the use of herbal treatments during pregnancy. We surveyed 210 pregnant women, 32 physicians, and 12 midwives to compare their perceptions of the risks related to treatment during pregnancy with herbal, over-the-counter (OTCs) and prescription medications. The medications included in the questionnaire belonged to one of three groups: anti-emetic, sedative or anti-inflammatory agents. Pregnant women generally perceived the risks associated with OTC and prescription medicines to be higher than the health care providers did. The magnitude of the risk perception of all groups of respondents was high for many OTC and prescription medicines that have been shown by scientific study to be relatively safe when used in pregnancy. All three respondent groups perceived many herbal treatments as low risk despite the lack of scientific evidence to support this conclusion. Our results highlight the lack of awareness of the potential risks of herbal medicines amongst pregnant women and their health care providers. There is a definite need for scientific research evaluating the safety and efficacy of herbal medicines. Having information on the safety or risks of herbal treatments in pregnancy is important for patient counselling.

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Molecular analysis of the SHOX gene in a series of 100 patients with short stature. *C. Huber[1], S. Ipsas-Jouron[2], M. Rosilio[2], C. Salaun-Martin[2], A. Munnich[1], V. Cormier-Daire[1].* 1) Department of Medical Genetics, Necker Enfants Malades Hospital, Paris, France; 2) Lilly France, Paris, France.

Leri-Weill Dyschondrosteosis (LWD) is an autosomal dominant form of mesomelic dysplasia associated with short stature and Madelung deformity. LWD has been ascribed to large scale deletions and point mutations of the SHOX gene on the pseudoautosomal region 1 of the X and Y chromosomes (PAR1). We report here the molecular analysis of SHOX in 100 patients (63 females and 37 males) included in GeNeSIS, the International observational study conducted by Eli Lilly and Company. They all presented with short stature (-2.0 to -2.5 SD). Madelung deformity was clinically present in 28/100 patients. The molecular study included the segregation of three extragenic microsatellite DNA markers of PAR1 (CASHOX, DXYS233, DXYS234) and two intragenic markers (GASHOX, CTSHOX). When a deletion of the SHOX gene was excluded by this analysis, direct sequencing of the six SHOX exons was performed. We found SHOX abnormalities in 28/100 patients (4 point mutations, 24 deletions). Among the 28 patients presenting with LWD, SHOX abnormalities were observed in 12 cases (42.8%) including one point mutation, one partial deletion (for DXYS233, suggesting a positional effect) and 10 deletions of the PAR1 region. Among the 72 patients with isolated short stature, SHOX abnormalities were observed in 16 cases (22%): three point mutations, one partial deletion (of the intragenic CTSHOX repeat) and 12 deletions of the PAR1 region. This incidence is higher than previously reported in patients with idiopathic short stature. However, an extensive radiological assessment of the 72 patients with no clinical evidence of LWD needs to be performed. These results show that partial deletions of SHOX may account for a fraction of LWD and emphasize the importance of studying intragenic microsatellite DNA markers. In addition, the observation of SHOX anomalies in short patients with no clinical evidence of LWD (especially in young children) illustrates the phenotypic variability of LWD and the importance of long-term follow up and repeated radiological assessment.

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Program Nr: 188 from the 2004 ASHG Annual Meeting

Microduplication 22q11.2 causes isolated cognitive and/or behavioral disability. *M.J. Somerville, W. Morrison, J. Christiansen, M. Lilley, K.A. Sprysak, H. McDermid, M. Hicks, R. Tomaszewski, B.G. Elyas, S.M. Haase, L.M. Vicen-Wyhony.* Dept Medical Genetics, Univ Alberta, Edmonton, AB, Canada.

Intellectual disability (ID) has a prevalence of about 1/100, but the cause is unknown in more than half of all cases. Chromosome 22q11.2 duplications have been reported in individuals with a range of features including palate and urogenital abnormalities, heart defects, hearing loss, and dysmorphia, with cognitive and/or behavioral defects (ID). Expression of these features has been variable, with a wide range of severity. The most consistent finding has been some degree of intellectual disability, indicating that 22q11.2 duplications could contribute to a significant proportion of ID cases. A real-time PCR array was used to measure relative dosage of 2 genes (HIRA and COMT) at the chromosome 22q11.2 locus in 98 unrelated females with ID. Females were selected to reduce the contribution of X-linked conditions to this analysis. These cases were drawn from a patient population that had been referred for fragile X syndrome testing, with no predefined referral criteria. All selected cases did not have an FMR1 expansion. A total of 2 of these 98 cases (2%) were found to carry a 22q11.2 duplication. No 22q11.2 duplications were detected in 160 normal unrelated females ($p < 0.04$). One patient has numerous abnormalities including heart and hearing defects, in addition to a cognitive deficit. However, the other patient has no clinical features other than Attention Deficit Hyperactivity Disorder (ADHD). This individual is a mother of 2 male children which were subsequently tested and were also found to carry the 22q11.2 duplication. One of these children has a significant cognitive deficit and the other has marked behavioral problems. Both have no other clinical features and were previously referred for fragile X syndrome testing. In summary, we have found that approximately 2% of females with unexplained cognitive or behavioral defects carry duplications of genes at the 22q11.2 locus which segregate with isolated ID. This is similar to the fragile X syndrome positive rate, and may warrant consideration for routine screening of idiopathic mental retardation referrals.

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22q, IRF6 and FGFR1 deletion study in a series of consecutive newborns with cleft lip and palate. *T.M. Felix[1], B.M. Riley[1], N. Miwa[1], E. Wettig[2], G. Montalvo[3], M. Jewtuskyk[4], S. Rueda[5], I.M. Orioli[6], E.E. Castilla[7], J.C. Murray[1].* 1) Department of Pediatrics, University of Iowa, Iowa City, IA; 2) Hospital Puerto Montt, Puerto Montt, Argentina; 3) Hospital Carlos Andrade Marin, Quito, Ecuador; 4) Hospital Narciso Lopez, Buenos Aires, Argentina; 5) Maternidade Natalio Aramayo, La Paz, Bolivia; 6) Department of Genetics, Universidade Federal do Rio de Janeiro, Brazil; 7) ECLAMC, Latin American Collaborative Study of Congenital Anomalies, Instituto Oswaldo Cruz, Brazil.

Cleft lip and palate (CL/P) is one of the most common birth defects in humans. It is multifactorial and many genes have been implicated to this malformation. The critical region at 22q11.2 can be deleted in cases of CL/P and point mutations or deletions in the genes IRF6 and FGFR1 can also result in clefts as part of the phenotype. We monitored 139,921 consecutive births in 38 hospitals from 7 countries in South America during the year 2003. 194 CL/P patients were born and we collected blood for DNA and karyotype from 108 newborns. 73 % was isolated cases and 27% at least one other anomaly was associated. Quantitative RT-PCR was performed for deletions on 22q and in the IFR6 and FGFR1 genes with the probes for UFDL1, exon 4 and exon 9, respectively. The preliminary results showed three cases of 22q, two cases of IRF6 and one case of FGFR1 were positive for deletions. All the cases have normal banded karyotypes. The phenotype of two out of the three cases with 22q and all IRF6 deletion were isolated CL/P. One 22q deletion, in addition to CL/P, died at 5 days of age with a complex cardiac anomaly. The FGFR1 deletion case had a brother with CL/P, microphthalmia, coloboma of the iris and pre auricular tag. Additional FISH and Southern blot and family studies are underway to confirm the deletions. RT-PCR can provide rapid assessments of deletions and small deletions appear to underlie a substantial portion of unselected cases of CL/P.

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Mutations in SOX2 cause Rogers Syndrome (Anophthalmia, Tracheo-Esophageal Fistula and Genitourinary anomalies). *D.R. FitzPatrick[1], A. Magge[2], Z. Fiedler[3], P. Turnpenny[4], A. Schneider[5], M. Messina[6], A. Hever[1], K.A. Williamson[1], V. van Heyningen[1]*. 1) Dept Cell & Molecular Gen, MRC Human Genetics Unit, Edinburgh, Lothian, United Kingdom; 2) Regional Genetics Service, Belfast City Hospital Trust, Belfast, United Kingdom; 3) Department of Medical Genetics, University Hospital Hradec Kralove, Czech Republic; 4) Institute of Biomedical and Clinical Science, Peninsula Medical School, Exeter, UK; 5) Dept of Clinical Genetics, Albert Einstein Medical Center, Philadelphia, USA; 6) Section of Pediatric Surgery, Department of Pediatrics, Obstetrics and Reproductive Medicine, University of Siena, Italy.

In 1988 Rogers reported a 11-month-old child with bilateral anophthalmia, tracheo-esophageal fistula and hypospadias. Since then nine very similar cases have been reported, all of which have been sporadic. *SOX2* is a highly-conserved transcription factor that shows site- and stage-specific expression in the developing brain and eye. Recently we identified heterozygous *de novo* , loss of function mutations in *SOX2* as a major cause of severe bilateral eye malformations. On reviewing the published expression data on *SOX2* we noticed it was highly expressed in the developing esophagous in chick embryos. We screened DNA samples from two cases with Rogers syndrome using DHPLC analysis (Transgenomic) and have demonstrated heterozygous, probable loss of function mutations in *SOX2* . The first mutation (R74P) was a non-conservative amino acid substitution in the middle of the HMG domain that would be predicted to ablate the DNA binding. The second mutation (Q55X) was predicted to truncate the protein at the beginning of the HMG domain. In both cases the parents sequence was clearly wild-type. These data strongly support a partially redundant role for *SOX2* during canalisation of the trachea and esophagus during human development. Why don't all embryos who are haploinsufficient for *SOX2* have the same major malformations? To date, severe eye malformations have been seen in all cases. This may reflect a bias in case selection and it would be useful to sceen a large cohort of isolated tracheoesophageal fistula cases.

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Program Nr: 191 from the 2004 ASHG Annual Meeting

Revisiting the Craniosynostosis-Radial ray hypoplasia association: Baller-Gerold syndrome caused by mutations in RECQL4 helicase gene. *L. Van Maldergem[1], A. Megarbane[2], A. Siitonen[3], M. De Roy[1], C. Fourneau[1], N. Jalkh[2], E. Chouery[2], Y. Gillerot[1], A. Verloes[4].* 1) Ctr Genetique Humaine, Inst Pathology & Genetics, Loverval, Belgium; 2) Service de Genetique, Faculte de Medecine St Joseph, Beyrut, Lebanon; 3) Department of Molecular Medicine, National Institute of Public Health, Helsinki, Finland; 4) Centre de Genetique Humaine, CHU du Sart-Tilman , Liege, Belgium.

Some time ago, we described independently two unrelated patients with Baller-Gerold syndrome (J Med Genet 29:266-68,1992;Clin Dysmorphol 9:303-6, 2000) which is a rare autosomal recessive disorder associating radial hypo/aplasia and craniosynostosis (OMIM 218600). Similarities between our patients and those reported with the anecdotal association of Rothmund-Thomson syndrome (OMIM 268400) with radial-ray hypoplasia, or, more recently with RAPADILINO syndrome (OMIM 266280) are striking. These two conditions may be caused by mutations in RECQL4. Therefore, we decided to look for disease-causing mutations in our families, apart from the reported patient. In the first family where the index patient was already reported, two subsequent affected pregnancies were terminated and the parents decided, after ultrasound detection of upper limb shortening during second trimester of a fifth pregnancy to maintain it. An affected baby girl was born. Her clinical course indicated stunted growth and poikiloderma. Compound heterozygosity for a R1021W) and g.2886delT frameshift mutation of exon 9 (a classical RTS mutation) were found.In the second family, the affected patient was found to harbour a homozygous splice site mutation (IVS17-2A>C) determining a set of different transcripts. Our results suggest that Baller-Gerold may arise from RECQL4 mutations and can be integrated in a spectrum of clinical manifestations that includes Rothmund-Thomson syndrome and RAPADILINO syndrome. Multiple malformation syndromes where craniosynostosis and/or radial ray aplasia and/or poikiloderma are a component should be investigated for RECQL4 mutations when no obvious other cause could be demonstrated.

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Barth Syndrome (x-linked cardiac and skeletal myopathy, neutropenia and organic aciduria): rarely recognised, frequently fatal. *R.A Newbury-Ecob*[1], *R.P. Martin*[2], *A.M. Hayes*[2], *M.M. Williams*[3], *L.A. Tyfield*[3], *C.G. Steward*[4]. 1) Clinical Genetics, Royal Hosp Child, Bristol, UK; 2) Paediatric Cardiology, Royal Hosp Children, Bristol, UK; 3) Molecular Genetics Unit, Southmead Hospital, Bristol, UK; 4) Paediatric Haematology, Royal Hosp Child, Bristol, UK.

Barth syndrome (BTHS) is an X-linked disorder characterised by cardiac and skeletal myopathy, severe and cyclical neutropenia, early growth retardation and excessive excretion of urinary organic acids (notably 3-methylglutaconic acid). Caused by mutations in the G4.5 (TAZ) gene at Xq28 leading to defective cardiolipin metabolism (L4-CL), BTHS mimics respiratory chain disease since L4-CL is a common species in mitochondria. Mortality is high due in early life to fulminant cardiac failure and/or severe sepsis and in adolescence or beyond to unexplained arrhythmia. Some pedigrees contain an excess of early and late fetal death. Only 50 affected families were documented worldwide by November 2002 and BTHS is therefore thought to be rare. We challenge this view, however, being aware of 10 unrelated families affected by BTHS in South-West England and South Wales alone. All had cardiomyopathy, intermittent neutropenia, organic aciduria. TAZ gene mutations were identified in all cases. At least 10 males are thought to have died of the disease in recent generations of these families. The commonest presentation is with an acute and unpredictable cardiomyopathy in the first decade, usually in infancy. This is easily mistaken for viral cardiomyopathy, especially as neutropenia may suggest viral bone marrow suppression. Other presentations include motor delay, myopathy, neutropenia and recurrent sepsis. Infection, which presents a particular threat because of the extra load placed on a failing heart, may be prevented by administration of G-CSF. Hence BTHS patients may present to a number of paediatric specialities. We propose that lack of awareness of BTHS has led to underdiagnosis of the condition. The genetic implications for families and potential for treatment make early diagnosis vital. We believe that BTHS should be recognised as an important cause of sudden infant death in boys.

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Further Delineation of Genotype-Phenotype Correlations in Tuberous Sclerosis Complex. *A. Williams, K.-S. Au, H. Northrup.* Ped/Medical Gen, Univ Texas-Houston Medical Sch, Houston, TX.

Tuberous sclerosis complex (TSC) is an autosomal dominant neurocutaneous disorder marked by hamartoma growth in multiple organ systems. Significant dermatologic, renal and neurologic morbidity are frequently observed in affected patients. The majority (70-80%) of affected individuals have identifiable mutations in either the TSC1 gene or the TSC2 gene. Past efforts to determine genotype-phenotype correlations have been hampered by marked allelic heterogeneity, limiting the ability to provide prognostic information. Previous genotype-phenotype studies conducted primarily on sporadic cases suggested increased morbidity from neurological and renal lesions associated with TSC2 mutations. We describe our analyses of genotype-phenotype patterns for 490 sporadic and familial TSC individuals. Through SSCP and direct PCR sequencing, subtle mutations were identified in the TSC1 gene (22%) and TSC2 gene (55%) leaving 23% TSC-affected individuals with no mutation identified (NMI). Almost all TSC1 and 70-80% of TSC2 mutations are predicted to prematurely terminate translation. Presence or absence of clinical symptoms most commonly observed in TSC patients were analyzed according to a) gene loci mutated, b) missense or protein truncating mutation, c) familial or sporadic TSC. Chi-square analyses yielded several findings. Individuals who have TSC2 mutations tend to have more symptoms in all major organ systems (facial angiofibromas, subependymal nodules, renal angiomyolipomas and cysts, and mental deficiency) with ungual fibromas more prominent in individuals with TSC1 mutations. Individuals who have protein truncation mutations were more likely to have facial angiofibromas and ungual fibromas while individuals who have missense mutations were more likely to have Confetti skin lesions and cerebral white matter radial migration lines. The general trends noted above held true for both familial and sporadic TSC patients. This study provides further information regarding genotype-phenotype correlations in a highly unpredictable genetic disease.

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Expanding the phenotype of otopalatodigital syndrome spectrum disorders in females. *S. Robertson[1], T. Morgan[1], S. Aftimos[2], A. Fryer[3], O. Quarrell[4].* 1) Dept Paediatrics, Otago Medical School, Dunedin, New Zealand; 2) Northern Regional Genetics Service, Auckland, New Zealand; 3) Regional Genetics Service, Alder Hey Children's Hospital, Liverpool, UK; 4) Regional Genetics Service Sheffield Children's Hospital, Sheffield, UK.

Clustered mutations in the X-linked gene *FLNA* that preserve the translational reading frame lead to a spectrum of disorders characterized by skeletal deformities and defects in multiple organ systems in affected males. Loss-of-function mutations in *FLNA* result in periventricular nodular heterotopia (PVNH) in females. The conditions characterized by skeletal abnormalities and organ malformations are collectively termed otopalatodigital syndrome spectrum disorders, span a broad range of severity and include otopalatodigital syndromes type 1 and 2, frontometaphyseal dysplasia and Melnick Needles syndrome. We have studied the entire coding region of *FLNA* by DHPLC in 102 patients with clinical presentations ranging from those typical for an OPD-spectrum disorder to possible variant phenotypes. Among those with identified mutations we have observed a frameshift mutation in an individual with short stature, delayed epiphyseal bone age, developmental delay, small hands and feet, bicoronal craniosynostosis, PVNH and a 47,XXX karyotype. Her mother had PVNH and normal stature but no acral or craniofacial anomalies, a normal karyotype and the same mutation. A second patient presented with multisutural craniosynostosis, developmental delay multiple flexion deformities of the limbs and campomelia. Molecular analysis revealed a 28 bp *de novo* deletion spanning the exon 28/intron 28 boundary. The third female patient presented with micrognathia, deafness, campomelia, and developmental delay; a phenotype very similar to males with OPD2. A complex *de novo* insertion/deletion mutation was detected within exon 29. These results demonstrate the existence of a wider phenotypic spectrum (including developmental delay and craniosynostosis) than has previously been appreciated in females with OPD spectrum disorders.

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Neoplasia in Costello Syndrome: Additional cases and update on the tumor screening protocol. *K.W. Gripp[1], B. Kerr[2], T. Kurczynski[3], M. Stephan[4], C. Tifft[5], D. Viskochil[6], L. Nicholson[1]*. 1) A.I. duPont Hospital for Children, Wilmington, DE; 2) Manchester Children's University Hospital, England; 3) Mercy Children's Hospital,Toledo,OH; 4) Madigan Army Medical Center, Tacoma, WA; 5) Children's National Medical Center, Washington, DC; 6) University of Utah, Salt Lake City, UT.

Costello syndrome is a rare congenital anomaly and tumor predisposition syndrome in which rhabdomyosarcoma (RMS; 12 cases), bladder carcinoma and neuroblastoma are the most common malignancies. We report 4 additional RMS and 1 neuroblastoma. The tumor risk led to proposal of a screening protocol, consisting of ultrasound (US) and urine studies. The suggestions were based on reasonable clinical practice, acknowledging that benefit remains to be documented. We follow a cohort of 29 patients. **Results:** Because the majority of RMS originated from the abdomen or pelvis, we proposed screening US. 10 of 16 eligible pts (<10 years; no prior RMS) had US; no RMS was found. 1 RMS originating from the prostate presented 3 months after a normal US. Urinalysis for hematuria due to bladder carcinoma was performed in 3 of 12 pts(>10 years; no prior bladder cancer) with normal results. Urine VMA/HVA levels are used to screen for neuroblastoma. 6 of 10 pts (<5 years) had these studies and all had abnormal results. On clinical, imaging study and/or lab follow up no neuroblastoma was identified. The pathophysiology for the catecholamine metabolite abnormality remains unclear. Possible explanations include increase in the amount of sympathetic nervous tissue, chronic physical stress causing sympathetic activation, or neuroendocrine tumors too small to be detected on imaging. No neuroblastoma occurred in any study pt.

Conclusions: The newly reported malignancies underscore the tumor risk. Data is insufficient to evaluate a possible benefit of tumor screening for RMS and bladder carcinoma. In contrast, screening for neuroblastoma resulted in uniformly abnormal results, not identifying neuroblastoma, but leading to anxiety and additional studies. Thus we recommend cessation of the urine VMA/HVA assay as screening test.

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Program Nr: 196 from the 2004 ASHG Annual Meeting

Clinical characterization of atypical cleidocranial dysplasia with parietal foramina, imperforate anus and porokeratosis mapping to chromosome 22q. *R. Mendoza-Londono*[1], *E. Lammer*[2], *R. Watson*[3], *A. Hatamochi*[4], *P. Hermanns*[1], *D. Napierala*[1,5], *K. Wakui*[1], *S. Carter*[1,5], *D. Nguyen*[1], *D.W. Stockton*[1], *B. Lee*[1, 5]. 1) Dept. of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) CHORI, Oakland,CA; 3) Dept. of Dermatology, Our Lady's Hosp for Sick Children,Crumlin, Dublin, Ireland; 4) Dept. of Dermatology ,Dokkyo University School of Medicine, Japan; 5) Howard Hughes Medical Institute.

We describe the clinical characterization, molecular analyses, and genetic mapping of an atypical form of cleidocranial dysplasia (CCD). We have identified 7 patients in 4 families from different geographic regions and ethnic backgrounds with this phenotype. Affected individuals present at birth with wide-open fontanels and variable degrees of craniosynostosis, as well as parietal foramina. In addition some patients have developmental delay, hypoplastic clavicles, a characteristic skin rash, urogenital malformations and an imperforate anus. This is a unique condition bringing together apparently opposing pathophysiologic and developmental processes, i.e., accelerated suture closure vs. delayed ossification. In one individual selected candidate genes including RUNX2, CBF beta, MSX-2, ALX-4, and Dermo 1 were screened for mutations by direct sequencing, and for microdeletions by FISH. No mutations or microdeletions were detected in any of the coding regions of the genes analyzed. Simulation analysis for the power to detect linkage estimated the maximum lod score was 2.3 with the available individuals. We performed genome wide screen with a panel of polymorphic markers distributed at an average spacing of 10 cM throughout the genome. The maximum Lod score was +2.38 for markers D22S283 and D22S274. Haplotype analysis narrowed the region of interest to a 34 cM interval between D22S1163 and D22S1170. We present the largest series of patients with this unique phenotype reported to date, and evidence suggestive of linkage to a 34cM region on chromosome arm 22q. The identification of the gene defect in this condition may elucidate a novel context-dependent regulation of RUNX2 during osteoblast differentiation and craniofacial morphogenesis.

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Program Nr: 197 from the 2004 ASHG Annual Meeting

Identification of the gene underlying an inherited disorder of pain sensation. *C.R. Fertleman, M. Rees, K.A. Parker, E. Barlow, R.M. Gardiner.* Paediatrics and Child Health, RFUCMS, London, United Kingdom.

Objective: To identify the gene underlying the autosomal dominant condition familial rectal pain (FRP). Design: A systematic genome wide search using highly polymorphic microsatellite markers was undertaken in a single large pedigree with familial rectal pain [OMIM 167400] of sufficient power to provide independent evidence for linkage. Following identification of a region of interest, analysis of the region was undertaken in additional families and a candidate gene was screened for mutations.Patients: Individuals affected with familial rectal pain present in infancy with severe pain after defecation followed by flushing of the lower body or one half of the body (harlequin phenomenon). In later life, ocular and maxillary crises predominate. Most affected individuals respond well to the anti-epilepsy drug carbamazepine. Although the phenotype is highly specific, there is no known pathophysiological basis of this disease.Results: 11 pedigrees were ascertained containing 55 individuals affected with FRP. DNA samples were obtained and individuals from the largest pedigree were genotyped for 455 microsatellite markers. Significant linkage was found on chromosome 2q31 with a pairwise LOD score of 4.99 at marker D2S2330 (theta = 0). This region was investigated in the rest of the family resource and resulted in a total LOD score of 9.68 at D2S2330. A candidate gene in the region, SCN9A, encoding the Nav1.7 voltage-gated sodium channel Nav1.7 was screened for mutations by sequencing. To date five different missense mutations have been found. These are now being subject to functional analysis to determine their precise effect on the biophysical properties of the channel.Conclusions: It is anticipated that the identification of the defect underlying this genetic condition will be of relevance to the understanding of pain pathways and may allow the development of novel approaches to the prevention and treatment of pain. It is noteworthy that mutations in this gene have been reported in a similar phenotype, that of primary erythermalgia [OMIM 133020].
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RAI1 is a PHD domain-containing transcriptional factor associated with Smith-Magenis syndrome (SMS). *W. Bi[1], T. Oyama[1], M. Saifi[1], J. Visvanathan[1], C. Shaw[1], K. Walz[1], P. Fonseca[1], M. Wilson[4], L. Potocki[1, 3], J. Lupski[1, 2, 3]*. 1) Departments Molecular & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Pediatrics, Baylor College of Medicine, Houston, TX; 3) Texas Children's Hospital, Houston, TX; 4) Department of Clinical Genetics, The Children's Hospital at Westmead, Sydney, Australia.

Smith-Magenis syndrome (SMS) is a mental retardation/multiple congenital anomalies disorder associated with a heterozygous ~ 4 Mb deletion in 17p11.2. Recently, point mutations in the *RAI1* gene, which lies within the SMS critical interval, were identified in three nondeletion SMS patients. We report two novel *RAI1* point mutations in nondeletion SMS patients. Comparisons of the clinical features suggest that the majority of the clinical features in SMS result from *RAI1* haploinsufficiency despite phenotypic variability. Little is known about the function of RAI1. We identified a PHD domain in the C-terminal of both the mouse and human RAI1, suggesting that RAI1 might be involved in chromatin remolding. Through transfection of a GFP-Rai1 construct into Hela cells, we showed that Rai1 is localized to the nuclear and it has transactivation activity. We have generated a potential null *Rai1* allele by gene targeting. A *lacZ* reporter gene was inserted into *Rai1* locus and a 4 kb fragment was deleted. Three ES cell lines have successfully given germline transmission. X-gal staining of the *Rai1* heterozygous mice recapitulated the endogenous expression pattern of *Rai1* gene. A fraction of *Rai1*$^{+/-}$ mice exhibited craniofacial abnormalities that were observed in SMS mouse model *Df(11)17*, which harbor a deletion of the syntenic region of SMS common deletion. Homozygous mice died during late embryonic stage or within a few days after birth, with very few surviving up to several weeks. The *Rai1*$^{-/-}$ mice that survive are significantly smaller than their wild-type and heterozygous littermates. Histopathological examination of these mice is ongoing. Our data indicated that RAI1 is a transcriptional factor, and the analyses of Rai1 mutant mice will help elucidate the function of RAI1 in human and its role in the pathophysiology of SMS.

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Molecular basis of a temperature-sensitive PEX13 mutation of peroxisomal biogenesis disorder (1) : Immunohistochemistry and protein analyses. *K. Hashimoto[1], Z. Kato[1], T. Nagase[1], N. Shimozawa[1], K. Kuwata[2], K. Omoya[1], A. Li[1], E. Matsukuma[1], Y. Yamamoto[1], H. Ohnishi[3], H. Tochio[3], M. Shirakawa[3], Y. Suzuki[4], R. Wanders[5], N. Kondo[1]*. 1) Department of Pediatrics, Gifu University School of Medicine ; 2) Department of Biochemistry and Biophysics, Gifu University School of Medicine; 3) Graduate School of Integrated Science, Yokohama City University0; 4) Medical Education Development Center, Gifu University School of Medicine ; 5) Department of Clinical Chemistry and Pediatrics, Academic Medical Center, University of AmsterdamC.

Peroxisomal biogenesis disorders include Zellweger syndrome (ZS), neonatal adrenoleukodystrophy (NALD) and infantile Refsum disease. We have revealed that two patients belonging to complementation group H with the ZS and NALD phenotypes carried a nonsense and a missense mutation of PEX13, respectively. The patient with the milder form, NALD, had a missense mutation with substitution of isoleucine 326 by threonine, I326T, within a SH3 domain, and showed normal development just before a sudden deterioration with a high fever due to a respiratory infection. Fibroblasts from the NALD patient showed a temperature-sensitive phenotype in peroxisome assembly detected with anti-catalase antibody, indicating clinical temperature-sensitive phenotype could be due to the insufficiency of the peroxiisomal assembly at high fever. However, the precise molecular mechanisms between genotypes and clinical phenotypes remain to be clarified. To address the issue, we have analyzed the protein phenotype of the human Pex13p-SH3 domain using immunohistochemical and biochemical analyses The results of the immunohistochemistry using anti-Pex13p and anti-Pex14p antibodies showed that the Pex13p expression and localization with temperature-sensitive manner should be primary to those of Pex14p. The recombinant Pex13p-SH3 protein, which had been confirmed as the binding domain against the Pex14p, had a marked susceptibility to protease cleavage compared to wild type protein at higher temperatures. In addition, the conformational changes with urea gradient showed similar sigmoidal transition curves for wild and mutant, but the half point of the conformational transition in I326T was significantly lower compared that of wild. These fragile phenotypes of the mutant protein indicate that the protein stability of the human Pex13p with TS gene mutation, I326T, should be the direct cause of the TS cell phenotype leading clinical deterioration of the patient at fever. These findings could give the avenues for better understanding and therapeutic approaches of the other diseases with TS phenotype. (See also 3D-structure and folding by Kato et al. ASHG 2004).

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Program Nr: 200 from the 2004 ASHG Annual Meeting

Alternative splicing suggests extended function of PEX26 in peroxisome biogenesis (PB). *S. Weller*[1], *I. Cajigas*[2], *S.J. Gould*[3], *D. Valle*[1,4]. 1) Inst of Genetic Medicine; 2) MSIP; 3) Dept of Biological Chemistry; 4) Howard Hughes Medical Inst, Johns Hopkins Univ, Baltimore, MD.

PEX26 is responsible for the most recently explained complementation group (CG8) of PB disorders (Zellweger spectrum) and encodes an integral peroxisomal membrane protein of 305 aa with a single C-terminal transmembrane domain (Matsumoto et al, 2003). PEX26 binds two interacting AAA ATPases, PEX1 and PEX6, through direct interaction with PEX6. Together the genes encoding these 3 proteins account for ~70% of all Zellweger spectrum disorders. Based on these properties PEX26 was proposed to function as a docking factor for the recruitment of PEX1 and PEX6 to peroxisomal membranes. In studies aimed at understanding the role of PEX26 in PB, we found extensive alternative splicing of *PEX26* in RNA isolated from fibroblasts and various human tissues. In addition to the full length transcript, we found prominent amounts of spliceforms skipping exons 2, 5, or 4+5. Interestingly, the exon 5-less transcript (PEX26-ex5) maintains frame and encodes a protein that lacks the transmembrane domain of full-length PEX26 (PEX26-FL). To test the function of PEX26-ex5, we expressed it in *PEX26* null cells and found that it had 80-100% activity in restoring PB as compared to PEX26-FL. By immunofluorescence and confocal microscopy, we confirmed the predicted cytosolic localization of PEX26-ex5. Thus, *PEX26* produces soluble and membrane bound isoforms both of which rescue PB in *PEX26* deficient cells. Moreover, testing a series of deletion mutants we identified a minimal N-terminal fragment of PEX26 (aa 29-174) with both complementing and PEX6 binding activity. Patient missense mutations in this fragment disrupt binding and function. Thus, our results show that the PEX6 binding and PB activity of PEX26 reside in the same N-terminal half of the protein and that peroxisomal localization of PEX26 is not required for its function. We suggest an alternative model of PB in which PEX26 functions not as a docking factor but as an assembler and/or activator of the PEX1-6 heterodimer, a function that can occur at an extraperoxisomal location.

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Program Nr: 201 from the 2004 ASHG Annual Meeting

Molecular pathogenesis of PINK1 parkinsonism. *P.M Abou-Sleiman[1], M.M.K. Muqit[1,2], A. Saurin[3], S. Jain[1], S. Ghandi[1], K. Harvey[6], R. Harvey[6], A.R. Clarke[5], N. McDonald[4], P. Parker[3], D.S. Latchman[2], N.W. Wood[1].* 1) Department of Molecular Neuroscience, Institute of Neurology, London WC1N 3BG, United Kingdom; 2) Medical Molecular Biology Unit, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK; 3) Protein Phosphorylation Laboratory, Cancer research UK, 44 Lincoln's Inn Fields, London WC2 3PX, UK; 4) Structural Biology Laboratory, Cancer research UK, Lincoln's Inn Fields, London WC2 3PX, UK; 5) Department of Biochemistry, School of Medical Sciences, University of Bristol, University Walk, Bristol BS8 1TD, UK; 6) Department of Pharmacology, The School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX, UK.

We have previously shown that mutations in PINK1, a protein kinase which co-localizes to the mitochondria, are causal in autosomal recessive early-onset Parkinsons disease (PARK6 locus). In addition to the two reported familial mutations we have identified a further seven separate missense mutations affecting highly conserved amino acids of the kinase domain and a nonsense mutation which truncates the predicted C-terminal regulatory region of the protein. The identification of Mendelian mutations in a mitochondrial protein kinase finally demonstrates a direct link between mitochondrial dysfunction and Parkinsons disease. We present a mutation analysis of the gene in our cohort of approximately 810 PD patients, identifying seven novel mutations. Furthermore, we provide functional data on the mutations identified in both cell models and an in silico protein model. The cell models provide evidence a protective effect which is abrogated by the mutations. While the function of PINK1 remains to be determined, we present data on the cellular and subcellular localisation of the protein and evidence of two novel interactions. These data provide evidence of a novel pathway in the aetio-pathogenesis of PD. For the first time the involvement of a protein kinase, direct involvement of the mitochondria, and an interaction with the PKC signal transduction pathway has been demonstrated.

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Program Nr: 202 from the 2004 ASHG Annual Meeting

Examination of *ATP1A3* the gene causing rapid onset dystonia parkinsonism: genotype/phenotype correlation.

L.J. Ozelius[1], P. de Carvalho Aguiar[1,2], K.J. Sweadner[3], J.T. Penniston[3], J. Zaremba[4], L. Liu[1], S. Gollamudi[1], G. Senthil[1], A. Rabinowitz[1], G. Linazasoro[5], M.A.J. Tijssen[6], S.B. Bressman[7], W.B. Dobyns[8], A. Brashear[9]. 1) Dept Mol Genetics, Albert Einstein College of Medicine, Bronx, NY; 2) Department of Neurology and Neurosurgery, Federal University of So Paulo, So Paulo-SP, Brazil; 3) Neuroscience Center, Massachusetts General Hospital, Boston, MA; 4) Department of Genetics, Institute of Psychiatry and Neurology, Warsaw, Poland; 5) Centro de Neurologia y Neurocirugia, Clinica Quiron, San Sebastian, Spain; 6) Department of Neurology, University of Amsterdam, Amsterdam, The Netherlands; 7) Department of Neurology, Beth Israel Medical Center, New York, NY; 8) Department of Human Genetics, Neurology and Pediatrics, University of Chicago, IL; 9) Department of Neurology, Indiana University School of Medicine, Indianapolis, IN.

Rapid-Onset Dystonia-Parkinsonism (RDP, *DYT12*) is an autosomal dominant movement disorder with variable expressivity and reduced penetrance characterized by abrupt onset of dystonia usually accompanied by signs of parkinsonism. The sudden onset of symptoms over hours to a few weeks, often associated with physical or emotional stresses, suggests a trigger initiating a nervous system insult resulting in permanent neurologic disability. We report six missense mutations in the gene for the Na^+,K^+-ATPase alpha 3 subunit (*ATP1A3*), in eight unrelated families with RDP. Functional studies and structural analysis of this pump, a crucial protein responsible for maintaining the electrochemical gradient across the cell membrane, suggest that these mutations impair enzyme activity or stability. Screening of additional patient samples will allow for the assessment of genotype:phenotype correlations and the determination of genetic heterogeneity.

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Program Nr: 203 from the 2004 ASHG Annual Meeting

Dysfunctional FOXH1 transcriptional activity is associated with midline anomalies of the holoprosencephaly spectrum, or alternatively with congenital cardiac malformations, including Tetralogy of Fallot. *E. Roessler*[1], *M. Ouspenskaia*[1], *J.D. Karkera*[1], *E. Zackai*[2], *E. Goldmuntz*[2], *P. Bowers*[3], *J.A. Towbin*[4], *J. Belmont*[4], *B. Feldman*[1], *M. Muenke*[1]. 1) NHGRI, NIH, Bethesda, MD; 2) Children's Hospital of Philadelphia, Philadelphia, PA; 3) Yale University, New Haven, CT; 4) Baylor College of Medicine, Houston, TX.

Holoprosencephaly (HPE) or laterality defects (such as heterotaxy or structural heart defects associated with mutations in CFC1) are hypothesized to be caused by defects in the formation or function of the axial midline during early gastrulation. These events depend on key Activin-like signals (such as Nodal and other TGFbeta secreted factors) mediated by the transcription factor FOXH1. We performed a mutational analysis of 454 HPE cases and 389 cases of congenital cardiac disease, including a wide spectrum of malformations, and identified 24 individual sequence variations not seen in normal controls (16 in HPE, 5 in the cardiac cohort, and 3 in both groups). In particular, two variations occur in the DNA-binding domain, 5 affect highly conserved residues important for interactions with the co-factor SMAD2, and three mutations cause frameshift extensions of the carboxy-terminus of the protein. In addition, we detected a highly variable segment of three codons at the end of the SMAD-interaction domain of unknown significance, yet extensively polymorphic. A zebrafish rescue assay was developed to determine the functional effects of the variations detected in either/both patient groups. Three of the first seven sequence variations tested demonstrate partial or complete failure to rescue the abnormal phenotype of a zebrafish embryo depleted for FoxH1 with an antisense morpholino oligonucleotide. In contrast, the intact human or zebrafish gene could correct the gastrulation defects. Here we describe a loss-of-function mutation in an autosomal dominant HPE family. Two additional mutations demonstrating decreased FOXH1 activity were detected in unrelated cases of Tetralogy of Fallot. These findings extend the hypothesis that gastrulation factors can play an etiological role in diverse birth defect phenotypes.

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Genetic predisposition in dogs to Lafora disease, a fatal form of human epilepsy. *H.T. Lohi[1], E.J. Young[1,3], S. Fitzmaurice[5], C. Rusbridge[6], E.M. Chan[1], M. Vervoort[1], J. Turnbull[1], L. Ianzano[1], A.D. Paterson[1,3], N. Sutter[7], E.A. Ostrander[7], C. Andre[8], D.G. Shelton[9], C.A. Ackerley[4], S.W. Scherer[1,3], B.A. Minassian[1,2,3].* 1) Genetic and Genome Biology, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Department of Pediatrics (Neurology),The Hospital for Sick Children, Toronto, Ontario, Canada; 3) Institute of Medical Sciences, University of Toronto, Ontario, Canada; 4) Department of Pathology, The Hospital for Sick Children, Toronto, Ontario, Canada; 5) Wey Referrals Woking, Surrey GU21 5BP, UK; 6) Stone Lion Veterinary Centre, Wimbledon, London SW195AU, UK; 7) Clinical Research and Human Biology Divisions, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA; 8) UMR6061, CNRS Genetique et Developpement, Faculte de Medecine, 35043 Rennes Cedex, France; 9) Department of Pathology, University of California, SanDiego 92093, USA.

Lafora disease (LD) is a rare (one per million), fatal, teenage-onset form of progressive myoclonus epilepsy (PME) with unique starch-like cellular accumulations. It is caused by recessively inherited mutations in the EPM2A or the recently identified EPM2B (NHLRC1) genes encoding the laforin starch-binding phosphatase and the malin E3 ubiquitin ligase, respectively. LD has also been reported in dogs, surprisingly frequently. Presently, a PME is common (~5%) in the popular Miniature Wirehaired Dachshund (MWHD) breed in the United Kingdom. We characterize the clinicopathologic phenotype of these dogs and show them to have LD. We map the underlying disease locus and identify the specific disease-associated mutation: the first coding dodecamer repeat expansion in any species and the first disease-causing tandem repeat expansion outside human. We show that the expansion mutation is recurrent, affecting epileptic dogs other than MWHD and demonstrate that it arises from a sequence variation particular to dogs (and other canids), predisposing the species to LD. Finally, we devise a test to detect expanded alleles in carrier and presymptomatic animals and allow eradication of the disease from MWHD and future affected canine populations.

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Program Nr: 205 from the 2004 ASHG Annual Meeting

Heterozygosity for a truncation allele of sodium channel SCN8A in a family with ataxia and cognitive impairment. *M.M. Trudeau[1], J.D. Dalton[2], J.W. Day[2], L.P.W. Ranum[2], M.H. Meisler[1]*. 1) Department of Human Genetics, University of Michigan, Ann Arbor, MI; 2) Institute of Human Genetics, University of Minnesota, Minneapolis MN.

Mutations in the neuronal voltage-gated sodium channel *SCN8A* (Nav1.6) are responsible for movement disorders in the mouse, including congenital ataxia. We screened 150 patients with sporadic and inherited ataxia in order to detect coding and splice site mutations of human *SCN8A*. The patients had been pre-screened for mutations in known ataxia genes. The proband, a 9 year old boy with ataxia and mental retardation, was heterozygous for a dinucleotide deletion in exon 24 that results in a frameshift mutation. The mutation is located within the pore loop of domain 4 of the channel protein, and the truncated protein is predicted to be inactive. The mutation was not detected in 179 control individuals or 200 ataxia patients. Three members of the proband's family who exhibit cognitive disabilities without ataxia are also heterozygous for the mutant allele. There is a history of cognitive dysfunction and incoordination in several additional family members who have not been tested. The data suggest that heterozygosity for a null allele of *SCN8A* is associated with variable cognitive disability and incompletely penetrant congenital ataxia. The apparent haploinsufficiency for *SCN8A* in this family is consistent with the previously demonstrated haploinsufficiency for the related sodium channel *SCN1A* in patients with Severe Myoclonic Epilepsy of Infancy (SMEI). Mice heterozygous for a null allele of *Scn8a* have reduced channel protein and a mild gait abnormality. Cognitive testing of heterozygous mice is in progress. Although there have been 4 spontaneous disease mutations in mouse *Scn8a* during the past 40 years, this is the first report of a mutation in human *SCN8A*.

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Infantile onset symptomatic epilepsy syndrome caused by a homozygous loss of function mutation in GM3 synthase. *M.A. Simpson*[1], *H.A. Cross*[2], *C. Proukakis*[1], *D.A. Priestman*[3], *D.C.A. Neville*[3], *G. Reinkensmeier*[3], *H. Wang*[5], *M. Wiznitzer*[4], *M.A. Patton*[1], *R.A. Dwek*[3], *T.D. Butters*[3], *F.M. Platt*[3], *A.H. Crosby*[1]. 1) Dept Medical Genetics, St Georges Hosp, London, United Kingdom; 2) Department of Ophthalmology, University of Arizona School of Medicine, 655 N. Alveron Way, Tucson, USA; 3) Glycobiology Institute, Department of Biochemistry, University of Oxford, Oxford, OX1 3QU, UK; 4) Rainbow Babies and Childrens Hospital, Cleveland, USA; 5) Das Deutsch Clinic, Geauga County, Ohio, USA.

We have identified an autosomal recessive infantile onset symptomatic epilepsy syndrome associated with developmental stagnation and blindness. Assuming a founder effect within a large Old Order Amish pedigree, we conducted a genome-wide screen for linkage and identified a single region of homozygosity on chromosome 2p12-p11.2 spanning 5.1cM (maximum LOD score 6.84). Sequencing of genes in the region identified a nonsense mutation in the SIAT9 gene, which is predicted to result in the premature termination of the GM3 synthase enzyme (CMP-NeuAc: Lactosylceramide -2,3 sialyltransferase, EC 2.4.99.9). GM3 synthase is a member of the sialyltransferase family and catalyses the initial step in the biosynthesis of the majority of complex gangliosides from lactosylceramide (LacCer). Biochemical analysis of plasma glycosphingolipids (GSLs) confirmed a lack of GM3 synthase activity in affected individuals. There was a complete absence of GM3 ganglioside and its biosynthetic derivatives, and an increase of LacCer and its alternative derivatives in affected cases. While the relationship between defects in ganglioside catabolism and a range of lysosomal storage diseases is well documented this is the first proven report of disruption of ganglioside biosynthesis associated with human disease.

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Mutations in SLC6A19 are Associated with the Hartnup disorder. *R. Kleta[1], E. Romeo[2], T. Ohura[3], C. Stuart[1], M. Arcos-Burgos[1], C.A. Wagner[2], S. Inoue[3], N. Matsuura[3], A. Helip-Wooley[1], D. Bockenhauer[4], R. Warth[5], I. Bernardini[1], G. Visser[6], T. Eggermann[7], P. Lee[8], A. Chairoungdua[9], Y. Kanai[9], F. Verrey[2], W.A. Gahl[1], A. Koizumi[3].* 1) MGB, NHGRI, NIH; 2) University of Zurich; 3) Kyoto University; 4) Yale University School of Medicine; 5) University of Regensburg; 6) University Hospital Utrecht; 7) Aachen University Hospital; 8) The National Hospital for Neurology and Neurosurgery; 9) Kyorin University School of Medicine.

Despite molecular characterization of other transporters, the neutral amino acid carrier defective in Hartnup disorder has resisted genetic identification. Hartnup disorder, an autosomal recessive defect named after an English family described in 1956, results from impaired transport of neutral amino acids across renal proximal tubules and intestinal mucosa. Symptoms include transient manifestations of pellagra, which can be life threatening and involve rashes, cerebellar ataxia, and psychosis. These findings are thought to result from impaired intake of one neutral amino acid, tryptophan, a precursor in the synthesis of niacin. We performed homozygosity mapping on 10 members of two consanguineous families, i.e., the original Hartnup sibship (A) and a United States sibship (B), both having typical neutral aminoaciduria. Data revealed linkage to 5p15 in Family A, with a maximum combined multipoint LOD score of 2.31 at 11.24 cM (p=0.01). This region is syntenic to the area of mouse chromosome 13 on which a sodium-dependent amino acid transporter, B0AT1, resides. We first demonstrated luminal expression of B0AT1 specifically in mouse proximal tubule and small intestine. We isolated the human homologue of B0AT1, called SLC6A19, and determined its correct size and molecular organization. We then identified mutations in both alleles of members of the Hartnup family and of three Japanese families. We also demonstrated that the protein product of SLC6A19 is expressed primarily in human intestine and in the proximal tubule and functions as a neutral amino acid transporter. Family B disclosed a LOD score of -2.40 at 15.81 cM and showed no mutations in SLC6A19, indicating locus heterogeneity.

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The gene for Hartnup disorder displays allelic heterogeneity and incomplete penetrance. *J.A. Cavanaugh[1], H.F. Seow[2], S. Broer[3], S.J. Potter[2], H.J. Rodgers[1], C.G. Bailey[2], J.E.J. Rasko[2].* 1) Medical Genetics Research Unit, ANU Medical School, Australian National University, Canberra, Australia; 2) Centenary Institute of Cancer Medicine & Cell Biology, University of Sydney, Australia; 3) Department of Biochemistry and Molecular Biology, Australian National University, Canberra Australia.

Hartnup disorder is an autosomal recessive abnormality of renal and gastrointestinal amino acid transport with estimates of incidence varying from 1 in 14,219 births in the USA, to 1 in 33,000 births in Australia. The disorder is diagnosed by characteristic increases in the urinary excretion of neutral amino-acids and delta/epsilon carboxyamides. Individuals who exhibit typical Hartnup aminoaciduria may be asymptomatic, although some develop a photosensitive pellagra-like rash, attacks of cerebellar ataxia and other neurological or psychiatric symptoms. This variable disease expression has led some authors to suggest that there may be multiple loci underlying disease etiology. Parametric linkage analyses of six Australian Hartnup pedigrees placed the gene for Hartnup disorder at a locus on chromosome 5p15 and dismissed other candidate loci containing previously identified neutral amino acid transporters. We have recently identified a novel cDNA at this position (designated SLC6A19) that we have shown is a Na^+ dependent neutral amino acid transporter in system B^0. SLC6A19 is expressed predominately in kidney and intestine. We have identified six mutations in SLC6A19 that co-segregate with disease in the predicted recessive manner, with the majority of affected individuals being compound heterozygotes. The D173N allele is the most frequent allele detected in probands as well as in the healthy population (frequency = 0.007). Haplotype analyses demonstrates that all D173N alleles detected to date share a common origin in Caucasians. This allele displays incomplete penetrance that may be a consequence of its partial transport activity. These data may explain the enigmatic nature of clinical presentation and the variable estimates of disease frequency.

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Transaldolase deficiency : an inborn error of the pentose phosphate pathway associated with a severe phenotype and multiorgan involvement including hydrops foetalis, cutis laxa, hepatic failure and hemolytic anemia. *V. Valayannopoulos*[1], *N. Verhoeven*[2], *G. Salomons*[2], *N. Biebyuck*[3], *K. Mention*[1], *JM. Saudubray*[1], *P. de Lonlay*[1], *C. Jakobs*[2]. 1) Metabolic Unit Necker-Enfants Malades Hospital,149, Rue de Svres, 75015 Paris, France; 2) Metabolic Laboratory, Department of Clinical Chemistry, VU University Medical Center, 1081 HV Amsterdam, The Netherlands; 3) Pediatric Nephrology, Necker-Enfants Malades Hospital, 149, Rue de Svres, 75015 Paris, France.

Transaldolase deficiency has been reported in a single patient presenting with liver failure and cirrhosis. We report here four additional patients born from the same consanguineous couple.All patients share common clinical features beginning in the antenatal period and present at birth including: hepatomegaly, splenomegaly, hepatic failure, hemolytic anemia, thrombopenia, genitourinary malformations, dysmorphic features, cutis laxa and hypertrichosis. The clinical outcome is variable: the first child died at age of four months from severe hepatic failure; the second pregnancy was terminated because of hydrops foetalis, fetal ascitis and pericarditis at 28 weeks of gestation. Pathology examination showed hepatic fibrosis. The third child survived and is doing well at age 5 years in spite of a liver fibrosis and mild renal failure. All other symptoms regressed spontaneously. The fourth child is 4 months old and has hepatosplenomegaly, mild anemia and thrombopenia, a micropenis and dysmorphic features. Cutis laxa, hypertrichosis and liver failure disappeared progressively. Polyols assessment in urine in the two surviving patients showed elevation of erythritol, arabitol and ribitol consistent with transaldolase deficiency. Enzyme studies in erythrocytes revealed deficient transaldolase activity. DNA analysis of the TALDO1 gene is pending. In conclusion, transaldolase deficiency is a new inherited metabolic disorder in the pentose phosphate pathway responsible for a broad clinical spectrum of severe multi organ involvement beginning antenatally and resembling to those seen in congenital disorders of glycosylation or respiratory chain defects.

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Hypoglycosylation of -dystroglycan, due to UDP-GlcNAc 2-epimerase/ManNAc kinase mutations, causes the muscle destruction of Hereditary Inclusion Body Myopathy. *M. Huizing[1], P. Savelkoul[1], MS. Sun[1], G. Rakocevic[2], S. Sparks[1], M. Dalakas[2], D. Krasnewich[1], W. Gahl[1].* 1) NHGRI; 2) NINDS, NIH, Bethesda, MD.

Hereditary inclusion body myopathy (HIBM), an adult onset neuromuscular disorder characterized by progressive myopathic weakness and atrophy, is caused by *GNE* defects. *GNE* encodes the rate-limiting enzyme of sialic acid synthesis, UDP-GlcNAc epimerase/ManNAc kinase. We documented decreased epimerase and kinase activities in HIBM fibroblasts, explaining impaired sialic acid production and glycoconjugate sialylation, but it was unclear why this leads to muscle destruction. A clue came from recent findings of abnormal glycosylation of -dystroglycan (DG) in other muscular dystrophies, e.g., Muscle-Eye-Brain disease (MEB) and Walker-Warburg syndrome (WWS). DG is a critical component of the dystrophin-glycoprotein complex and contains abundant oligosaccharides, including rare tissue-specific O-mannosylated glycans. We examined the glycosylation status of -DG in quadriceps muscle of 4 unrelated HIBM patients. All 4 biopsies showed absent or markedly reduced signals by immunohistochemistry and immunoblotting with the antibodies VIA4 and IIH6, which are thought to detect O-mannosylated epitopes of -DG. Antibodies to the core -DG protein, -DG and laminin-2 showed normal patterns. These findings, indicating that aberrant O-mannosylation of -DG underlies the muscle weakness of HIBM, are supported by additional data: 1.We found normal serum transferrin isoforms in HIBM patients, indicating normal N-glycosylation. 2.We showed residual GNE activity in HIBM myoblasts, which may produce a large enough sialic acid pool to preferentially sialylate other (N- and O-GalNAc linked) glycoconjugates over the very rare O-mannosylated glycans. 3.HIBM skeletal muscle tissue is hyposialylated (Nishino et al). 4.Abnormal O-mannosylation causes other muscular dystrophies: MEB and WWS are caused by UDPGlcNAc:Man -O1 GlcNAc transferase and O-mannosyltransferase I defects, respectively. Further understanding of -DG modifications, especially via O-linked mannosylation, is essential for development of diagnostic tests and therapies for HIBM and other muscular dystrophies with similar pathology.

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New Acyl-CoA Dehydrogenases: Redefining Long Chain Fatty Acid -oxidation in Humans. *M. He[1], R.E. Ensenauer[2], J. Vockley[1].* 1) Children's Hospital of Pittsburgh, University of Pittsburgh, School of Medicine, Pittsburgh, PA; 2) Department of Medical Genetics, Mayo Medical School, Rochester, MN.

Very long chain fatty acids are traditionally thought to be oxidized exclusively by peroxisomes, even though mitochondrial dysfunction has been reported in patients with peroxisomal defects. The ACDs are a family of flavoenzymes involved in mitochondrial -oxidation of fatty acids. Nine members of this family have previously been identified, but none of them utilize acyl-CoAs with carbon chain > 20. Recently, we identified two new potential ACD family members, *ACDX1* and *ACDX2*, which are widely conserved across evolution. They are 20-30% identical to the other ACDs, while human *ACDX1* and *ACDX2* share 46%; homology with each other. We have now definitively characterized *ACDX2* as a long chain ACD, and name it *ACADH10*. The *ACADH10* locus is a complicated one with multiple predicted coding domains. The transcript from the ACD-portion of this gene is efficiently imported into mitochondria when translated in vitro, and processed to a mature form. Expression of the mature intra-mitochondrial form of human ACADH10 in E.coli produces an enzyme with maximal ACD activity towards C22-CoA. It has relative activities towards C23-, C24-, and C26-CoA of 60%, 15%, and 15% respectively compared to C22-CoA. Computer modeling of the structures of ACADH10 and ACDX1 predicts that their catalytic bases are aspartic acid instead of the glutamic acid present in other members of this gene family. The substrate specificity of ACDX1 remains under study, and we predict that it will be similar to ACADH10. Alternative forms of the ACDXs have only a partial ACD domain at the C terminus, but have a predicted aminoglycoside phosphotransferase domain at the N terminus that may serve to target them to different subcellular locations. In conjunction with our recent characterization of ACADH9 as an enzyme involved in the mitochondrial catabolism of unsaturated long chain substrates, the finding of ACADH10 rewrites our view of long chain fatty acid -oxidation and raises new questions about the interrelationaship of mitochondria and peroxisomes in very long chain fat catabolism.

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Program Nr: 212 from the 2004 ASHG Annual Meeting

G_{M1}-gangliosidemediated activation of the unfolded protein response (UPR) causes neuronal apoptosis in G_{M1}-gangliosidosis. *A. D'Azzo, A. Tessitore.* Department of Genetics , St Jude Children's Research Hospital, Memphis, TN.

G_{M1}-ganglioside (G_{M1}) is a major sialoglycolipid of neuronal membranes that, among other functions, modulates calcium homeostasis. Progressive accumulation of G_{M1} due to deficiency of lysosomal -galactosidase (-gal) characterizes the neurodegenerative disease G_{M1}-gangliosidosis. Children with this disorder suffer from a generalized CNS involvement, which results in profound neurological deterioration, mental retardation and early death. The murine model of G_{M1}-gangliosidosis closely resembles the early-onset form of the disease. *-gal*$^{-/-}$ mice develop a severe CNS condition, which is associated with massive, age-dependent accumulation of G_{M1} and is accompanied by gradual deterioration of motor functions. We have investigated whether the abnormal intracellular concentration of G_{M1} could be directly responsible for the CNS pathogenesis in the mouse model. The activation of the unfolded protein response (UPR) was tested as a putative cause of neurodegeneration. We demonstrate that in *-gal*$^{-/-}$ spinal neurons activation of the UPR leads to the up-regulation of the ER chaperone BiP and the proapoptotic transcription factor CHOP, followed by the activation of the kinase JNK2 and the ER resident caspase-12. The combined induction of these effectors culminates with neuronal apoptosis. G_{M1}-loading of wild-type neurospheres activated this pathway by depleting calcium from the ER and induced cell death. The activation of ER stress-regulated apoptotic pathways did not occur in mice deficient for both -gal and ganglioside synthase, *-gal*$^{-/-}$*/GalNAcT*$^{-/-}$, which do not accumulate G_{M1}. These findings point to a novel mechanism of neuronal apoptosis, and have significant implications for therapy of this disorder.
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Program Nr: 213 from the 2004 ASHG Annual Meeting

Global vacuolar dysfunction resulting from the absence of a single lysosomal enzyme: studies in Pompe disease.

T. Fukuda, K. Zaal, E. Ralston, P. Plotz, N. Raben. NIAMS, NIH, Bethesda, MD.

Pompe disease, a deficiency of lysosomal acid alpha-glucosidase (GAA), results in accumulation of glycogen in lysosomes and manifests as cardiomyopathy and autophagic myopathy. Therapy with recombinant human GAA (rhGAA) has shown only a modest effect on glycogen clearance in skeletal muscle in both clinical trials and preclinical studies in the KO mice. The therapy relies on M6P-receptor mediated endocytosis and trafficking of the rhGAA to the acidic milieu of lysosomes. The limited effect of the therapy may be related to a perturbation and/or abnormal acidification of the vacuoles along the lysosomal degradative pathway. We have analyzed endocytic and autophagic vacuoles in a model system - human fibroblast cells from unaffected individuals and patients with Pompe disease. Confocal microscopy of cells transfected with GFP-tagged late endosomal/lysosomal (lgp120) or early endosomal (Rab5) markers and immunostained with anti-M6P-receptor antibody showed a significant expansion of all the endocytic vacuoles, redistribution of early endosomes from the periphery to the perinuclear area in some cells, and increased co-localization of the M6P-receptor with late endosomes. Furthermore, real time imaging showed significantly decreased movement of late endocytic compartments in Pompe cells. There was also a striking increase in the number and size of autophagic vacuoles in GFP-LC3 (autophagosomal marker) transfected cells; the autophagosomes extensively co-localized with both early endosomes in the perinuclear region and with late endosomes in the periphery suggesting an increased formation of amphisomal structures in diseased cells. In addition, we have developed a novel method for pH measurement in vacuoles of the endosomal/lysosomal system in live cells by using a combination of fluorescently-tagged dextrans which allowed us to identify a subset of lysosomes with pH above 5.0. Thus, a profound secondary impairment of vacuolar membrane trafficking of the endocytic-autophagic pathway in Pompe cells may result in the inability of the rhGAA to dissociate from the M6P- receptor in the amphisomes or the inability of the enzyme to function properly in the lysosomes.

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Unraveling lysosome-related organelle biogenesis through the cell biology of Hermansky-Pudlak syndrome. *A. Helip Wooley[1], H. Dorward[1], H. Stanescu[1], R. Hess[1], W. Westbroek[1], R. Boissy[2], W. Gahl[1], M. Huizing[1].* 1) NHGRI, NIH, Bethesda, MD; 2) University of Cincinnati, OH.

Hermansky-Pudlak syndrome (HPS) is a disorder of lysosome-related organelle biogenesis, characterized by albinism, a bleeding diathesis and occasional colitis or pulmonary fibrosis. Seven human HPS subtypes are now identified. All HPS subtypes are caused by genes of unknown function, except HPS-2, which is caused by a defective 3A subunit of adaptor complex-3 (AP3). Some HPS proteins interact with each other in BLOCs: biogenesis of lysosome-related organelle complexes. We performed confocal microscopy on affected cells to study the cell biology of HPS. First, using AP3 deficient HPS-2 melanocytes, we showed that AP3 recognizes tyrosinase, but not TYRP1, and that these two proteins travel to melanosomes by different routes. Next, we studied HPS3, and showed that GFP-HPS3 localizes to small, perinuclear vesicles, partially co-localizing with LAMP1, CD63, and clathrin in melanocytes. Clathrin was immunoprecipitated by HPS3 antibodies from normal but not HPS-3 null melanocytes. Immuno-EM confirmed co-localization of HPS3-GFP and clathrin. Early endosome and lysosome markers show a clustered, perinuclear distribution in HPS-3 fibroblasts compared to a normal more uniform distribution. This clustering in HPS-3 cells was corrected by transfection with GFP-HPS3 in affected cells. HPS3 interacts with HPS5 and HPS6 in BLOC-2. In fibroblasts of two patients with homozygous missense changes in HPS5, we showed an abnormal LAMP3 distribution similar to that found in patients with severe HPS5 defects, thereby confirming the HPS-5 diagnosis in these two patients. We are now studying HPS5 and HPS6 function and localization in normal and HPS cells. Additional studies on HPS1 and HPS4, which interact in BLOC-3, have shown that GFP-LAMP1 or GFP-CD63 accumulate in large membranous structures in HPS-1 and HPS-4 cells. Normal LAMP1 distribution was restored in HPS1 deficient cells by transfection with GFP-HPS1. These studies demonstrate the strength of patients' cultured cells in the study of HPS protein function, and contribute to a better understanding of the pathophysiology of HPS.

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Variable expressivity of the clinical and biochemical phenotype associated with the 149Leu mutation within the Apolipoprotein E mutation. *L. Duvillard[1], P. Saugier[2], B. Verges[3], B. Couret[4], B. Lorcerie[5], MT. Vannier[6], C. Thauvin[7], F. Charbonnier[2], F. Huet[7], P. Gambert[1], T. Frebourg[2], L. Faivre[7]*. 1) INSERM U498, Hopital le Bocage, Dijon, France; 2) Genetique Moleculaire, Faculte de Medecine, Rouen, France; 3) Endocrinologie, CHU Dijon, France; 4) Medecine Interne, Hopital Purpan, Toulouse, France; 5) Medecine Interne, CHU Dijon, France; 6) Fondation Gillet, CHU Lyon Sud, France; 7) Centre de Genetique, CHU Dijon, France.

Splenomegaly with sea-blue histiocytes, thrombocytopenia and hypertriglyceridemia is a very rare association which has been described in only one report to date. The molecular defect consists in a deletion of leucine at position 149 in the receptor-binding region of the ApoE molecule. This mutated ApoE binds lipoproteins with high affinity to macrophages, which explains the development of splenomegaly with sea-blue histiocytes. Here we report on another family in whom the proband and his brother were diagnosed with splenomegaly, thrombocytopenia and hypertriglyceridemia. A storage disease such as Niemann-Pick type B or C was first ruled out. A 149Leu ApoE mutation was found in the brothers as well as a large band in the VLDL fraction and an elevated VLDL cholesterol to plasma triglyceride ratio in the proband. Their mother, presenting with hypertriglyceridemia only, also carried the same 149Leu mutation. The reasons of the variable expressivity of the 149Leu ApoE mutation are discussed. The coexistence of factors facilitating the development of hypertriglyceridemia and/or low HDL cholesterol level, such as metabolic syndrome or gender, could explain why the proband and his brother developed a splenomegaly with thrombocytopenia, whereas the mother did not. Moreover the presence of an ApoE2 allele in the proband likely explains the more severe phenotype we observed in this subject. In conclusion, the 149Leu ApoE mutation results in a very striking phenotype including one or all symptoms among splenomegaly, thrombocytopenia and hypertriglyceridemia, and should be considered as a differential diagnosis of storage disorders in the causes of splenomegaly with sea-blue histiocytes.

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Oxidative stress causes aldehyde adduction of proteins in cystathionine beta-synthase deficient homocystinuric mice. *K.N. MacLean, B.R. Ickes, R.W. Powers, L.S. Greiner, K.H. Overdier, J.P. Kraus, V. Kozich, L.S. Crnic, D.R. Petersen.* Sch Medicine and Pharmacy, C-233, Univ Colorado Health Sci Ctr, Denver, CO.

Cystathionine beta-synthase (CBS) catalyzes the condensation of homocysteine (Hcy) and serine to cystathionine during cysteine synthesis. CBS deficient homocystinuria (CBSDH) is accompanied by mental retardation, thrombosis and connective tissue disorders. The pathogenic mechanisms that underlie CBSDH are poorly understood. Analysis of our new mouse models of CBSDH indicate that many aspects of the pathology of this disease are due to oxidative stress which can cause the autocatalytic process of lipid peroxidation, the principal alpha, beta-unsaturated aldehydic products of which, 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA) can be cytotoxic. Plasma levels of MDA in CBSDH mice are significantly higher than those recorded in control animals. MDA and 4-HNE can impair protein function by forming covalent adducts with solvent accessible cysteine, histidine and lysine residues. We performed Immuno detection of MDA and 4-HNE adducted proteins using immunoglobulin G-purified polyclonal rabbit antibodies specific for either 4-HNE or MDA-adducted protein epitopes. We found striking evidence of both 4-HNE and MDA adducted proteins in the tissues of CBS deficient mice. The MDA and 4-HNE adducted proteins show a different intracellular distribution from each other indicating that each aldehyde is adducting different populations of proteins in these tissues. No detectable aldehyde adduction was found in wild type mice controls. The degree of adduction found in the livers of moribund CBSDH mice has never been reported previously and is so severe that it is very likely to be contributing to the profound hepatopathy incurred by these mice. The susceptibility of cysteine, lysine and histidine residues to aldehyde adduction indicates that proteins involved with cross-linking such as Fibrillin-1 and collagen could be particularly vulnerable to impairment by chronic exposure to MDA or 4-HNE adduction. Our data represents the first definitive proof of oxidative stress in CBSDH and suggests a novel mechanism for the connective tissue disturbances typically observed in this disorder.

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Initial functional characterization of conserved non-genic sequences (CNGs) on chromosome 21. *C. Attanasio[1], R. Lyle[1], E.T. Dermitzakis[2], A. Reymond[1], S.E. Antonarakis[1].* 1) Department of Genetic Medicine and Development, University of Geneva Medical School, Geneva, Switzerland; 2) The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK.

Recent comparative analysis of the human, mouse and other genomes brought to light a large number of conserved non-genic sequences (CNGs) (for example Dermitzakis et al. Nature 2002, Thomas et al. Nature 2003). Although their conservation is high enough to strongly support their functionality, the function of theses sequences remains largely unknown. We hypothesize that CNGs may be involved in transcription regulation, chromatin structure modulation, or as yet unidentified functions. To first evaluate their potential contribution in the regulation of gene transcription, we performed luciferase expression assays in 293T and HuH7 cell lines. We selected a set of 52 CNGs (200 bp, 85% ungapped identity) and 20 control sequences (single-copy non-conserved) on chromosome 21. Although a few CNGs displayed an enhancer activity, there is no statistical difference between CNGs and control sequences. This suggests that the role of the majority of CNGs may not be cis- transcriptional regulation. To then investigate if CNGs occur in regions of chromatin accessibility in different cell lines, we evaluated the DNaseI hypersensitivity of the 52 CNGs in 293T, HuH7, and K562 cells. Interestingly, our results show that some CNGs are hypersensitive, which strongly suggest they may function through binding with proteins. Moreover, some of them show cell-type dependent hypersensitivity, suggesting a tissue specific role. To further explore the function of CNGs, additional experiments in progress focus on the identification of bound proteins (footprinting, bandshifts and yeast 1-hybrid assay), chromatin structure and epigenetic features (DNA methylation and histone modification).

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Program Nr: 218 from the 2004 ASHG Annual Meeting

Sequencing the short arm of human chromosome 21. *R. Lyle[1], K. Osoegawa[2], B. ten Hallers[2], B. Zhu[2], M. Cruts[3], C. van Broeckhoven[3], C. Bird[4], J. Rogers[4], P. de Jong[2], S.E. Antonarakis[1].* 1) Genetic Medicine and Develop, Centre Medicale Univ, Geneva, Switzerland; 2) 2Children's Hospital Oakland Research Institute, 747 52nd Street, Oakland, CA 94609-1673, USA; 3) 3Department of Molecular Genetics, University of Antwerp, Universiteitsplein 1, B-2610 Antwerpen, Belgium; 4) Wellcome Trust Sanger Institute, Cambridge CB10 1SA, UK.

Although most of the human genome has been sequenced, large regions of the genome, notably the short arms of the acrocentric chromosomes (13, 14, 15, 21 and 22) remain to be sequenced. The acrocentric chromosomes are of great interest as they are involved in many translocations causing human genetic disease. Human chromosome 21 has special significance because of its involvement in Down syndrome. The sequence of the short arm of Hsa21 (21p) would thus be important not only for understanding chromosome 21 genetics and disease, but an important step in the characterisation of these unexplored regions of the genome and toward the completion of the human genome project. We have recently constructed a BAC library containing human sequence from only Hsa21 and shown that it contains clones from 21p. Preliminary sequence of 8 BAC clones reveal that these sequences have the characteristics of gene-containing euchromatic sequence. So far we have generated approximately 750kb of new sequence from 21p, estimated to be 7-15% of the total. In this project, we aim to produce a sequence contig of 21p and characterise 21p with respect to genes and repeat content.

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Program Nr: 219 from the 2004 ASHG Annual Meeting

Identification of a common and large inversion on chromosome 17q21.31 accompanied by a large segmental duplication or triplication. Expression of genes within the boundaries of the inverted region is strongly correlated with haplotype background. *H. Stefansson, V. Steinthorsdottir, G. Masson, A. Jonasdottir, A. Baker, A. Olafsdottir, B. Birgisdottir, A. Ingason, V.G. Gudnadottir, N. Desnica, K. Agnarsson., G. Thorleifsson, J. Sainz, J. Gulcher, T.E. Thorgeirsson, A. Kong, K. Stefansson.* Decode Genetics, Reykjavik, Iceland.

By isolating and genotyping clones from the RP11 library, chromosome specific clone contigs have been generated for the 17q21.31 locus. Microsatellite genotypes were essential for the assembly which was complemented by fingerprint data, BAC end alignments and available sequences from clones in the region. The assembly clearly demonstrates that the locus is inverted on one of the RP11-chromosomes from Mb 44.1 to Mb 45.0 in Build 34 of the human genome. Furthermore, gene dosage analysis reveals that copy numbers for the different genes within the locus vary from one to three per chromosome which is in line with expression analysis of genes within the inverted segment showing significantly different expression of genes with respect to haplotype orientation. Pulsed-field southern blot analysis of the locus is in keeping with the dosage analysis demonstrating at least five chromosomal variants with considerable variation in size at this locus. Markers on the inverted region are in strong LD, no recombination event between chromosomes of different orientation have been detected. Two haplotypes, H1 and H2, have been described within this locus overlapping the MAPT gene. One of the RP11 chromosomes carries the H2 haplotype and represents the inverted chromosome. H2, the inverted chromosome, is in higher frequency in Caucasians than in other ethnic groups and is in low frequency in Asians. Interestingly, although there are many tagging SNPs on H2, distinguishing H2 from H1, there is very little diversity among the H2 chromosomes based on the HapMap project and our own data although H2 is accounting for 17.5% of chromosomes in Caucasians. Inversion polymorphism and other genomic rearrangements in varying frequencies may contribute to the LD structure of the genome and may promote the formation of disease causing variants through various mechanisms.

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Program Nr: 220 from the 2004 ASHG Annual Meeting

The recurrent DNA rearrangement present in Williams-Beuren syndrome patients affects the level of expression of the non-hemizygous flanking genes. *A. Reymond[1,2], R. Lyle[2], C. Howald[2], C. Wyss[2], M.T. Zabot[3], S.E. Antonarakis[2], G. Merla[2,4]*. 1) Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland; 2) Dpt of Genetic Medicine, University of Geneva Medical School, Geneva, Switzerland; 3) Hospices Civils de Lyon, Hpital Debrousse, Lyon, France; 4) Servizio Genetica Medica, IRCCS, San Giovanni Rotondo, Italy.

An increasing number of human diseases, like the Williams-Beuren contiguous gene syndrome (WBS, OMIM#194050), are recognized to result from recurrent DNA rearrangements involving unstable genomic regions. Rearrangements are facilitated by the presence of region-specific low-copy repeats and result from nonallelic homologous recombination. It is often assumed that these aneuploidies will lead to underexpression of genes mapping to the commonly deleted region. Furthermore, it is conceivable that the large chromatin rearrangement harbored by WBS patients influences the transcription levels of genes that map centromerically or telomerically to the critical region and the repeats, even if these genes are present in two copies. To test this latter hypothesis we have used quantitative real-time PCR to accurately measure the expression of the genes mapping to the 7q11.23 region. We studied in 20 WBS patients and 10 controls the relative expression of a total of 41 genes mapping to the WBS critical region, its flanking repeats and neighboring regions in two different cell lines (skin fibroblasts and lymphoblastoids). As anticipated almost all of the genes mapping to the common deletion interval show relative levels of expression decreased by two fold in the WBS population. A decrease in relative expression, albeit not as large, is measured for the non-hemizygous genes that map on both sides of the common deletion region. This decrease is proportional to the distance relative to the chromosomal rearrangement. Our results suggest that in genomic disorder not only the aneuploid genes but also the genes that map close to the rearrangement should be considered as candidate genes for the specific features of these respective pathologies.

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Hypertension: Risk in Williams Syndrome is determined by gender and parent of origin. *H.J. Antoine*[1], *H. Wijesuriya*[1], *M. Appelbaum*[2], *X-N. Chen*[1], *A. Simon*[1], *F. Rose*[2], *J.R. Korenberg*[1]. 1) Med Genet, Cedars-Sinai/UCLA, Los Angeles, CA; 2) UCSD/Salk Inst, La Jolla, CA.

Hypertension (HTN) is the leading independent risk factor for stroke and cardiovascular disease. Despite intensive study, the genetic origins of essential hypertension are largely unknown although it accounts for more than 95% of cases. We now report that the elevated risk of HTN in Williams Syndrome (WS) is significantly modified by both gender and parental origin of the deletion. We initially determined the parental origin of the deletion and HTN in 53 families with WS (ages 12-51y) and 32% reported HTN. We found HTN in 21% (6/29) of those with maternally derived deletions in contrast to 46% (11/24) of those with paternally derived deletions. To better model the HTN risk factors found in the normal population, we reasoned that gender might also affect the risk of HTN in WS, independent of parental origin of the deletion. Unexpectedly, we found a highly significant effect in females with 0% (0/17) HTN in those with maternally derived deletions vs 40% (6/15) in those with paternally derived deletions. In contrast, males had an increased risk of HTN regardless of the parental origin of the deletion with HTN found in 56% (5/9) with paternally derived and 50% (6/12) with maternally derived deletions. To test the significance of these findings, we examined a second independent population of 30 WS families and observed a similar trend: in females, only 10% (1/10) of maternal but 29% (2/7) of paternal deletions had HTN in contrast to 67% (2/3) of paternal and 40% (4/10) of maternal males. Combining both populations in a multivariate model, we find that the risk of HTN is independently related to gender ($p=0.0002$), age ($p=0.0002$), and parental origin of the deletion ($p=0.0107$). In summary, the risk of HTN appears to be dramatically affected by gender and the parental origin of the deletion in WS. This suggests an imprinted gene(s) in the WS region affecting HTN as well as a potential role for gender specific epigenetic imprinting in HTN. We propose that incorporating this in current models may improve the ability to define genes for essential HTN in the normal population.

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Program Nr: 222 from the 2004 ASHG Annual Meeting

Impact on gene expression of a transgene insertion and 5 Mb deletion in a mouse model of Prader-Willi and Angelman syndromes (PWS and AS). *K.C. Claiborn[1], M. Stefan[1], E. Stasiek[2], J-H. Chai[1], T. Ohta[1], T. Portis[3], R. Longnecker[3], J.M. Greally[2], R.D. Nicholls[1].* 1) University of Pennsylvania, Philadelphia, PA; 2) Albert Einstein College of Medicine, The Bronx, NY; 3) Northwestern University, Chicago, IL.

PWS and AS patients typically have a 5 Mb deletion of human chromosome 15q11-q13, that differs in parental origin due to loss of imprinted genes. We previously described a transgene insertion-deletion ($Tg^{ins-del}$) of chromosome 7C in a mouse model of PWS and AS. Here, we characterize the deletion endpoints and describe the impact of the $Tg^{ins-del}$ on expression of flanking genes. Allele-specific imprinting, quantitative (Q) RT-PCR, fluorescence *in situ* hybridization (FISH), and brain microarray data demonstrate that 13 imprinted and 9 non-imprinted genes are included in the Tg^{del}, equivalent to class I deletions in PWS and AS. In contrast, microarray or QRT-PCR on Tg^{PWS} *vs.* wildtype (WT) mouse brain for 4 genes in a gene desert extending 6.1 Mb centromeric and 16 genes in the 5.6 Mb region telomeric of the deletion indicate normal expression levels, demonstrating that the 80 copies of the *Lmp2a* Tg do not induce silencing and that there are no additional linked rearrangements. QRT-PCR studies of 5' *Luzp2* suggest a proximal breakpoint of the $Tg^{ins-del}$ within the 3' end of this 600 kb locus, which is being further refined by FISH and single nucleotide polymorphism inheritance analyses. Most intriguingly, QRT-PCR data for 5' *Chrna7* indicate 0.5-fold levels in Tg^{PWS} and Tg^{AS} *vs.* WT brain, suggestive of deletion, whereas similar analyses on liver show a 15-fold increase in expression. Control mice with B-cell lineage expression of *Lmp2a* show normal expression of *Chrna7*. These results are explained by a model positing a telomeric $Tg^{ins-del}$ breakpoint within a 60 kb region in 3' *Chrna7* with deletion of a neuronal transcriptional enhancer for this schizophrenia-susceptibility candidate gene, while the *Chrna7* promoter is upregulated by the Tg immunoglobulin enhancer active in B cells of the neonatal liver. Consistent with this model, we detect a fusion *Chrna7-Lmp2a* RNA in Tg^{AS} but not WT liver.

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Identification of a Novel Component of the PWS Imprinting Center. *S. Rodriguez-Jato[1], R.D. Nicholls[2], D.J. Driscoll[1], T.P. Yang[1].* 1) University of Florida, Gainesville, FL; 2) University of Pennsylvania, Philadelphia, PA.

The Prader-Willi/Angelman syndrome (PWS/AS) region encompasses a cluster of imprinted genes that are coordinately regulated by a bipartite imprinting center (IC). The PWS-IC appears to be required for somatic maintenance of the paternal epigenotype, is located in the 5' region of the *SNURF-SNRPN* gene, and is associated with two candidate regulatory regions defined by paternal-specific DNase I hypersensitive sites: DHS1 at the *SNURF-SNRPN* promoter, and DHS2 located within intron 1. *In vivo* footprint analysis of the promoter identified multiple allele-specific footprints, including footprints at potential NRF-1, CTCF, and E2F binding sites on the paternal allele. Analysis of DH2 by transient expression assays identified a novel activator function that activated the *SNURF-SNRPN* promoter. This *cis*-acting intronic activator is highly conserved between humans and rodents and contains potential binding sites for SP1, NRF-1 and YY1. ChIP assays confirmed binding of all three transcription factors in this region only on the paternal chromosome, and histones associated with the activator on the paternal chromosome showed modifications characteristic of regulatory regions in active chromatin. ChIP analysis also demonstrated the strong association of unphosphorylated RNA polymerase II with the activator on the paternal allele, suggesting a role for this activator in recruiting RNA pol II to the *SNURF-SNRPN* locus and potentially transferring it to the transcriptionally active promoter. Based on these data and comparison with existing mouse models for PWS, we propose that the activator associated with DHS2 is a newly identified functional component of the PWS-IC. We postulate that this intronic activator, in conjunction with the *SNURF-SNRPN* promoter region, is involved in the generation of an active chromatin hub (ACH) similar to that proposed for the -globin locus. This ACH would consist of regulatory elements within DHS1 and DHS2 that cluster and interact with the promoters of paternally expressed genes on the paternal chromosome to form a holocomplex that maintains the paternal epigenotype and a transcriptionally active chromatin environment.

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Aberrant Methylation at HOXA10 May Be Responsible for Its Aberrant Expression In the Endometrium of Women with Endometriosis. *S. Guo[1], Y. Wu[1], G. Halverson[2], Z. Basir[3], E. Strawn[2], P. Yan[4].* 1) Dept of Pediatrics, Box 26509, Med Col Wisconsin, Milwaukee, WI; 2) Dept of OB/GY, Box 26509, Med Col Wisconsin, Milwaukee, WI; 3) Dept of Pathology, Med Col Wisconsin, Milwaukee, WI; 4) Division of Human Cancer, Cancer Center, Genetics,Ohio State.

There is mounting evidence suggesting that the eutopic endometrium in women with endometriosis has various alterations as compared with those from women without. These reported aberrant alterations in the endometrium of women with endometriosis beg for an obvious question: What cause these alterations? Epigenetic alterations, especially methylation changes, provide a flexible yet stable mechanism that may be potentially responsible for these alterations. However, the epigenetic role in the pathogenesis of endometriosis so far has received little attention. In this study, we investigated patterns of methylations in the transcription start sites of HOXA10, based on the previous report that the expression of HOXA10 gene in the endometrium of women with endometriosis failed to show the equivalent up-regulation at the time of implantation. HOXA10 expression peaks during the window of implantation, suggesting a possible role in uterine receptivity. In 6 women with endometriosis and 9 women without, we found by bisulfite sequencing aberrant methylations in various regions of HOXA10 gene in the endometrium from women with endometriosis as compared with normal controls, and the difference in methylation patterns is statistically highly significant. Methylation specific PCR assays also confirmed our finding. In addition, quantitative real-time RT-PCR analysis showed, as reported, reduced expression at HOXA10 in endometrium from women with endometriosis as compared with those without. These results suggest that aberrant methylation at HOXA10 is responsible for its aberrant expression in the endometrium from women with endometriosis. In addition, the results suggest that endometriosis may be also an epigenetic disease.

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Molecular dissection of the events leading to inactivation of the *FMR1* gene. *G. Neri[1], E. Tabolacci[1], R. Pietrobono[1], F. Zalfa[2], I. Zito[1], A. Terracciano[1], C. Bagni[2,3], B.A. Oostra[4], P. Chiurazzi[1]*. 1) Istituto di Genetica Medica, Universit Cattolica, Rome, Italy; 2) Istituto di Farmacologia, Fondazione Santa Lucia IRCCS, Rome, Italy; 3) Department of Biology, University of Roma Tor Vergata, Rome, Italy; 4) Department of Clinical Genetics, Erasmus Medical Center, Erasmus University, Rotterdam, The Netherlands.

Through the analysis of a cell line derived from a rare individual of normal intelligence with an unmethylated full mutation of the *FMR1* gene, we present a detailed description of the molecular events leading to the inactivation of the gene and to fragile X syndrome. We found that complete demethylation of the entire promoter region, including the expanded CGG repeat, correlates with methylation of lysine 4 residue on the N-tail of histone H3 (H3-K4), as in a wild-type control. Slightly elevated levels of *FMR1* mRNA were detected by real-time fluorescent RT-PCR (1 to 1.5 times compared to wild-type), while translational efficiency was clearly reduced (-40%), as judged by polysome profiling, and the FMRP protein was likewise reduced to approximately 30% of a control. These results underline once more that CGG repeat amplification *per se* does not prevent *FMR1* transcription and FMRP production in the absence of DNA methylation. Even more strikingly, we found by chromatin immunoprecipitation (ChIP) that this cell line has deacetylated histones H3 and H4 as well as methylated lysine 9 on histone H3 (H3-K9) as in the inactive methylated full mutation born by fragile X patients, showing that these two epigenetic marks can coexist with active gene transcription. Our observations also suggest that the molecular pathways causing DNA demethylation and H3-K4 methylation are independent from those leading to histone deacetylation and H3-K9 methylation.

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Downstream effects of MECP2 mutations in the frontal cortex of patients with Rett syndrome. *J. Gibson*[1, 2], *J. Stern*[3], *B. Slobedman*[3], *J. Christodoulou*[1, 2]. 1) Western Sydney Genetics Program, Children's Hospital Westmead, Sydney; 2) School of Paediatrics and Child Health, University of Sydney; 3) Centre for Virus Research, Westmead Millennium Institute, Sydney.

Rett syndrome (RTT) is an X linked neurodevelopmental disorder with characteristic features including severe cognitive impairment, stereotypic hand movements and breathing irregularities. The RTT brain shows regional pathology such as a decrease in the size of individual neurons, an increase in the packing density of neurons and a decrease in dendritic branching. Frontal, motor and inferior temporal cortices are affected to a greater extent than the occipital cortex, which appears to escape neuropathological changes. Most cases of RTT are caused by mutations in the transcriptional repressor MECP2 (methyl-CpG binding protein 2) gene. This raises the question of whether MeCP2 is a global transcriptional repressor or whether it targets a specific subset of genes.We have used a combination of cDNA microarray and siRNA technology to evaluate aberrant gene expression in RTT post mortem patient brains and neuron-like cell lines (respectively). Multi-factorial microarray experiments were used to compare affected (frontal cortex) with unaffected (occipital cortex) regions of RTT and non-RTT brains. We also transfected a neuroblastoma cell line with dsRNA that has homology to MECP2 and obtained greater than 80% knockdown. A small number of genes were differentially expressed, and this was confirmed using Real-Time PCR, and protein expression was evaluated using Western blot analysis.One of the genes we found to be overexpressed in RTT is a secreted glycoprotein associated with neurodegenerative disorders of both development and aging. Other genes have also been identified with roles in synaptic vesicle recycling and transmembrane signalling.Our approach of comparing affected and unaffected brain regions from the same RTT individual has allowed us to show for the first time in humans that there may be a specific subset of genes that are involved in RTT. Characterisation of this subset of genes will help elucidate the pathways that may contribute to this phenotypically complex disorder.

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Minding your LOD's and q's: How Linkage Effect Size Bias Can Contribute to the Winner's Curse in Replication Association Studies. *B.F. Voight, N.J. Cox.* Department of Human Genetics, Univ. of Chicago, Chicago, IL.

A major challenge to the successful identification of genetic variation for complex traits is the replication of an initially significant linkage or association signal. The largest of the linkage mapping signals (those most likely to lead to positional cloning efforts) are rarely replicated, and most follow-up association studies fail to reproduce the initial signal (Hirschhorn *et al*., 2002). These findings appear related to the phenomenon of the *winner's curse*: an initial finding overestimates the true effect size, and follow-up studies, powered to replicate effects based on this overestimate, are underpowered to detect more modest actual effects. A contributing factor for the curse can arise when studies take advantage of linkage-based case samples. Consider the situation when a family-based sample provides strong evidence for linkage in a region, and cases for a replication study include a single affected chosen from each pedigree used in the linkage study. While design is powerful for detecting associations at susceptibility loci in the vicinity of the initial linkage signal (Fingerlin *et al*., 2004), if cases for the replication study are chosen from a family-based sample with evidence for linkage *other* than at the region identified in the first study, there may be substantially less power to replicate the initial association.

We investigated the magnitude of this effect under models of locus heterogeneity, estimating allele frequencies at all causal loci conditional on a single locus showing evidence for linkage at a pre-specified threshold. We found that the allele frequencies at other susceptibility loci decreased steadily, proportional to the magnitude of linkage conditioned upon and to the case sample size. We observed a sizable decrease in the expected allele frequencies for other susceptibility loci that sometimes placed the expected frequency for the linkage-based selection strategy lower than the expected frequency for a population-based (as well as to a family-history positive) case selection strategy. Our results provide an additional perspective on the winner's curse, and may suggest compensatory strategies to minimize the cursing.

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Program Nr: 228 from the 2004 ASHG Annual Meeting

Efficient Study Designs for Test of Association using Sibship Data and Unrelated Controls. *M. Li, M. Boehnke, G.R. Abecasis.* Dept Biostat, Univ Michigan.

Mapping of complex diseases often involves linkage analysis, followed by association studies between phenotypes and marker genotypes. Association studies can use case-control or family-based designs that are based on transmission disequilibrium. Given a fixed amount of genotyping resources, it is important to know which study design is most efficient. To address this problem, we extended the likelihood-based method of Li et al. (2003), which assesses whether there is disease locus-SNP linkage disequilibrium (LD), to analysis of sibships of arbitrary size and phenotype configuration. Here, LD suggests the SNP accounts in part for the linkage signal. Our method seeks the overall evidence of association and is more powerful than TDT tests. Consider scenarios where genotypes are available for unrelated cases, affected sib pairs (ASPs), or only one sib per ASP. We construct designs that use cases only, and others that use unaffected sibs or unrelated unaffecteds as controls. Different combinations of case and control types result in 3x3=9 study designs.To compare the efficiency of these designs, we fixed the total number of SNP genotypes and simulated data under a variety of genetic models. Our results indicate that for a broad class of genetic models: (1) the design using one case per ASP together with unrelated controls (D1) is the most efficient; (2) the case-control design is less efficient than the ASP-control design (D2); and (3) sib controls are less helpful than unrelated controls so that for most models, the discordant sib pair (DSP) design is the least powerful. For example, for a disease with 5% prevalence, allele freq .3, and $_s$=1.02, power is 40% for =0.01 when the disease-SNP r^2=.5 and the sample size is 1000 for the case-control design, and increases to 60% for the D2 design and 80% for the D1 design. We also compared the power of our likelihood ratio test with standard tests for case-control design, DSP design, and affecteds only design. Results indicate that our test is more powerful in most cases. Further, there is a useful improvement of power of our test by including flanking markers. Our findings should help researchers to design association studies efficiently in terms of power and genotyping resource allocation.

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Selecting tag single-nucleotide polymorphisms to capture common variation in the human genome. *P.I.W. de Bakker*[1,2,3], *I. Pe'er*[3,4], *S.F. Schaffner*[3], *S.B. Gabriel*[3], *M.J. Daly*[3,4], *D. Altshuler*[1,2,3]. 1) Department of Molecular Biology, Massachusetts General Hospital, Boston, MA; 2) Department of Genetics, Harvard Medical School, Boston, MA; 3) Broad Institute of Harvard and MIT, Cambridge, MA; 4) Whitehead Institute for Biomedical Research, Cambridge, MA.

Linkage disequilibrium (LD) between single-nucleotide polymorphisms (SNPs) can be exploited for identifying disease associations. Given the HapMap resource, a major challenge is the selection of markers (tag SNPs) that capture common genetic variation both efficiently and powerfully.
Important questions related to tag SNP selection include: What is the power and efficiency of pairwise relative to multi-marker (haplotype) approaches? What is the impact of sample size and ascertainment in the initial HapMap on the performance of tag SNPs in association studies? How many tag SNPs are necessary to capture the common variation comprehensively across the genome? How well will tag SNPs chosen in one set of DNAs transfer to independent DNA samples (either a second sample from the same population, or across different populations)?
We describe a novel tagging strategy that combines the simplicity of pairwise methods with the potential efficiency of multimarker approaches. We avoid overfitting and unbounded haplotype tests in the association phase by (a) using only those multiallelic combinations in which the alleles are themselves in strong LD, and (b) explicitly recording the allelic hypotheses that are to be tested in the subsequent analysis.
We present the performance of our approach in comparison to simple pairwise tagging in the HapMap ENCODE data, comprising 500-kb regions obtained by resequencing in 48 and genotyping in 270 individuals. The complete nature of these data allows us to estimate the number of tag SNPs for a whole-genome scan. We also evaluate the effect of increasing marker density in the HapMap on tagging performance. Finally, we assess the tag SNPs selected from HapMap samples in other DNA samples: one additional sample set drawn from the same population, and a second set of DNAs from independent sources.

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Program Nr: 230 from the 2004 ASHG Annual Meeting

Demonstrating stratification in a European-derived population. *J.N. Hirschhorn[1,2,3], C. Campbell[1,2,3], E. Ogburn[1,2], H. Lyon[1,2,3], M. Freedman[2,4], L. Groop[5], D. Altshuler[1,2,4], K. Ardlie[6]*. 1) Genetics, Harvard Med Sch, Boston, MA; 2) Broad Institute of MIT and Harvard, Cambridge, MA; 3) Genetics and Endocrinology, Children's Hosp, Boston, MA; 4) Molecular Biology, Mass General Hosp, Boston, MA; 5) Endocrinology, Lund Univ, Malmo, Sweden; 6) Genomics Collaborative, Cambridge, MA.

Population stratification due to ethnic admixture may represent an important source of false positive associations, but empiric studies using large sets of random markers have found little evidence of stratification in well-matched studies of European-derived populations. We studied adult height, a phenotype that varies widely across Europe and thus might be particularly prone to stratification. We genotyped over one hundred random, unlinked SNPs in European-American individuals in the 5th-10th percentile (n=176) and 90th-95th percentile (n =192) for height; these individuals were chosen to be representative of a larger set used for association studies of height (n = 2288). We observed no evidence for stratification using these SNPs: the mean ^{2} was 0.94 (expected for no stratification, 1.00) and STRUCTURE did not reveal evidence of population substructure. However, we then typed the -13910C/T SNP from the *LCT* gene, which varies widely in frequency across Europe (the frequency of the T allele is 5-40% in Italy but 40-80% in Northern Europe). In the larger sample (n=2288), we observed a strong association with height (p 10^{-6}). This apparent association was likely due to stratification: we used grandparental country of origin to re-match the 902 individuals with at least 1 non-US-born grandparent, and this greatly reduced the apparent association. Furthermore, the *LCT* marker was not associated with height in a similar panel from Poland or in a family-based study from Scandinavia. Taken together, our results suggest that European-American populations can be susceptible to stratification, and that typing unusual markers that vary widely in frequency across Europe will be helpful to detect stratification. Conversely, apparent associations are more likely due to stratification if the associated marker itself varies in frequency across Europe.

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Effect of population stratification on case-control association studies: false-positive rates and their limiting behavior as number of subpopulations increases. *P. Gorroochurn*[1], *S.E. Hodge*[1,2], *G. Heiman*[1], *D.A. Greenberg*[1]. 1) Division of Statistical Genetics, Department of Biostatistics, Columbia University, New York, NY; 2) New York State Psychiatric Institute, New York, NY.

There has been considerable debate in the literature concerning bias in case-control association mapping studies due to population stratification (Wacholder, Rothman, and Caporaso 2000; Wacholder, Rothman, and Caporaso 2002; Thomas and Witte 2002). Recently, there has been a growing perception that the effect of stratification might have been previously exaggerated. We perform a theoretical analysis of the effects of population stratification by measuring the inflation in the test's Type I error. Using a model of stratified sampling, we derive an exact expression for the Type I error as a function of population parameters and sample size. We give necessary and sufficient conditions for the bias to vanish when there is no statistical association between disease and marker genotype in each of the subpopulations making up the total population. We also investigate the variation of bias with increasing subpopulations and show, both theoretically and by using simulations, that the bias can sometimes be quite substantial even with a very large number of subpopulations. We show that the bias does not decrease with an increasing number of subpopulations when disease prevalence is inversely related to subpopulation size, and that bias decreases only very slowly with an increasing number of subpopulations when the ranges of marker genotype frequency or disease prevalence are wide.

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Measurement of linkage disequilibrium (LD) parameter D' for complex traits via the posterior probability of LD (PPL-LD) changes the LD picture within CAPON, a large candidate gene for schizophrenia. *VJ. Vieland[1], X. Yang[1], M. Shi[1], A. Bassett[2], L. Brzustowicz[3].* 1) Univ Iowa, Iowa City, IA; 2) Univ Toronto, Toronto, ON; 3) Rutgers Univ, Piscataway, NJ.

The evaluation of LD between a putative disease locus and a set of markers in or around a candidate gene is a crucial step in gene discovery for complex diseases, but the unknown parameters of the trait model complicate analysis and interpretation of LD data. One approach is to forego estimation in favor of a test of significance, but significance levels can vary from one marker to another within a small region for reasons having little to do with LD per se. We have developed an approach to estimating the LD coefficient D' from family data, based on the Bayes Ratio (BR), which involves integrating out the nuisance parameters of the trait model while allowing for locus heterogeneity. The result is a marginal posterior density either in the recombination fraction and D', or in D' alone. The mode of the BR is a point estimator of D', while the integral is the posterior probability of LD (or LD and linkage). The method can be applied to arbitrary pedigree structures and multiallelic markers, and is readily extended to measurement of marker-marker disequilibrium based on the full likelihood for all data; but it involves substantial computational burden. We have thus far implemented an efficient version for SNPs, and we show here simulation results for variably-sized nuclear families. The estimate of D' behaves quite well even in relatively small (N=50) heterogeneous samples. We have applied the method to a set of SNPs within CAPON, a large (~300 kb genomic extent) previously mapped candidate gene on 1q22 showing LD with schizophrenia, in order to more accurately determine the most promising regions of the gene for molecular focus. Our PPL-LD estimates of LD differ from previously published results, indicating even stronger LD with schizophrenia and a different pattern of LD across the gene.

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Program Nr: 233 from the 2004 ASHG Annual Meeting

Exhaustive Allelic TDT: How to use haplotypes for disease association studies at genomic scales. *D.J. Cutler, A. Chakravarti, S. Lin.* McKusick-Nathans Institute of Genetic MedicineJohns Hopkins University School of MedicineBaltimore, MD 21205.

We describe a novel, computational method which makes it feasible to analyze and interpret genome-scale data for disease association mapping. Along the way, we dispel several myths, among them: 1) one cannot use SNPs in whole genome association studies, because the multiple test correction will destroy power, 2) one cannot use haplotypes in association studies since there are so many of them and one cannot determine a priori which ones to test, 3) one cannot use haplotypes in association studies because the multiple test correction will destroy power, 4) common SNPs cannot be used to find rare disease alleles. Our computational method incorporates haplotype data, accounts for multiple testing, and runs very efficiently in real-time. We test this method on simulated and real data. We conclude that applying our algorithm to genome-scale SNP data generated by extant technologies can allow for the detection of disease mutations of small affect. In essence, we are announcing that the promises of association studies discussed by Lander (1996) and Francis Collins, Mark Guyer and Aravinda Chakravarti (1997) are finally realizable. Moreover, our results shows that we can detect both rare and common disease alleles by association, effectively making the debate between the common disease common variant and common disease rare variant hypotheses moot. These results have major implications for the conduct of association studies and more broadly, genetic study designs.

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Program Nr: 234 from the 2004 ASHG Annual Meeting

Screening Large-Scale Association Study Data: Exploiting Multi-Gene Interactions.
K.L. Lunetta, J. Segal, B. Hayward, P. Van Eerdewegh. Oscient Pharmaceuticals, Waltham, MA.

Genome-wide and candidate-region association studies for complex phenotypes may produce genotypes on hundreds of thousands of single nucleotide polymorphisms (SNPs). A logical approach to dealing with massive numbers of SNPs is to first screen the data using some test: for example, rank SNPs by p-value, and retain those with the lowest p-values for subsequent study. SNPs having large interaction effects on a disease but small marginal effects in a population are unlikely to be retained when univariate tests are used for screening, and model-based screens that pre-specify interactions are impractical for data sets with thousands of SNPs. Random forest analysis (RFA; Breiman 2001) is an alternative method that produces a single measure of importance, *I*, for each predictor variable that takes into account interactions among variables without requiring model specification. Interactions increase *I* for each interacting variable, making them more likely to be ranked high relative to other variables. We test the performance of RFA as a screening procedure using several complex disease models with sib recurrence risk $_S$=2 and up to 32 risk-associated SNPs (rSNPs) in 32 genes. **Results:** The larger the number of rSNPs interacting together, the better RFA performs compared to univariate analysis in identifying rSNPs. For example, in a model consisting of 8 independent systems of 4 interacting rSNPs, we screen data consisting of 4 of the rSNPs and 96 unassociated SNPs. For the 4 rSNPs, we chose 1) a single rSNP from each of 4 independent systems, 2) pairs of interacting rSNPs from two systems, 3) three interacting rSNPs from one system and one rSNP from a second system, or 4) 4 rSNPs from one system. Using RFA, the number of top-ranked SNPs that must be retained to have 80% probability of including all 4 rSNPs is 50, 34, 22, and 5, respectively for the 4 scenarios; using the Fisher Exact test all scenarios require an average of 43 SNPs retained. Results from other models are similar. RFA is a significantly more efficient screening tool than the univariate Fisher Exact test when unknown interactions among SNPs or SNPs and environmental covariates increase disease risk.

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Program Nr: 235 from the 2004 ASHG Annual Meeting

MDR Reveals Gene-Gene Interactions Associated with Multiple Sclerosis. *A.A. Motsinger*[1], *D. Brassat*[2], *J.R. Oksenberg*[2], *K. Walker*[3], *L.L. Steiner*[3], *H.A. Erlich*[3], *L. Barcellos*[4], *M.A. Pericak-Vance*[5], *S.L. Hauser*[2], *J.L. Haines*[1], *M.D. Ritchie*[1]. 1) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN; 2) Department of Neurology, School of Medicine, University of California at San Francisco, CA; 3) Department of Human Genetics, Roche Molecular Systems, Alameda, CA; 4) Division of Epidemiology, School of Public Health, UC Berkeley, Berkeley, CA; 5) Center for Human Genetics, Duke University Medical Center, Durham, NC.

Multiple Sclerosis (MS) is one of many common diseases with a complex mode of inheritance and a lack of consistent single locus association results. Due to the complexity of this autoimmune disorder, many genes and/or environmental factors may play a role in disease susceptibility. The goal of this study was to determine whether interactions among immune system genes are associated with MS. We applied the Multifactor Dimensionality Reduction (MDR) method to detect interactions in a sample of 570 African Americans: 359 MS cases and 211 unrelated healthy controls. We also applied MDR to a sample of 100 African American discordant sib pairs (DSP). MDR is a powerful new statistical approach used to detect gene interactions in the absence of main effects. We selected 51 candidate genes, all of which function in the immune system. In the case-control analysis, we detected a single locus association of IL4R (Q551R) that was able to predict disease status with a prediction accuracy of 75.61% ($p<0.01$). We also detected a statistically significant interaction between IL4R (Q551R), CD14 (-260), and IL5RA (-80), which had a prediction accuracy of 76.10% ($p<0.01$). These results indicate that while there is a main effect of IL4R, there is also an interaction including IL4R that improves the ability to predict disease status. In the DSP analysis, we detected a four locus interaction between IL4R (I50V), CCR5 (promoter), IL1A (-889), and CSF2 (I117T) which was able to predict disease status with a prediction accuracy of 71.65% ($p<0.01$). This study illustrates the importance of considering high-order gene interactions in addition to single locus association in studies of complex diseases such as MS.

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Estimation of the Location of Susceptibility Genes by Incorporating Parent-of-Origin Effects into Multipoint Affected Sib Pair Analyses. *W.M. Chen[1], M.D. Fallin[2], V.K. Lasseter[3], A.E. Pulver[3], K.Y. Liang[1]*. 1) Dept. of Biostatistics, Johns Hopkins Univ., Baltimore, MD; 2) Dept. of Epidemiology, Johns Hopkins Univ., Baltimore, MD; 3) Dept. of Psychiatry, Johns Hopkins Univ., Baltimore, MD.

Parent-of-origin effects may be an important mechanism for several complex diseases. We present a new method for taking parent-of-origin effect into account when estimating the location of a susceptibility gene. In contrast to traditional estimation procedures via LOD scores, our approach estimates the location of susceptibility gene by use of Generalized Estimating Equations (GEE) based on all available sibling IBD sharing from paternal chromosome and maternal chromosome respectively. We performed extensive computer simulations, comparing the performance of our method with a GEE approach that does not include parent-of-origin effects, a conditional-logistic model approach (as implemented in LODPAL), and a non-parametric linkage approach (NPL). We found in the presence of parent-of-origin effect, our proposed approach provides the most precise location estimate, and the associated 95% CI (through bootstrap) has ~95% coverage probability in all scenarios being investigated. In comparison, the confidence intervals given by the conditional-logistic model approach have a very low coverage probability, and the confidence intervals obtained by the NPL approach are too wide. Comparing to the GEE approach that does not include parent-of-origin effects, the 95% CI provided by our new method is much shorter (up to 1.4 times shorter according to analytical calculations) in the presence of parent-of-origin effect, while being similar in the absence of parent-of-origin effect. We have implemented our approach in the GENEFINDER software package (Liang et al. 2001). We applied our method to data from a genome scan for bipolar disorder. Our analyses agree with previous findings on the parent-of-origin effect on chromosome 18, but with a different location estimate and a narrower 95% CI.

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Program Nr: 237 from the 2004 ASHG Annual Meeting

The Y deletion gr/gr confers susceptibility to testicular germ cell cancer. *K.L. Nathanson[1,4], P.A. Kanetsky[3,4], R. Hawes[5], R. Letrero[1], D. Dudakia[5], K. Robertson[1, 4], L. Johnson[5], D. Vaughn[2,4], M.R. Stratton[5], E.A. Rapley[5], International Testicular Cancer Linkage Consortium.* 1) Dept Medicine, Med Genetics; 2) Hem-Onc; 3) Dept Biostatistics and Epidemiology; 4) Abramson Cancer Center, Univ of Pennsylvania School of Medicine, Philadelphia PA; 5) Institute of Cancer Research, Section of Cancer Genetics, Sutton, Surrey UK.

Testicular germ cell cancer (TGCT) is the most common cancer in men ages 20-40. Multiple epidemiological studies have linked male infertility and testicular germ cell cancer. TGCT patients often have abnormal semen characteristics in excess of what can easily be explained by a local tumor or general effect of cancer. Increased risk of TGCT has been associated with fewer than expected children (subfertility) and with abnormal spermatogenesis. Presumably the common occurrence of testicular cancer and male infertility is related to impaired germ cell differentiation. Recently, a novel deletion in the Y chromosome has been described (*gr/gr*), which is a risk factor for spermatogenic failure. The *gr/gr* deletion demonstrates incomplete penetrance and hence can be transmitted from father to son. Based on these data, we undertook a study to determine whether the *gr/gr* deletion also is associated with an increased risk of developing testicular germ cell cancer. Cases were comprised of US (n=111) and UK (n=356) hospital series and probands (n=341) from International Testicular Cancer Linkage Consortium (ITCLC) families. The majority of controls (n=911) were population-based from the US and UK. Overall, the *gr/gr* deletion was seen in 3.3% of cases as compared to 1.2% of controls, p=0.004. The frequency of the *gr/gr* deletion was the same in both the hospital based series without a family history of TGCT and the probands from the ITCLC. The Y deletion *gr/gr* appears to confer susceptibility equivalently to both sporadic and familial testicular cancer, suggesting that it acts in concert with other factors in the development of testicular cancer. Our preliminary data suggests that the Y deletion *gr/gr* is the first potential genetic variant to 1) link male infertility and TGCT, and 2) confer susceptibility to TGCT. We are following up with further studies.

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Program Nr: 238 from the 2004 ASHG Annual Meeting

Molecular genetic profiling of uveal melanoma reveals clinically significant molecular subclasses. *M. Abdel-Rahman*[1, 3], *L. Shen*[1], *D. Jones*[2], *E. Craig*[1], *F. Davidorf*[1], *C. Eng*[3]. 1) Ophthalmology, The Ohio State Univ, Columbus, OH; 2) Div. of Epidemiology and Biometrics, The Ohio State Univ, Columbus, OH; 3) Human Cancer Genetics Program, The Ohio State Univ, Columbus, OH.

Uveal melanoma (UM) is the most common primary intraocular malignancy in adults. UM and cutaneous melanomas (CM) have similar embryological origin from the neural crest, but the 2 tumors differ in their biological behavior and associated chromosomal abnormality. However, none of the chromosomal alteration are exclusive to either UM or CM. The aims of our study are to: identify molecular genetic alterations associated with UM that differentiate them from metastatic CM; and to identify potential molecular alterations associated with metastatic UM. We used a combination of comparative genomic hybridization (CGH) and gene expression profiling to generate global DNA and RNA profiles for 9 primary UMs and 3 metastatic CMs. All UMs (8) with available CGH data showed alterations of chromosome 3, 2 with partial gains of 3p and 6 with complete loss of whole arm. Only 1/3 CM showed alteration of chromosome 3. Chromosome 6 changes were seen in both UM (4/8) and CM (2/3). Molecular profiling differentiated CMs from primary UMs and detected 2 molecular subtypes of UMs unrelated to the status of chromosome 3. One of those UM subtypes showed partial similarity to the molecular pattern of CMs. The expression of genes coding for major histocompatibility cluster (MHC) and extra-cellular matrix, and confirmed by immunohistochemistry on 62 primary tumors, was significantly higher in metastatic CMs relative to UMs. This may explain some of the biological differences between UMs and CMs. Further, metastatic UMs and CMs showed a significant ($p=0.02$) under-expression of PTEN mRNA relative to non-metastatic tumors. The expression of PTEN was validated using quantitative RT-PCR. In conclusion, our data suggest an important role of extracellular matrix and MHC expression in the pathogenesis of metastatic CMs. Our data also suggest a role for PTEN down-regulation in the progression of both CM and UM.

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Program Nr: 239 from the 2004 ASHG Annual Meeting

Fluorescence in situ hybridization (FISH) targeting plasma cells improves the detection of multiple myeloma: phenotype/genotype correlation in residual disease detection. *M.L. Slovak, V. Bedell, K. Pagel, K.L. Chang, D. Smith, G. Somlo.* City of Hope Natl Medical Ctr, Duarte, CA.

Standard FISH (SF) detects karyotypic anomalies in multiple myeloma (MM) when tumor burden exceeds 20%; however, the detection of residual MM by SF post treatment correlates poorly with pathology assessment. Using a combined May-Grunwald morphology/FISH approach targeting plasma cells, we investigated 115 samples from 100 MM patients (pts) receiving diverse therapies and at various stages in their treatment using FISH probes to detect immunoglobulin heavy-chain (IGH) rearrangements, -13/13q-, 17p deletions, or hyperdiploidy in plasma cells. Target FISH results were correlated with cytogenetics (CG), SF, and pathology. Target FISH identified MM aberrations in 51/115 (44%) cases, a frequency comparable to pathology, 50/110 (45%), with IGH in 38 cases (74.5%), del(13q)/-13 in 20 cases(39%), hyperdiploidy in 13 cases (25.5%), and 17p deletions in 5 cases (9.8%). The presence of plasma cells as low as 0.1% on the cytospin slide allowed for the positive identification of FISH abnormalities and was sensitive enough to detect an IGH rearrangement in a sample with 1% plasma cells by morphologic examination. SF using 3-probes in the first 54 pts detected residual disease in 3/54 (5.5%) compared to 23/54 (42.5%) by target FISH. The level of detection by SF was 3.3%-3.8%(just above background) but target FISH detected IGH rearrangements in 53-100% of plasma cells. CG identified residual MM in 11/111 cases (10%) and other hematological disorders in five patients. One case showed the co-existence of CLL, 3 pts showed cytogenetic evidence of MDS without evidence of MM [del(20q), del(13q), and dup(1q)] and 2 pts had both MDS and MM. Because del(13q) and dup(1q) are common in both MM and MDS, genotype/phenotype correlation underscores patient care concerns, especially in a setting of autologous vs. allogeneic stem cell transplant. These findings indicate target FISH improves residual disease detection, identifies cell-lineage involvement of cytogenetic abnormalities by genotype/phenotype, and provides new strategies for investigating unrelated clonal populations in hematologic malignancies.

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Mutation analysis of the *HOX* paralogous 4-13 genes in children with acute lymphoid malignancies. *V. vanScherpenzeel Thim*[1], *J. Picard*[3], *G. Cornu*[2], *F. Gofflot*[3], *R. Rezsohazy*[3], *C. Verellen-Dumoulin*[1]. 1) Center for Human Genetics,UCL, Brussels, Belgium; 2) Department of Pediatric Hematology and Oncology, UCL, Brussels, Belgium; 3) Developmental Genetics Unit, UCL, Brussels, Belgium.

The molecular basis of susceptibility to childhood malignant hemopathy remains largely unknown. Interestingly, an excess of skeletal congenital anomalies has been reported among children with hematological malignancy and points towards the involvement of developmental genes, like those belonging to the *HOX* gene family. In addition to their role in embryogenesis, HOX transcription factors are also known to be important regulators of proliferation and differentiation of hematopoietic cells. The aim of this study was to explore the possibility that germline alterations of the *HOX* genes might be involved in childhood acute lymphoid malignancy. We therefore selected 20 young patients, out of a cohort of 86 acute lymphoid malignancy cases, which also presented a congenital anomaly of the skeleton. Firstly, we screened for nucleotide changes throughout the *HOX* genes of paralogous groups 4 to 13 in these 20 patients with skeletal defects. Subsequently, we extended the *HOX* mutation screening to the other 66 children having a lymphoproliferative disorder, but without skeletal anomalies. In total, 16 germline mutations were identified, 13 novel *HOX* variants and 3 previously described polymorphisms. *HOX* mutations were found in children with and without skeletal defects. While 11 changes were also observed in 100 healthy controls, 5 *HOX* variants were exclusively found in acute lymphoid malignancy cases. These comprised [*HOXD4*:p.Glu81Val] and [*HOXB7*:p.Tyr78Cys] missense mutations, the silent [*HOXB7*:c.144C>G] variant and [*HOXD10*:c.1078G>T] and [*HOXD10*:c.1099G>C] non-coding mutations. Among the 86 children with acute lymphoid malignancy, 7 unrelated patients carried one of these unique *HOX* germline mutations. Furthermore, [*HOXD4*:p.Glu81Val] and [*HOXB7*:p.Tyr78Cys] mutations were each found in association with other specific *HOX* variants, defining unique haplotypes. Our findings strongly suggest that these unique *HOX* sequence variants might be implicated in the occurrence of malignant lymphoproliferative disorders.

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Identification of transcripts modulated by ETV6, a candidate tumor suppressor gene. *G. Boily, J. Larose, S. Langlois, D. Sinnett.* Division of Hemato-oncology, Research Center, Sainte-Justine Hospital, University of Montreal, Montreal, Quebec, Canada.

Hemizygous deletions at chromosome 12p12-13 are observed in approximately 30 % of childhood pre-B acute lymphoblastic leukemia (ALL) cases, suggesting the presence of a tumor suppressor gene at this locus. Accumulating genetic and functional evidence suggest that ETV6 is the most probable candidate tumor suppressor gene. ETV6 is a ubiquitously expressed transcription factor of the ETS family with very few known targets. In order to understand its function and to elucidate the impact of its absence in leukemia, we conducted a study to identify targeted genes. We generated ETV6-inducible HeLa clones using the Tet-On system. ETV6 expression was induced with doxycycline and global expression was evaluated at different time points (0h, 4h, 12h and 48h) using DNA microarrays (Affymetrix HG-U133A). Initial in silico analyses lead to the identification of 64 modulated genes. The modulation of 11 candidate genes was then validated using real-time RT-PCR. All were shown to be significantly modulated, validating our approach for gene selection. To evaluate whether these candidate genes could be targeted by ETV6 in leukemia, we assessed their expression as well as that of ETV6 in up to 15 pre-B ALL patients. Preliminary results show that PTGER4/EP4, TP53, LUM and DUSP1 present a significant positive correlation with ETV6 expression levels. No obvious link between LUM and leukemia has been reported until now. TP53 is frequently inactivated in most cancers and is associated with poor prognosis in leukemia. DUSP1 is a MAPK phosphatase (inhibitory effect), a pathway involved in cell proliferation and stress response. PTGER4/EP4 is a PGE(2) receptor and it is known that this prostaglandin can inhibit proliferation and induce apoptosis in immature B lymphocytes. Our results support the hypothesis that the inactivation of ETV6 in lymphocyte precursors promotes proliferation and apoptosis resistance, hence promoting leukemia development. Future work will enable us to determine whether these modulated genes are actual targets and to evaluate the functional impact of their modulation in leukemia.

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A novel DNA/RNA FISH X inactivation assay reveals the nonrandom duplication of the active X chromosome in childhood hyperdiploid acute lymphoblastic leukemia (ALL). *O.A. Haas, P. Zeitlhofer, M. Knig, S. Strehl, A. Weinhusel.* CCRI, Vienna, Austria.

The most common numerical chromosome aberration in childhood ALL is the gain of an extra X in both male and female patients. It is not yet known whether the active and inactive X chromosomes are affected in a random or nonrandom fashion. Although in female patients either the active or inactive X may be duplicated in a random or nonrandom fashion, in male patients the only active homologue can be copied. Moreover, a duplicated active X might subsequently also become de novo inactivated in both sexes. The inactivation status of acquired X chromosomes can be evaluated by methylation-specific PCR (MS-PCR), which allows the simultaneous quantification of allele-specific methylation patterns of polymorphic sequences. However, MS-PCR has its limitations, especially in cases with low blast cells numbers. To overcome this problem, we have developed a simultaneous dual-color DNA/RNA FISH assay that enables the enumeration of active and inactive X chromosomes on a single cell level. FISH was performed with probes specific for the X centromere and the XIST RNA, which is exclusively expressed from and covers vast parts of the inactive X in human interphase cells. Following the successful evaluation of the assay on methanol/acetic acid-fixed cells that were obtained from 10 healthy individuals and 23 cases with various constitutional X chromosome aneuploidies, we analyzed 54 hyperdiploid cases of childhood ALL (24 males with two X, 23 females with three X and seven females with four X). In contrast to all constitutional control samples, which revealed only one active X, two of the three X in leukemic cell samples were active. In cases with four X, however, both the active and inactive X chromosomes were duplicated. These findings prove that irrespective of the sex of the patient, the active X is exclusively duplicated in cases with three X chromosomes. The consistent gain of both the active and inactive X in cases with four X, on the other hand, further corroborates previously established evidence that the hyperdiploid karyotype derives from a single abnormal non-disjunction event.

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A novel 1p36.2 located gene, APITD1, with tumor suppressive properties and a putative p53 binding domain, shows low expression in neuroblastoma tumors. *C. Krona[1], K. Ejeskar[1,2], H. Caren[1], F. Abel[1], R.M. Sjoberg[1], T. Martinsson[1]*. 1) Dept Clin Genetics, Inst Health Women & Children, Gothenburg University, Sweden; 2) Cell Gene Therapy Group, Murdoch Childrens Research Institute, Parkville, Australia.

Neuroblastomas generally lack TP53 mutations and no other tumor suppressor gene consistently inactivated has yet been identified in this childhood cancer. Characterization of a new gene, denoted APITD1, in the neuroblastoma tumor suppressor candidate region in chromosome 1p36.22 reveals that APITD1 contains a predicted TFIID-31 domain, representing the TATA box binding protein associated factor, TAFII31, which is required for p53 mediated transcription activation. The genomic organization of APITD1 was determined, and two different transcripts were shown to be ubiquitously expressed, one with an elevated expression in fetal tissues. Primary neuroblastoma tumors of different stages showed either weak or no APITD1 expression. DNA sequencing of the coding regions and the promoter region in 44 neuroblastoma tumors did not reveal any mutations indicating that the coding sequence of APITD1 is well conserved. APITD1 was functionally tested by adding APITD1 mRNA to neuroblastoma cells (SK-N-AS and SK-N-BE), which reduced the cell growth by 90 % as compared to control cells, suggesting APITD1 to have a role in a cell death pathway. We suggest that low expression of this gene, in defective cells, impair the ability for apoptosis through the p53-pathway. Based on its cytogenetic location, 1p36.2, and its biological features in primary neuroblastoma tumors, APITD1 could therefore be considered as a candidate tumor suppressor gene. Further functional studies of the APITD1 RNA and protein, and possible interaction with other genes, are ongoing.

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Program Nr: 244 from the 2004 ASHG Annual Meeting

Sequence analysis of the protein kinase gene family in human breast cancer. *R. Wooster, P.J. Stephens, H.R. Davies, C. Hunter, G.R. Bignell, R. Smith, M.R. Stratton, P.A. Futreal.* Cancer Genome Project, Wellcome Trust Sanger Institute, Hinxton, Cambs, United Kingdom.

Protein kinases are comparatively frequent targets for mutation in cancer and attractive for therapeutic intervention. To identify mutations in human cancer we have resequenced the entire protein kinase family in a series of breast cancer samples comprising of 18 primary carcinomas and 9 cell lines derived from primary tumours (all with a matched constitutional DNA sample). The annotated human genome contains 518 kinase genes. In total 35 megabases of DNA sequence was screened (1.3Mb/sample) from this set of breast cancers making this the most in-depth sequence analysis of human cancer to date. We identified seventy-seven somatic (tumour specific) mutations that altered either protein coding sequence or consensus splice-junction sequences. Additionally, we discovered 14 silent somatic mutations along with 1892 germline polymorphisms. No frequently somatic point-mutated protein kinase was identified in the samples sequenced. The breast cancers in this study had a diverse mutation spectrum and produced substantial evidence for a specific, but as yet un-attributed, mutator phenotype.

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High incidence of skewed X inactivation in young patients with familial non-BRCA1/BRCA2 breast cancer. *M. Kristiansen[1], G.P. Knudsen[2], P. Maguire[3], J. Pedersen[2], A. Lindblom[3], K.H. rstavik[1,2].* 1) Department of Medical Genetics, Rikshospitalet, Oslo, Norway; 2) Faculty Division Rikshospitalet, Faculty of Medicine, University of Oslo, Norway; 3) Department of Molecular Medicine, Karolinska Institutet, Stockholm, Sweden.

One of the two X chromosomes in female mammalian cells is inactivated in early embryonic life. Females are therefore mosaic for two different cell types, cells with the paternal X chromosome as the active X, and cells with the maternal X chromosome as the active X. The distribution of the two cell types normally approximates 50:50. Skewed X inactivation is often defined as a pattern where 90% or more of the cells have a preferential inactivation of one of the two X chromosomes. We have previously reported that a consecutive series of young patients with breast cancer had a higher frequency of skewed X inactivation compared to controls. In this study we have investigated 292 patients with sporadic breast cancer, 143 patients with familial non- BRCA1/BRCA2 breast cancer and 35 patients with BRCA1/BRCA2 mutations. Controls were 437 previously analyzed females. X inactivation pattern was determined by polymerase chain reaction (PCR) of the highly polymorphic CAG repeat in the androgen receptor (AR) gene. Methylation of HpaII sites in close proximity to this repeat correlates with X chromosome inactivation.Young familial non- BRCA1/BRCA2 breast cancer patients (<55 years) had a higher frequency of skewed X inactivation (15%) than young controls (2%) (P=0.002). No such association was found for the sporadic or BRCA1/BRCA2 patients. However, the number of BRCA1/BRCA2 patients was low and needs further study. The discrepancy between the familial non-BRCA1/BRCA2 breast cancer and the sporadic breast cancer may reflect different genetic background or tumor pathways between these two kinds of breast cancer. Skewed X inactivation may therefore be a risk factor for the development of breast cancer in familial breast cancer and may indicate an effect of X linked genes.

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Cancer risks in BRCA2 families: Estimates for sites other than breast and ovary. *C.J. Van Asperen[1], R.M. Brohet[3], E.J. Meijers-Heijboer[2], N. Hoogerbrugge[2], S. Verhoef[2], H.F. Vasen[2], M.G. Ausems[2], F.H. Menko[2], E.B. Gomez Garcia[2], J.G. Klijn[2], F.B. Hogervorst[2], J.C. Van Houwelingen[2], L.J. Van 't Veer[3], M.A. Rookus[3], F.E. Van Leeuwen[3], on behalf of the Netherlands Collaborative Group on Hereditary Breast Cancer (HEBON).* 1) Clinical Genetics, Leiden Univ Medical Ctr, Leiden; 2) HEBON; 3) Epidemiology, The Netherlands Cancer Institute, Amsterdam, the Netherlands.

Background In BRCA2 mutation carriers increased risks have been reported for several cancer sites besides breast and ovary. Since most of the families included in earlier reports were selected on the basis of multiple breast/ovarian cancer cases, it is possible that risk estimates may differ in mutation carriers with a less striking family history.
Methods 139 BRCA2 families with 66 different pathogenic mutations were included in a nationwide study in the Netherlands. To avoid testing bias, we chose not to estimate risk in typed carriers, but in male and female family members with a 50% a priori probability of being a carrier (n=1811). The relative risk (RR) for each cancer site with the exception of breast and ovarian cancer was determined by comparing observed numbers with numbers expected on the basis of Dutch cancer incidence rates. *Results* We observed an excess risk for four cancer sites, i.e., pancreas (RR 5.9; 95% CI 3.2-10.0), prostate (RR 2.7; 95% CI 1.7-4.1), bone (RR 14.4; 95% CI 2.9-42.1) and pharynx (RR 7.3; 95% CI 2.0-18.6). A small increase was observed for cancer of the digestive tract (RR 1.5; 95% CI 1.1-1.9). Histological verification was available for 45% of the tumors. Nearly all increased risks were significant for males only. Cancer risks tended to be stronger increased before age 65. Moreover, families with mutations outside the previously defined ovarian cancer cluster region (OCCR) tended to have a higher cancer risk.
Conclusions We found that BRCA2 carriers are at increased risk for cancers of the prostate, pancreas, bone and pharynx. Larger databases with extended follow-up are needed to provide insight into mutation specific risks of selected carriers in BRCA2 families.

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Program Nr: 247 from the 2004 ASHG Annual Meeting

Joint EU US proficiency testing for cystic fibrosis: quality evaluation of data interpretation and reporting practices. *E. Dequeker[1], I.M. Lubin[2,3], E. Girodon[4], M. Schwarz[5], M. Stuhrmann[6], M.M. McGovern[7], J. Amos[8,3], J.J. Cassiman[1]*. 1) Department of Human Genetics, University of Leuven, Belgium; 2) Division of Laboratory Systems, CDC, Atlanta, GA, USA; 3) Association for Molecular Pathologists; 4) Service de Biochemie et Genetique Molculaire, Creteil, France; 5) Paediatric Genetics Unit, NW Regional Molecular Genetics Laboratory, Manchester, United Kingdom; 6) Human Genetics, Hannover, Germany;; 7) Mt. Sinai School of Medicine, New York, NY, USA; 8) Specialty Laboratories, Inc., Santa Monica, CA, USA.

Errors in the molecular genetic test reporting process can include incorrect laboratory results and inaccurate interpretation of the data. Such errors need to be minimized since they can lead to inappropriate use of the test result. Proficiency testing (PT) schemes for cystic fibrosis (CF) in Europe have included an assessment of result reporting. In contrast, the CAP PT, in which a majority of US laboratories participate, does not review reports. In this study, we compared result reports from twenty-seven US laboratories with others (210) participating in the European PT scheme to determine if international guidelines for result reporting may be beneficial. A set of 6 blinded DNA samples containing common CFTR mutations was sent to clinical laboratories for analysis. Laboratories reported results using their own report form. Reports were evaluated and participants received general and individual performance evaluations. Errors in reports from US and other laboratories were similar in type and proportion. Overall, 18% of laboratories reported an incorrect test result. Thirty-one percent of laboratories provided reports with one or more mistakes. Twenty percent of reports contained administrative errors , 2% had errors in risk calculation, and 9% included errors in interpretation of technically correct results. Overall, there was considerable variation in the format, content, and quality of the written reports for CF molecular genetic tests. These observations suggest the need for development and uniform implementation of practice guidelines to assure high quality CF reporting practices.

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Fragile X testing: To Southern blot or not? *L.R. Burney[1], S. Bhatt[1], L. Rosenblum-Vos[2], S. Hallam[2].* 1) Clinical Genetics, Genzyme Genetics, Orange, CA; 2) Molecular Genetics, Genzyme Genetics, Westborough, MA.

Testing for fragile X syndrome is performed in our laboratory by both PCR and Southern blot techniques. These methods have differing strengths and weaknesses. PCR is less expensive and more rapid, but cannot amplify large alleles. Southern blotting can assess the methylation status and the allele size, but is more labor intensive, requires more genomic DNA and is less precise in determining allele size. Retrospective data analysis of 21,031 specimens was undertaken to determine: 1- What is the rate of false negative results if Southern blot is performed only for males with no normal allele or for females with only one normal allele by PCR? 2- If PCR is the primary assay, in what circumstances would Southern blot be required to minimize the number of false negative results? 3- Could the screening protocol differ from the diagnostic protocol? PCR analysis was performed using the method of Brown et al, 1993, allowing detection of alleles up to 200 CGG repeats. Southern blot analysis was performed by the method of Rousseau et al, 1991. Of 8944 samples received for suspected diagnosis of fragile X, 6803 were male and 2141 were female. Two males (0.03%) were found to be mosaic for both a normal and a full mutation allele. An additional 2 males with both an intermediate and a full mutation allele were also identified. The full mutation alleles were only detected by Southern blot. Two of the four males were karyotyped as 46,XY. This gives a potential false negative rate of 0.03%, or 0.06% if detection of an intermediate allele would also not lead to Southern blot analysis. Among the 12,087 individuals studied for carrier testing, the result interpretation would have been the same using either PCR only or PCR plus Southern blot testing. Based on these data, fragile X testing by PCR first may be adequate for carrier screening; however normal/full mutation mosaic females may exist. Missing a full mutation in a woman of childbearing age could have serious consequences. In cases referred for a suspected diagnosis of fragile X syndrome, both PCR and Southern Blot should be performed to increase diagnostic sensitivity.

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Program Nr: 249 from the 2004 ASHG Annual Meeting

Predictors of prenatal test use: Beyond race/ethnicity and socioeconomics. *M. Kuppermann[1], L. Learman[1], E. Gates[1], S. Gregorich[1], V. Gildengorin[1], J. Lewis[2], A. Washington[1]*. 1) UCSF, San Francisco, CA; 2) Kaiser, San Francisco, CA.

Purpose. Racial/ethnic and socioeconomic variation exists in prenatal testing for chromosomal disorders, yet its underlying basis is poorly understood. We explored the extent to which test utilization can be predicted by individual values and preferences.
Methods. Cross-sectional interview study of 959 socioeconomically diverse English-, Spanish-, and Chinese-speaking pregnant women, interviewed by their 20th gestational week and at 30 weeks. We used logistic regression analysis to analyze predictors of undergoing versus forgoing testing (women 35) and for invasive testing versus screening versus no testing (women 35).
Results. No significant racial/ethnic differences emerged in the use of testing by women 35. For women 35, a bivariate analysis found significant racial/ethnic differences in testing strategy (p=.002). However, in multivariate analyses, sociodemographic factors including race/ethnicity did not predict prenatal test use. For women 35, knowing someone with DS (OR=0.44; 95% CI .21-.95), perceived autonomy in health care decisions (OR=0.67; 95% CI .45-.99), and greater acceptance of Gods will or ones destiny (faith/fatalism scale; OR=0.38; 95% CI .20-0.74) predicted lower rates of test use, whereas willingness to have an abortion for a DS-affected fetus predicted higher rates (OR=4.0; 95% CI 1.4-11.2; P.05 for all ORs). Among women 35, feeling that a miscarriage would be worse than a DS-affected child (OR=0.14; 95% CI .04-.52; p=.003), low perceived miscarriage risk (OR=0.06; 95% CI .01-.26; p=.0002), high perceived DS risk (OR=9.9; 95% CI 1.7-.59; p=.01), less autonomy (OR=0.6; 95% CI .36-.89; p=.01), less fatalism (OR=0.5; 95% CI .31-.87; p=.01), and high value placed on testing information (OR=2.3; 95% CI 1.4-3.8; p=.002) independently predicted invasive test use.
Conclusion. Decisions regarding prenatal testing are driven by values, preferences and, for women 35, perceived risk of procedure-related miscarriage and DS. Testing practices should attend to values and preferences and focus on clear communication of risks.

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Program Nr: 250 from the 2004 ASHG Annual Meeting

Emerging Challenge in Public Health: Integrating Human Genomics into Acute Public Health Investigations. *M. Lindegren, C.A. Moore, L. Nguyen, S. Panse, M. Gwinn, M.J. Khoury.* Office of Genomics and Disease Prevention, Centers for Disease Control and Prevention, Atlanta, GA.

Acute public health investigations (APHI) are timely assessments of the cause and extent of acute health problems in a community using epidemiological and laboratory methods with implementation of prevention and control measures. These investigations have typically evaluated demographic, behavioral, and exposure-related risk factors; few have included an assessment of human genomic factors. With completion of the Human Genome Project, data are accumulating on the role of human genomics in disease causation and individual susceptibility and the importance of host factors in disease variability. Health investigations where human genomics may be important include infectious disease outbreaks, bioterrorism events, environmental exposures, disease clusters, and adverse events to therapeutics. Key applications of integrating genomics into the APHI include identifying genomic risk factors associated with susceptibility, resistance, severity, persistence, and therapeutic response; characterizing environmental exposures with signature exposure profiles using mRNA expression; and assessing variation in outcomes using protein expression. These components could aid in targeting public health interventions including vaccination, exposure reduction, behavioral intervention, and therapeutics. Based on recommendations from an expert panel, CDC and state partners are currently developing an agency-wide plan to address several core areas: 1) knowledge synthesis and management, e.g., candidate gene selection, bioinformatics; 2) laboratory science, e.g., collection and storage of specimens, genomic technologies; 3) epidemiology and statistics, e.g., study design, analysis; and 4) ethical, legal, and social implications, e.g., consent, confidentiality, security. Integrating human genomics into the APHI will require templates and protocols for informed consent and field and laboratory work. This new initiative will assess the usefulness of human genomic information in increasing our understanding disease, identify gene-environment interactions, and develop prevention and treatment interventions.

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Lessons from Genetic Integration into Universal Newborn Hearing Screening (UNHS). *J. Benkendorf*[1], *D. Beckett*[1], *J. Cooksey*[1,2], *D. Lea*[3], *H. Travers*[1], *J. Mansour*[2], *P. Flanagan*[1], *C. Gordon*[1], *M. Blitzer*[1]. 1) Univ Maryland Baltimore; 2) Univ Illinois at Chicago; 3) Foundation for Blood Research, Scarborough, ME.

The roles of genetics professionals and services in UNHS programs remain undefined. State-sponsored UNHS programs have embraced early detection, diagnosis, and intervention as recommended in the Children's Health Act [PL106-310]. There is no consensus on the roles of genetic evaluation and testing in the management of infants with severe hearing loss/deafness, even though 50% of cases have an underlying genetic cause. As part of a 3-year national study of genetic services we examined the integration of genetics into state-sponsored UNHS programs and into clinical assessment and management of infants with severe hearing loss. Our study describes state-level UNHS organization and oversight, audiologic and medical evaluation, counseling and interventions. Data from 40+ interviews (state and federal agencies, audiologists, otolaryngologists, clinical geneticists, genetic counselors, nurses and others) were analyzed using standard qualitative methods and QSR NVivo software. We found varied approaches to genetics integration in state programs and clinical evaluation, and identified factors promoting incorporation of genetics into these programs and services. Organizational proximity and other structural factors led to greater genetics involvement (eg, relationship of UNHS to state genetics program, advisory committees that included geneticists, combined UNHS and metabolic screening results reporting). Professional factors played a role; otolaryngologists' personal views of the value of genetic services/tests in diagnosis and management, and their access to geneticists, appeared critical. We observed that state UNHS programs, like other publicly funded detection and intervention programs for children (eg, birth defects surveillance or Children with Special Health Care Needs), generally omit genetics evaluation from program design, despite a high prevalence of underlying genetic etiologies. Continued tracking of these emerging models should provide insights for other public health population screening programs for heterogeneous conditions with genetic contributions.

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Program Nr: 252 from the 2004 ASHG Annual Meeting

An International Survey of Predictive Genetic Testing in Children for Adult Onset Disorders. *R.E. Duncan, J. Savulescu, L. Gillam, R. Williamson, M.B. Delatycki.* Murdoch Childrens Research Institute and the University of Melbourne, VIC, Australia.

Predictive genetic testing is offered to asymptomatic adults even when there is no effective prophylaxis or treatment available. However, testing of young people in similar circumstances is controversial and professional guidelines recommend against it. We collected detailed information from clinical geneticists in the UK, USA, Canada, Australia and New Zealand about predictive genetic tests they had performed on asymptomatic young people for conditions where no prophylaxis or treatment exists and onset is usually in adulthood. In particular we sought details of the psychosocial outcomes of testing.

Of 301 responses, we received details of 48 cases in which such testing had occurred. The most common condition tested for in young people was Huntington Disease (18 cases). Testing had been refused on over 800 occasions. We failed to find evidence of any catastrophic event affecting young people or their parents. Two adverse events occurred in young people who were tested in their later teenage years, but none for those tested at a younger age. This may be related to the fact that results were rarely disclosed to these younger individuals. Three instances of anxiety occurred in parents of young people who were tested, related to how and when to inform their child of the gene positive result. Consistent follow-up did not take place and our findings represent the minimum frequency of adverse events. Many respondents agree with existing guidelines in principle, but believe each case should be considered individually.

Although extensive theoretical literature speculates about the effects of predictive genetic testing in young people for adult-onset conditions, we are the first to report empirical evidence of outcomes, internationally. Our findings fail to support concerns raised in existing guidelines. This suggests a need to relax the absolute prohibition of testing children for adult-onset diseases and a need to leave greater latitude for practitioners.

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Program Nr: 253 from the 2004 ASHG Annual Meeting

Diagnosis and follow up of patients referred for genetic evaluation: The Harris County District Hospital experience. *F. Suárez[1], L. Potocki[2].* 1) Instituto de Genética Humana, Universidad Javeriana, Bogotá, Colombia; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Timely and accurate diagnosis of genetic disorders is essential to provide therapeutic intervention, proper anticipatory guidance, and appropriate counseling. Often it takes several visits to reach a definitive diagnosis. To determine the follow up pattern and diagnosis status of patients referred for genetics evaluation we conducted a review of 542 patients evaluated between 1981 and 2003. The patient population was 87.7% Hispanic. The age range was newborn-18y (mean 33.8m). 55.7% of the patients were given a specific diagnosis, and in 66.7% of the undiagnosed patients a genetic etiology was strongly suspected. Of those with a definitive diagnosis, 27.2% were diagnosed as a result of clinical impression or laboratory investigation obtained at the initial encounter, 68.9% following the 2nd, and 82.5% after the 3rd. Nearly 20% of patients required more than 3 visits for a diagnosis to be established. A diagnosis was rendered more often in patients referred with multiple congenital anomalies vs. those referred for minor anomalies, developmental delay, or mental retardation ($p=0.001$). The patients with a definitive diagnosis returned to the Genetics Clinic an average of 2.43 times, and the patients without a diagnosis returned an average of 2.05 times in a period of 12 months ($p=0.0016$). 62% of all patients were lost to follow up. Of these 41.4% were diagnosed and counseled, 14% were diagnosed but not counseled regarding the disorder, and 55.4% were undiagnosed. Our data show that a specific diagnosis is given in only 56% of patients referred to Genetics and that the majority of these patients are not diagnosed until after the first visit. Follow up and counseling is not always completed even when a diagnosis is reached, which can negatively affect health care in patients with genetic disorders. The causes of this lack of follow up will need to be determined, however, likely include issues regarding health care coverage, lack of perceived benefit on the part of the referring physician and/or parent, and poor communication between health care providers.

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Quantitative and Qualitative Assessment of Human Genetics Courses for Non-Science Majors. *B.L. Vice*[1], *J.A. Wagner*[1], *J.D. McInerney*[2], *C.A. Huether*[1]. 1) Univ Cincinnati, OH; 2) Natl. Col. for Health Prof. Ed. in Gen., Baltimore, MD.

Genetics education has been studied to some degree in grades kindergarten-12 and in medical schools, but little consideration has been given to genetics education at the undergraduate level. We studied college human genetics courses for non-science majors (NSM) by estimating the number of institutions offering such courses, and the number of students taking them. Between the years 2002-2004 an estimated 480 U.S. institutions of higher education (15.2%) offered a human genetics course for NSM. Only 8.4% of 1667 associate colleges were estimated to offer such a course in contrast to 32.9% doctoral, 25.3% masters, and 16.1% baccalaureate institutions, indicating a need to increase access to genetics education in two-year colleges. A survey of course instructors was administered, questioning course demographics, content, materials and approaches used. 63 instructors responded, reporting an average of approximately 95 students (range: <29 to >500) complete the course during an academic year. Accounting for multiple sections and frequency in which the courses are offered, approximately 35,000 students annually complete a human genetics course for NSM of the roughly 1.7 million students earning a college degree each year (2.1%). Course content was compared to a 2002 publication by the ASHG Human Genetics Education subcommittee that defines six concepts (each with several subconcepts) in genetics an undergraduate NSM should understand. Instructors indicated that 1/4 of class time was dedicated to issues of genetics and society and another 1/4 to transmission genetics. Instructors consistently rated many subconcepts significantly higher in importance than the emphasis placed on them in the course. Although time is a likely factor, instructors may need guidance on integration of the various subconcepts into their courses. Considering only 30.2% of the instructors reported they were trained in genetics (another 25.4% molecular/cell biology) and the small fraction of students currently completing NSM human genetics courses, these results demonstrate a considerable need for professional geneticists to become more involved in undergraduate human genetics education.

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Program Nr: 255 from the 2004 ASHG Annual Meeting

Misalignments in clients responses to reflective frames in counseling for Huntingtons Disease predictive testing.

A. Clarke[1], S. Sarangi[2], K. Bennert[2], L. Howell[2], P. Harper[1], J. Gray[1]. 1) Dept Medical Genetics, Univ Wales Col Medicine, Cardiff, United Kingdom; 2) Health Communication Research Centre, Cardiff University, Cardiff, United Kingdom .

The counseling encounter for HD predictive testing is framed like a scaffolding to allow clients to reflect on, among other things, the risks of knowing ones genetic status. Drawing on detailed transcripts of 24 HD consultations audio-recorded in South Wales as part of The Wellcome Trust funded project (2000-2004), we analyse 119 counselor-client question-response sequences with the tools of discourse analysis. Of the six types of counsellor-initiated reflective questions that can be identified, we focus on the two types which recur throughout the counseling protocol: (i) questions about clients decisions to have genetic testing and (ii) questions exploring the potential impact of a positive or negative test result. Our theoretical discussion is centred around the notion of alignment as a framework for locating the convergence and divergence of counselors and clients agendas in interaction. Preliminary coding of clients responses led us to identify three recurrent themes: (a) gaining knowledge as a basis for future action; (b) needing to know as a subjective necessity and (c) the downplaying of what can be known. In a further analysis of extended extracts, we draw attention to how clients display varying degrees of engagement with regard to the testing process and outcomes along the temporal and social axes. The analytic focus here is how clients often discuss coping with the onset of disease when they have been asked to discuss coping with genetic test results. Our findings show that, on the one hand, some clients take up the opportunity to engage in self-reflection and thus endorse the legitimacy of the reflective frame, while on the other hand, some clients implicitly or explicitly challenge the relevance of self-reflection. We suggest that clients' varied response behaviours result from the clients' perceived need to display their readiness for predictive testing -- an overarching meta-question posed by the very existence of the counseling protocol.

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Program Nr: 256 from the 2004 ASHG Annual Meeting

Efficacy of community-based group education for hereditary cancer. *N.G. Wolf*[1], *D. Culler*[1,2], *A. Sadeghpour*[1], *G.L. Wiesner*[1,2]. 1) Case Western Reserve University, Cleveland, OH; 2) University Hospitals of Cleveland, OH.

Genetic counseling for cancer susceptibility usually involves several lengthy face-to-face clinic visits with the at-risk patient. However, the increasing demand for genetic counseling and testing may limit this approach and has stimulated the development of alternative methods to bring these services to the community. **Goal**: To compare the effectiveness of group educational sessions to that of standard individual counseling sessions prior to genetic testing for inherited predisposition to cancer. **Methods**: Adults of Ashkenazi Jewish descent were (1) randomly assigned to either an individual or one of three group educational sessions to learn about their risk of carrying one of the ancestral mutations in the BRCA1, BRCA2, and APC genes, (2) offered free, confidential testing for these mutations, and (3) asked to complete questionnaires before, directly following, and six months subsequent to the sessions. **Results**: Of the 178 people who enrolled, 145/178 (81%) actually attended (75 group, 70 individual) and 140/145 (97%) underwent genetic testing. Of these, 15/140 (8%) had the I1307K mutation in the APC gene and 3/140 (2%) had the 185delAG mutation in the BRCA1 gene. Immediately following the educational sessions, knowledge scores increased significantly ($p<0.001$ for both session types) and most participants were satisfied with how the material had been presented (93% group, 100% individual). However, 26% of group session participants indicated they would have preferred an individual session, while only 6% of individual session participants would have preferred a group session ($p<0.001$). Follow-up surveys are currently being completed. **Conclusions**: A group session is an effective way to provide education regarding cancer predisposition risk and to offer genetic testing. For some individuals, however, one-on-one sessions might be a better way to meet their needs. Studies are ongoing to investigate factors that distinguish between those satisfied with a group session and those who would prefer a private session. This work was supported by the Mt. Sinai Healthcare Foundation, Mt. Sinai Community Partners, and the Michael and Anita Siegal Family Foundation.

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Program Nr: 257 from the 2004 ASHG Annual Meeting

Glycerol kinase: Role in nuclear translocation of the activated glucocorticoid receptor complex (GRC). *K. Eastman*[1], *J.A. Martinez*[1], *E.R.B. McCabe*[1,2]. 1) Pediatrics and; 2) Human Genetics, David Geffen School of Medicine at UCLA, LA, CA.

Glycerol kinase (GK) is an enzyme in glycerol metabolism, and has been identified as ASTP (ATP-stimulated translocation promoter) through interaction with the glucocorticoid receptor complex (GRC). When GRC is activated, GRC and GK accumulate in the nucleus (Okamoto et al, 1988). GK, in the presence of activated GRC, is associated with various histones (Okamoto et al., 1989). Our purpose was to examine GK-GRC interaction and nuclear uptake. Our methods included GK protein sequence analysis for motifs involved in nuclear receptor binding, and development of a cell-based system to investigate the role of GK in nuclear translocation of activated GRC. Sequence analysis revealed an LXXLL motif at residues 154-158 in the GK protein, which is a domain involved in nuclear receptor-co-regulator interactions. HepG2 cells were selected for GK-GRC interaction studies because they have been used previously to study GRC translocation. Immunohistochemistry of untreated HepG2 cells with goat purified polyclonal anti-GK antibody displayed cytoplasmic immunoreactivity primarily associated with mitochondria as shown by co-localization with voltage-dependent anion channel (VDAC) immunoreactivity and mitotracker dye. When treated with dexamethasone, nuclear translocation of GK and GR immunoreactivities was observed within 30 minutes. Concurrent staining with GK and GR antibodies displayed apparent nuclear co-localization of their immunoreactivities in the presence of dexamethasone. GK signal also co-localized with histone H1 and DNA staining. We conclude that GK has an LXXLL nuclear receptor co-regulator motif and GK protein translocates from the cytoplasm to the nucleus in response to a GR ligand. We speculate that the movement of GK from VDAC on the outer mitochondrial membrane to the nucleus in response to glucocorticoids has significant implications for cellular metabolism, and that mutations in patients with GKD alter nuclear translocation and binding of activated GRC complex by GK. Our cell-based system will permit further investigation of the GK-GRC interaction, its perturbation by mutations in the GK protein, and the function of GK as a putative GR co-regulator.

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Homozygous Nonsense Mutations in *TWIST2* Gene Cause Setleis Syndrome. *R.J. Desnick[1,2], T. Tukel[1,3], N. Diaz[4], L.I. Al-Gazali[5], R.W. Marion[6], R.D. Clark[7], C. Cadilla[4].* 1) Department of Human Genetics,Mount Sinai School of Medicine, New York, NY; 2) Department of Pediatrics,Mount Sinai School of Medicine, New York, NY; 3) Istanbul University, Institute for Experimental Medicine (DETAE), Department of Genetics, Istanbul, Turkey; 4) Department of Biochemistry, School of Medicine, University of Puerto Rico, San Juan, PR; 5) Department of Pediatrics, Faculty of Medicine and Health Sciences, UAE University, Al Ain, United Arab Emirates; 6) Department of Pediatrics, Montefiore Medical Center, Mamaroneck, NY; 7) Department of Pediatrics, School of Medicine, Loma Linda University, Loma Linda, CA.

Recessively inherited Setleis syndrome (OMIM 227260), also known as Focal Facial Dermal Dysplasia (FFDD) type II, is characterized by distinctive bitemporal scar-like depressions resembling forceps marks, lateral deficiency of the eyebrows, double-row eyelashes, and a course facial appearance. In two large Puerto Rican families a genome scan mapped the disease locus to microsatellite marker D2S1397 (2q37.3) with a LOD score of = 5.22 at = 0. With additional families and markers, the critical region was reduced to the 8 cM between markers D2S2949 and D2S2253. By sequencing the candidate genes in the region, two nonsense mutations (Q65X and Q119X) were identified in the first exon of the *TWIST2* gene, which codes for a basic-helix-loop-helix (bHLH) protein. The mutations truncated the entire bHLH domain plus the C terminus, and the C terminus of the protein, respectively. The TWIST2 protein is extremely conserved in mammals and also shows very high homology to the TWIST1 protein. TWIST2 has been shown to play a role in the regulation of cytokine gene expression by interacting with RelA and in bone formation by suppressing the activity of Runx2. *TWIST2* null mice have elevated expression of proinflammatory cytokines, resulting in perinatal death from cachexia. Contrary to the expectations from the KO mice, Setleis patients do not have severe immunological problems. Further studies of the role of *TWIST2* in humans may provide new insights into the targets of this transcription factor in man.

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ADAMTS 10 mutations in the autosomal recessive Weill-Marchesani syndrome. *N. Dagoneau[1], C. Benoist-Lasselin[1], C. Huber[1], L. Faivre[1], A. Mégarban[2], H. Dollfus[3], A. Alswaid[4], Y. Alembik[3], A. Munnich[1], L. Legeai-Mallet[1], V. Cormier-Daire[1]*. 1) Department of Medical Genetics and INSERM U393, Necker-Enfants Malades Hospital, Paris, France; 2) Unit of Medical Genetics, University Saint Joseph, Beirut, Lebanon; 3) Unit of Medical Genetics, Hautepierre Hospital, Strasbourg,France; 4) Clinical Genetics, Riyadh Armed Forces Hospital, Saudi Arabi.

Weill-Marchesani syndrome (WMS, MIM 277600) is characterized by the association of short stature, brachydactyly, joint stiffness, and eye anomalies including microspherophakia, ectopia of the lenses and occasionally heart defects. Despite clinical homogeneity, autosomal recessive (AR) and autosomal dominant (AD) modes of inheritance have been reported and we have recently identified an in frame deletion in the fibrillin-1 gene in one AD WMS family. Using an homozygosity mapping strategy in two consanguineous families from Lebanon and Saudi Arabia, we have reported linkage of the AR WMS gene to chromosome 19p13.3-p13.2 in a 12.4 cM interval. We then considered ADAMTS 10 (a disintegrin and metalloprotease with thrombospondin motifs), a member of the extracellular matrix proteases as a good candidate gene. Here, we present three distinct mutations in the ADAMTS 10 gene in these two families and in one sporadic WMS case including one stop mutation (R237X) and two splices mutations (1190+1GA, 810+1GA). In addition, we report the pattern of expression of ADAMTS 10 in human adult and fetal tissues. RT-PCR, Northern blot and dot blot analyses showed that ADAMTS 10 is expressed in skin, fetal chondrocytes and fetal and adult heart. Electron microscopy and immunological studies of the skin fibroblasts of patients confirm the impairment of the extracellular matrix. These data show for the first time the involvement of a member of the ADAMTS family in brachydactyly and microspherophakia.

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Program Nr: 260 from the 2004 ASHG Annual Meeting

Mutations in a novel gene, NPHP5, cause Nephronophthisis with early-onset retinitis pigmentosa. *E. Otto*[1], *B. Loeys*[2], *F. Fan*[3], *H. Khanna*[4], *U. Muerb*[1], *J. O'Toole*[1], *M. Attanasio*[1], *B. Utsch*[1], *A. Kispert*[5], *M. Tsuda*[6], *D. Williams*[7], *B. Margolis*[3], *A. Swaroop*[4], *F. Hildebrandt*[1, 8]. 1) Department of Pediatrics, University of Michigan, Ann Arbor, MI, USA; 2) Department of Human Genetics, Johns Hopkins University, Baltimore, MD, USA; 3) Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA; 4) Department of Ophthalmology, University of Michigan, Ann Arbor, MI, USA; 5) Institute of Molecular Biology, Medizinische Hochschule Hannover, Hannover, Germany; 6) Life Science, Himeji Institute of Technology, Hyogo, Japan; 7) Department of Pharmacology, University of California at San Diego, San Diego, CA, USA; 8) Departments of Human Genetics, University of Michigan, Ann Arbor, MI, USA.

Nephronophthisis (NPHP) is an autosomal recessive cystic renal disease. NPHP is the most common inherited cause of renal failure in childhood. Four causative genes (NPHP1-4) have been identified, three of which encode novel proteins (nephrocystin-1, -3, -4). Recently, we identified mutations in inversin as causing NPHP2 and localized inversin and nephrocystins to primary cilia of renal epithelial cells, thus linking NPHP to primary cilia, polycystic kidney disease, and to left-right axis development. 10% of patients with mutations in either NPHP gene have retinitis pigmentosa (RP), termed Senior-Loken syndrome (SLSN). We here performed a genome wide search for linkage and identified a new locus (NPHP5) for NPHP on chromosome 3q within a critical region of 7.8 cM. Maximum 2-point LOD score was Zmax=3.5 (=0). By directly sequencing cDNAs of 10 candidate genes we identified 8 distinct recessive mutations in a novel gene (NPHP5) in 15 patients with SLSN. All mutations were truncating. RP was present in 100% of cases. The NPHP5 gene product, nephrocystin-5 is highly conserved in evolution. It occurs in a complex with RPGR, which is mutated in the most frequent form of X-linked RP. Nephrocystin-5 localizes to primary cilia of renal epithelial cells and to the retina, thus explaining the renal-retinal phenotype. We thus identified mutations in a novel gene as the cause of NPHP and SLSN.

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Program Nr: 261 from the 2004 ASHG Annual Meeting

Human laminin 2 deficiency causes congenital nephrosis with mesangial sclerosis and distinct eye abnormalities (Pierson syndrome). *M. Zenker[1], T. Aigner[2], O. Wendler[3], V. Schumacher[4], P. Cochat[5], C. Kraus[1], I. Maruniak-Chudek[6], L.M. Neumann[7], A. Reis[1].* 1) Institute of Human Genetics, University of Erlangen, Germany; 2) Institute of Pathology, Erlangen; 3) Department of Otorhinolaryngology, University of Erlangen; 4) Institute of Human Genetics, University of Duesseldorf, Germany; 5) Hôpital Edouard-Herriot, Lyon, France; 6) University Childrens Hospital, Katowice, Poland; 7) Institute of Human Genetics, Charité Berlin, Germany.

Recently we delineated a new entity comprising severe congenital nephrotic syndrome with diffuse mesangial sclerosis and distinct eye abnormalities, clinically characterized by microcoria, but representing a complex ocular maldevelopment including anomalies of iris, ciliary body, lens, cornea, and retina (*Am J Med Genet* in press). The condition had been first described by Pierson et al. in 1963 (*J Genet Hum* 12, 184-213), but not recognized as a separate entity before. Homozygosity mapping in two consanguineous families with 11 affected offspring identified a candidate region on chromosome 3p flanked by markers D3S1768 and D3S1766. This region encompasses the *LAMB2* gene which was considered a strong candidate based on the laminin 2-deficient knockout mice which present with congenital nephrosis and abnormalities of neuromuscular junctions and retina (Noakes et al.: *Nat Genet* 1995; 10, 400-6; *Nature* 1995; 374, 258-62). In Pierson syndrome patients from five families we detected homozygous or compound heterozygous *LAMB2* mutations. Five of six disease causing alleles were truncating mutations. By immunohistochemical investigations and Western blotting we could demonstrate that the observed *LAMB2* mutations lead to lack of laminin 2 expression in the glomerular basement membrane and in other tissues studied. In unaffected controls we determined a specific ocular expression pattern of laminin 2 which is particularly abundant in the intraocular muscles and the lens capsule. These findings correspond to the morphological eye abnormalities in Pierson syndrome, characterized by severe hypoplasia of ciliary and dilatator pupillae muscles and lenticonus posterior.

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Program Nr: 262 from the 2004 ASHG Annual Meeting

Retinoschisin oligomerises, binds lipid and associates with the cell surface. Mutations causing X-linked retinoschisis interfere with these processes. *T. Wang[1, 2], A. Zhou[3], C.T. Waters[2], B.J. Peter[4], E. O'Connor[1, 2], H.T. McMahon[4], R.J. Read[3], D. Trump[1, 2]*. 1) Academic Unit of Medical Genetics, Centre for Molecular Medicine, University of Manchester, Oxford Road, Manchester M33 9PT, United Kingdom; 2) Department of Medical Genetics, Cambridge Institute for Medical Research, University of Cambridge, Cambridge CB2 2XY, United Kingdom; 3) Department of Haematology, Cambridge Institute for Medical Research, University of Cambridge, Cambridge CB2 2XY, United Kingdom; 4) MRC laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QQ, United Kingdom.

Retinoschisin is a single discoidin domain protein secreted by photoreceptors and bipolar cells. Mutations in RS1, which encodes retinoschisin, lead to the retinal dystrophy X-linked retinoschisis (RS, MIM#312700), which is characterised by splitting within the inner retinal layers, leading to visual deterioration. By site directed mutagenesis, we have expressed 24 disease-causing mutant forms of retinoschisin and found that many mutations lead to intracellular retention and degradation of mutant proteins, but a subset of mutant proteins were secreted. We then developed a structural model of the retinoschisin discoidin domain and show that most of the secreted mutants (F108C, R141H, R141G, R182C, H207Q, R209H) affect residues within surface loops at one end of the -barrel structure, indicating the importance of this region for interacting with other molecules rather than structural maintenance. By using solid phase ELISA, liposome lipid binding assay and cell surface biotinylation, we demonstrate that wild-type retinoschisin binds phospholipids and associates with the cell surface. Secreted mutations reduced lipid binding (C59S, R141G, R141H, R182C, H207Q, R209H, C219G, C223R) suggesting the loops are responsible for lipid binding. Using non-reducing polyacrylamide gel and gel filtration, we demonstrate that wild-type retinoschisin oligomerise as an octamer and a subgroup of mutants reduces oligomerisation (C59S, C219G, C223R). Our finding suggests that binding of oligomerised retinoschisin at the cell surface is important in its presumed role in cell adhesion.

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Program Nr: 263 from the 2004 ASHG Annual Meeting

A MUTATION IN ARL6 SEGREGATES WITH BARDET-BIEDL SYNDROME 3 IN A NEWFOUNDLAND FAMILY. *Y. Fan[1], M. Esmail[1], S.J. Moore[2], J.S. Green[2], P.S. Parfrey[2], M.R. Leroux[1], W.S. Davidson[1].* 1) Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, Canada; 2) Department of Medical Genetics and Clinical Epidemiology, Memorial University, St. John's, NF, Canada.

Bardet-Biedl syndrome (BBS [MIM 209900]) is a multisystemic disorder that is characterized by obesity, retinal degeneration, polydactyly, hypogenitalism in males, and renal anomlies. Eight loci (BBS1-8) are known to segregate with this disorder and genes for all of them except BBS3 have been identified. Genetic linkage and haplotype analyses showed that BBS3 maps to the centromeric portion of chromosome 3. To screen for candidate genes within the BBS3 critical region, a bioinformatic approach has been developed that takes advantage of the fact that C.elegans bbs genes all contain a common promoter element known to regulate the expression of cilia-specific genes. This approach identified three genes as candidates for BBS3. No mutations were observed in the exons or exon-intron boundaries of two of the candidate genes in an affected individual from a Newfoundland BBS3 family, NF-B2. However, all affected individuals in this family were homozygous for a G to C transversion at position 859 of ARL6 (GenBank # NM_032146) whereas the parents were heterozygotes and no unaffected siblings were homozygous for the mutation. This mutation, which was not observed in 100 chromosomes from ethnically matched controls, results in an amino acid substitution, G169A. ARL6 encodes an ADP-ribosylation factor-like protein that belongs to the Arf/Arl family of GTP-binding proteins. A multiple sequence alignment of ARL6 with other Arf/Arl family members indicates that Gly169 is highly conserved. These results indicate that a mutation in ARL6 causes BBS3. Research funded by Heart and Stroke Foundation (HSF) of B.C. & Yukon, HSF Canada, the Janeway Foundation, Memorial University Opportunities Fund, and CIHR.

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Program Nr: 264 from the 2004 ASHG Annual Meeting

The Bardet-Biedl protein BBS4 targets cargo to the pericentriolar region and is required for microtubule anchoring and cell cycle progression. *J.L. Badano*[1], *J.C. Kim*[2], *S. Sibold*[3], *M.A. Esmail*[2], *J. Hill*[3], *B.E. Hoskins*[3], *C.C. Leitch*[1], *K. Venner*[4], *S.J. Ansley*[1], *A.J. Ross*[3], *M.R. Leroux*[2], *N. Katsanis*[1,5], *P.L. Beales*[3]. 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Dept. of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby BC, Canada; 3) Molecular Medicine Unit, Institute of Child Health, University College London, London, UK; 4) Dept. of Electron Microscopy, Institute of Neurology, Queens Square, London, UK; 5) Wilmer Eye Institute, Johns Hopkins University, Baltimore, MD.

BBS4 is one of several proteins that cause Bardet-Biedl syndrome (BBS), a multisystemic disorder of significant genetic and clinical complexity. Here we show that BBS4 localizes to the centriolar satellites of centrosomes and basal bodies of primary cilia, where it functions as an adaptor of the $p150^{glued}$ subunit of the dynein transport machinery to recruit PCM1 (Pericentriolar Matrix 1 protein) and its associated cargo to the satellites. Silencing of *BBS4* induces PCM1 mislocalization and concomitant de-anchoring of centrosomal microtubules, arrest in cell division, and apoptotic cell death. Expression of two truncated forms of BBS4 that are similar to those found in some individuals with BBS had a similar effect on PCM1 and microtubules. Our findings indicate that defective targeting and/or anchoring of pericentriolar proteins and microtubule disorganization contribute to the BBS phenotype and provide new insights into possible causes of familial obesity, diabetes and retinal degeneration.

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Mkks-null mice have a phenotype closely resembling Bardet-Biedl Syndrome (BBS). *M.K. Tayeh[1], H. Yen[1], R.F. Mullins[1], D.Y. Nishimura[1], C.C. Searby[1,2], B. Yang[1], E.M. Stone[1,2], V.C. Sheffield[1,2].* 1) University of Iowa, Iowa City, IA; 2) Howard Hughes Medical Institute, Iowa City, IA.

McKusick-Kaufman syndrome (MKS) is an autosomal recessive disorder characterized by post-axial polydactyly, congenital heart defects and hydrometrocolpos, a congenital structural abnormality of female genitalia. The *MKKS* gene was initially mapped and identified by using a large Amish kindred. MKKS has sequence homology to the alpha subunit of the *Thermoplasma acidophilum* thermosome, a prokaryotic chaperonin complex with similarity to eukaryotic chaperonins. Mutations in the *MKKS* gene (also referred to as *BBS6*) have been shown to cause some cases of Bardet-Biedl Syndrome (BBS; characterized by obesity, pigmentary retinopathy, polydactyly, renal malformations and functional abnormalities, learning disabilities, and hypogenitalism). Mckusick-Kaufman syndrome patients are not obese, and do not develop retinopathy.

To further explore the pathophysiology of BBS and the related disorder, MKS, we created a knockout mouse model ($Mkks^{+/-}$). We demonstrate that the absence of Mkks leads to retinal degeneration through apoptosis, failure of spermatozoa flagella formation, and increased food intake. The phenotype of the $Mkks^{+/-}$ mice closely resembles the phenotype of other mouse models of BBS ($Bbs2^{+/-}$ and $Bbs4^{+/-}$), although the retinopathy is milder (later onset). These data demonstrate that a null mutation of the *Mkks/Bbs6* gene lead to BBS phenotypes in mice. These data indicate that the human MKS phenotype is likely to result from specific missense mutations in *MKKS/BBS6*.

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Bardet-Biedl Syndrome (BBS) genes play a role in vesicle trafficking and in nodal cilia function. *H.-J Yen*[1, 2], *M. Tayeh*[1,2], *R.F. Mullins*[1], *J. Wei*[1,2], *C.C. Searby*[1,2], *T. Westfall*[1], *E.M. Stone*[1,2], *D.C. Slusarski*[1], *V.C. Sheffield*[1,2]. 1) Univ Iowa, Iowa City, IA; 2) HHMI.

Bardet-Biedl syndrome (BBS) is a genetically heterogeneous disorder characterized by retinitis pigmentosa, obesity, polydactyly, cognitive impairment, and renal and cardiac anomalies. In addition, BBS patients have an increased incidence of hypertension and diabetes mellitus. To date, eight BBS genes have been identified. While the precise functions of the BBS proteins have yet to be determined, several pieces of data, including the conservation of BBS orthologs in ciliated organisms but not in non-ciliated organisms, indicate that BBS proteins play a role in cilia function. To further investigate the function of BBS proteins, we have identified and cloned four BBS genes from zebrafish (*zBbs2*, *zBbs4*, *zBbs6*, and *zBbs7*). Using Morpholino anti-sense oligonucleotides, we knockdown expression of the individual zBbs genes. We demonstrate that knockdown of BBS gene expression results in defective melanosome transport in zebrafish embryos. The defect in transport appears to be limited to retrograde movement. These data indicate that defective vesicle trafficking is a common feature involved in BBS pathophysiology. In addition, we observed a consistent defect in disruption of Kupffers vesicle formation and early heart laterality defects. Kuppfers vesicle is a ciliated transient structure in zebrafish that has been proposed to be functionally equivalent to the mouse node in relation to Left-Right patterning. These results indicate a role for BBS proteins in nodal cilia function.

The zebrafish system has also allowed us to investigate genetic interactions between BBS genes by co-injection of combinations of zBbs antisense MOs. These data suggest that BBS genes can modify the phenotype resulting from mutations at other BBS loci. In addition, analysis in melanosome transport phenotype can further elucidate cellular functions of BBS proteins.

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Program Nr: 267 from the 2004 ASHG Annual Meeting

A phase 3, randomized, double-blind, placebo-controlled, multicenter, multinational clinical study of recombinant human N-acetylgalactosamine 4-sulfatase (rhASB) in patients with Mucopolysaccharidosis VI (MPS VI). *P. Harmatz*[1]*, R. Giugliani*[2]*, I. Schwartz*[2]*, N. Guffon*[3]*, C. Sa Miranda*[4]*, E. Teles*[5]*, J.E. Wraith*[6]*, M. Beck*[7]*, J. Hopwood*[8]*, S. Swiedler*[9]. 1) Children's Hosp Oakland, CA; 2) Hospl de Clinicas de Porto Alegre, Brazil; 3) Hosp Edouard Herriot, France; 4) Instituto de Biologia Molecular e Celular, Portugal; 5) Hosp de Sao Joao, Portugal; 6) Royal Manchester Children's Hosp, England; 7) Children's Hosp, University of Mainz, Germany; 8) Women's and Children's Hosp, Adelaide, Australia; 9) BioMarin Pharmaceutical Inc, Novato, CA.

MPS VI (Maroteaux-Lamy syndrome) is a lysosomal storage disease caused by a deficiency of the enzyme N-acetylgalactosamine 4-sulfatase (arylsulfatase B or ASB), leading to a progressive disorder with multiple organ and tissue involvement. Weekly treatment with rhASB has been studied in 3 clinical trials. Phase 3 study data through Wk 24 are presented. Nineteen patients received 1mg/kg rhASB and 20 patients received placebo as a weekly intravenous infusion over 4 hrs. Efficacy (primary endpoint: 12 minute (min) walk test; secondary endpoints: urinary glycosaminoglycans (GAGs) and 3 min stair climb) and safety were evaluated. Patients receiving rhASB demonstrated significant improvement ($p=0.025$) in the 12 min walk test as compared to patients receiving placebo. The mean difference between the two groups after 24 wks was 92 meters. Patients receiving rhASB demonstrated a significant ($p<0.001$) reduction (75%) in urine GAGs as compared to patients receiving placebo. On the 3 min stair climb, patients receiving rhASB demonstrated approximately 6 stairs/min greater improvement ($p=0.053$). Infusions were generally safe and well-tolerated. More drug-related adverse events occurred in the rhASB group (92 vs 14), but most were of mild or moderate severity. In contrast, more serious adverse events occurred in the placebo group (12 vs 3). In conclusion, rhASB demonstrated statistically and clinically significant efficacy in primary and secondary endpoints evaluating endurance and urine GAG excretion confirming results from Phase 1/2 and 2 studies and rhASB demonstrated a favorable safety profile.

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Program Nr: 268 from the 2004 ASHG Annual Meeting

Immunotargeting to ICAM-1 provides binding, internalization and lysosomal delivery of acid sphingomyelinase.

S. Muro[1], *E. Schuchman*[3], *V. Muzykantov*[2]. 1) Institute for Environmental Medicine and; 2) Department of Pharmacology, University of Pennsylvania, Philadelphia, PA; 3) Department of Genetics, Mount Sinai School of Medicine, New York, NY.

Enzyme replacement therapy (ERT) is one of the most feasible means to treat lysosomal storage disorders (LSDs), and relies on the uptake of intravenously delivered enzymes via cell receptors recognizing carbohydrate moieties. However, abnormal or reduced enzyme glycosylation may lead to poor internalization and restricts uptake to cell types expressing these receptors. To develop an alternative delivery system for LSD ERT, we targeted acid sphingomyelinase (ASM), the lysosomal enzyme deficient in Types A & B Niemann-Pick disease (NPD), to InterCellular Adhesion Molecule (ICAM)-1, a glycoprotein involved in inflammation and expressed on a wide range of cell types. Previous studies showed that: i) targeting to ICAM-1 can be achieved by coupling enzymes with anti-ICAM antibodies; ii) the resulting conjugates were internalized by cells via a novel, clathrin-independent pathway (CAM-mediated endocytosis); and, iii) enzymes delivered in this manner accumulated in lysosomes. Recombinant ASM and anti-ICAM were coated onto fluorescent nanoparticles, and the resulting, enzymatically active conjugates were incubated with endothelial cells (HUVEC) and analyzed by fluorescence microscopy. Anti-ICAM/ASM (not ASM or control IgG/ASM) bound to the HUVEC surface at 4C (25949 vs. less than 10 conjugates/cell). Binding was inhibited by anti-ICAM in the media, but not mannose-6-phosphate (3.20.7% vs. 12215%), indicating that anti-ICAM was entirely responsible for targeting. HUVEC efficiently (833%) and rapidly (t1/2=25 min) internalized anti-ICAM/ASM at 37C. This was inhibited by amiloride, but not MDC (635% vs. 11415%), drugs that affect CAM and clathrin-mediated endocytosis, respectively. Anti-ICAM/ASM effectively trafficked to lysosomes (707%) in HUVEC, as well as ASM deficient NPD cells. Therefore, lysosomal enzyme targeting to ICAM-1 provides an alternative delivery method that may enhance the efficacy of ERT for certain LSDs, such as NPD. Funding: AHA 435481N (SM), NIH HD28607 (ES), NIH HL71175 (VM).

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Program Nr: 269 from the 2004 ASHG Annual Meeting

Long-term CNS and somatic correction of lysosomal storage in Mucopolysaccharidosis II mice by AAV-mediated gene transfer after pretreatment with mannitol. *J. Muenzer, J.S. Jennings, H. Fu.* Dept Pediatrics, Univ North Carolina, Chapel Hill, NC.

Mucopolysaccharidosis II (MPS II, Hunter syndrome) is due to the deficiency of the lysosomal enzyme iduronate sulfatase (Id-S). A major obstacle in developing therapies for the CNS disease in mucopolysaccharidoses is the presence of the blood-brain barrier (BBB). Therapeutic effects of AAV gene delivery on both somatic and the CNS features in adult MPS II mice were studied using IV injection after pretreatment with mannitol, to disrupt the BBB and facilitate the CNS entry of the vector. An AAV serotype 2 vector, AAV2-CMV-hIdS, containing a human Id-S cDNA (hIdS) driven by a human cytomegalovirus promoter (CMV) was used. A single dose of AAV2-CMV-hIdS viral vector (2-4×10^{11} viral particles in 200 l) was delivered into adult (4-6 week old) MPS II mice (n=20) by tail vein injection 10 minutes after an IV infusion of mannitol (1-2 mg/gm of body weight). Our results demonstrated correction of GAG storage in multiple organs/tissues. Complete correction of GAG accumulation in liver was achieved ($P<0.01$) and partial correction in kidney, heart, intestine and muscle was observed ($P<0.05$) at 3, 6, 9 and up to 17 months in one animal. No obvious decrease of GAG content was observed in the spleens of the mice after AAV injection. Id-S enzyme activity was detected in the injected mouse liver with complete correction of lysosomal storage, at a level of 10-50% of that in the liver of normal mice. Histopathology and transmission electron microscopy studies demonstrated clearance of lysosomal storage in liver. Decreased CNS lysosomal storage was shown by histopathology in Purkinje cells and also in the neurons of the hippocampus, thalamus and cerebral cortex after AAV-mediated gene transfer. Physical appearances of AAV injected animals, such as facial feature and spinal curvature, were improved, and the life span was prolonged, compared to that of non-treated MPS II mice. Our results suggest that IV administration of AAV vector following mannitol pretreatment may be a promising approach for treating both somatic and CNS disease in lysosomal storage disorders.

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Program Nr: 270 from the 2004 ASHG Annual Meeting

Intravenous retroviral gene therapy in neonatal mucopolysaccharidosis VI cats. *M. Haskins[1], T. O'Malley[1], P. O'Donnell[1], K. Cullen[1], G. Aguirre[1], Y. Liu[2], P. Wang[1], K.P. Ponder[2].* 1) Sch Vet Med, Univ Pennsylvania, Philadelphia, PA; 2) Sch Med, Washington University, St. Louis, MO.

Mucopolysaccharidosis (MPS) VI is a lysosomal storage disease caused by deficient activity of N-acetylgalactosamine 4-sulfatase (4S) resulting in an inability to degrade the glycosaminoglycan (GAG) dermatan sulfate. Clinical features include skeletal abnormalities, growth retardation, facial dysmorphia, and corneal clouding. Six MPS VI cats were injected intravenously at 4 days of age with a Moloney murine leukemia virus-based retroviral vector containing the human alpha-1-antitrypsin promoter, the feline 4S (f4S) cDNA (kindly provided by John J. Hopwood), and the WPRE posttranscriptional regulatory element. The kittens received 7.2x10E9 transducing units/kg of body weight. Stable f4S activity has been detected in serum for 11 months in 2 cats at 30-fold normal, while the remaining four cats had 4S activity 5-fold normal at 4 months. The f4S activity in liver biopsies at 5 months in the oldest cats was 40-fold normal and GAG in the biopsies was normal. The body weights of the two oldest treated cats at 6 months were 126% that of their untreated MPS VI littermates (average of 1.89 kg). There are profound differences in the appearance of all 6 treated cats. The MPS VI controls have short ears and tails, broad flat faces, joint stiffness, and major locomotor difficulties. The treated cats have mild facial dysmorphia, a much freer range of pelvic motion, near normal gait, and are more physically active than their MPS VI littermates. The cat with the highest f4S activity is nearly indistinguishable from a phenotypically normal cat except for the eyes, as there was no improvement in corneal clouding in any of the cats. MPS VI is particularly suited to liver-directed therapy because unlike other MPS disorders, CNS involvement is minimal. As MPS VI is a progressive disease, the full extent of the clinical benefit will only become evident over time. We will present a 15-month update of the oldest cats, including a video demonstrating mobility and radiographs comparing the skeletons of normal, treated, and untreated MPS VI cats. Supported by NIH grant DK25759.

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Program Nr: 271 from the 2004 ASHG Annual Meeting

Enzyme replacement therapy for mucopolysaccharidosis IVA: development of bone targeting system. *S. Tomatsu, M.A. Gutierrez, T. Nishioka, O. Pena.* Dept Pediatrics, Ped Res Inst, St Louis Univ, St Louis, MO.

Mucopolysaccharidosis IVA is an autosomal recessive disorder caused by a deficiency of N-acetylgalactosamine-6-sulfate sulfatase (GALNS), a lysosomal enzyme required for the degradation of keratan sulfate and chondroitin-6-sulfate. Deficiency of GALNS leads to the systematic skeletal dysplasia. To improve the quality of life, it is necessary to maximize to deliver the enzyme to the bones to clear the storage materials. The aims of this study were to compare efficiency of delivery of GALNS to the bone among native (simple recombinant), sulfatase modifier factor (SUMF1) co-expressed, and N-terminal bone targeting (NBT: six glutamines were attached to N-terminus of mature protein) enzymes and to assess pharmacokinetics and tissue distribution of purified enzymes and pathological improvements by using MPS IVA knockout mice. Methods: Three GALNS enzymes were expressed in stably transfected CHO-K1 cells and purified from the medium. Each purified GALNS was administered intravenously to 3 mo knockout mice at a single dose of 250 units/kg of body weight. Treated mice were examined by assaying the GALNS activity at 0, 2, 5, 10, 20, 30, 60, 120, and 240 min to assess clearance of the enzyme in the blood stream. The mice were sacrificed 2, 24, 72, 168 hr after infusion of the enzyme for studies of the tissue distribution pattern of the enzyme and pathological improvements.Results: Each GALNS was purified around 1000-fold with 40-70% yield by a column method. 1. All three enzymes cleared storage materials in visceral organs. 2. SUMF 1 co-expressed GALNS provided 6-12 times more specific activity than other two enzymes. 3. Native and SUMF-1 co-expressed GALNS had the same biphasic pattern of enzyme clearance in blood and tissue distribution while NBT GALNS had 10 times more prolonged clearance in blood in a monophasic manner. 4. NBT GALNS changed tissue distribution of enzyme with a long retention of enzyme activity in tissues including the bone.Conclusion: Combination of SUMF1 co-expressed GALNS and established bone-targeting system will be a potential treatment for MPS IVA and the current bone-targeting system can be widely used for other bone diseases.

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Program Nr: 272 from the 2004 ASHG Annual Meeting

Developmentally regulated Man6-P receptor-mediated transport of phosphorylated human -glucuronidase across the blood-brain barrier in newborn mice. *J.H. Grubb[1], A. Urayama[2], W.A. Banks[2], W.S. Sly[1]*. 1) Dept of Biochem & Molec Biol, St Louis Univ Sch of Med, St Louis, MO; 2) Dept of Int Med, St Louis Univ Geriatric Research, Education & Clinical Ctr, VA Med Ctr, St Louis, MO.

MPS VII is a lysosomal storage disorder resulting from an inherited deficiency of -glucuronidase (GUS) characterized by storage of glycosaminoglycans (GAGs) in most tissues, including brain. Enzyme delivery across the blood-brain barrier (BBB) is the main obstacle for enzyme replacement to correct lysosomal storage in the CNS. Prior studies suggested the brain is accessible to GUS in the first 2 weeks of life, but not later. To investigate a role for the Man6-P/IGF II receptor (M6P/IGF2R) in transport of GUS across the BBB in neonatal mice, we compared brain uptake of phosphorylated (P-GUS) and nonphosphorylated (NP-GUS) GUS in newborn and adult mice.

P-GUS and NP-GUS were labeled with [^{131}I]Na by the iodobead method. Male CD-1 mice received an i.v. injection of [^{131}I]P-GUS or [^{131}I]NP-GUS with [^{125}I]albumin (550,000 cpm each) into the superficial temporal (neonates) or jugular vein (adults). After injection, blood, brain, and other tissues were collected. Multiple time regression analysis was used to determine the brain influx rate (K_{in}).

[^{131}I]P-GUS, but not [^{125}I]albumin was transported across the BBB after i.v. injection in 2-day-old mice. The K_{in} of [^{131}I]P-GUS in 2-day-old mice was 0.21 l/g-min. By 7 weeks of age, transport of [^{131}I]P-GUS was not significant. Capillary depletion revealed that 62% of the [^{131}I]P-GUS in brain was in brain parenchyma in 2-day-old mice. The uptake of [^{131}I]P-GUS into brain was inhibited by co-injection of unlabeled P-GUS or M6P in a dose-dependent manner. The K_{in} of [^{131}I]NP-GUS (0.04 l/g-min) was significantly lower than that of [^{131}I]P-GUS in 2-day-old mice. Transcardiac brain perfusion confirmed that neither [^{131}I]P-GUS nor [^{131}I]NP-GUS crossed the BBB in adult mice. Thus, transport of [^{131}I]P-GUS into the brain parenchyma in early postnatal life is specifically mediated by the M6P/IGF2R, and this receptor-mediated transcytosis is not observed in adult mice.

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Program Nr: 273 from the 2004 ASHG Annual Meeting

The detection of enzyme deficiencies in dried blood spots by tandem mass spectroscopy. *C.R. Scott*[1], *Y. Li*[2], *F. Turecek*[2], *M.H. Gelb*[2,3]. 1) Dept. of Pediatrics, University of Washington School of Medicine, Seattle, WA; 2) Dept. of Chemistry, University of Washington, Seattle, WA; 3) Dept. of Biochemistry, University of Washington, Seattle, WA.

Enzyme replacement therapy (ERT) and bone marrow transplantation (BMT) offer clinical benefit for selected lysosomal storage disorders (LSDs), raising a compelling argument for presymptomatic detection by newborn screening. We have developed a technique to measure enzyme activity associated with LSDs for which ERT or BMT has shown promise. The technique uses dried blood samples (DBS) submitted for newborn screening, elution of lysosomal enzymes using a single buffer, a single 5 mm punch, overnight incubation with substrates, and injection into a mass spectrometer. The table summarizes our data for detecting specific LSDs.

Affected Patients (n)		Normal Infants
	range (/mol/h/L)	range (/mol/h/L)
Gaucher (5)	0 - 0.18	0.89 - 9.60
Niemann-Pick (5)	0 - 0.32	0.92 - 11.30
Krabbe (9)	0 - 0.20	0.42 - 1.53
Pompe (11)	0 - 0.33	0.93 - 7.33
Fabry (5)	0 - 0.17	0.77 - 5.65

There is no overlap between affected and normal infants or between affected and obligate carriers (data not shown). The technique is applicable to newborn screening programs and to other known genetic disorders that express enzyme deficiency in peripheral blood.

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Program Nr: 274 from the 2004 ASHG Annual Meeting

Correction of hypertriglyceridemia in lipoprotein lipase deficient cats by intramuscular administration of AAV1-LPL^{S447X}. *C.J.D. Ross[1], J. Twisk[2], J. Kuivenhoven[3], D. Verbart[2], M. Beetz[2], P. Dijkhuizen[2], W. Hermens[2], J. Kastelein[3], J. Meulenberg[2], M.R. Hayden[1].* 1) U.B.C., Canada; 2) Amsterdam Molecular Therapeutics; 3) University of Amsterdam.

Human lipoprotein lipase (LPL) deficiency is associated with hypertriglyceridemia and life-threatening pancreatitis. We have previously shown that administration of AAV serotype 1 encoding the human LPL^{S447X} cDNA to LPL deficient mice completely corrected the hypertriglyceridemia for over a year (dose: $8x10^{12}$ genome copies (gc)/kg). **AIM:** We now tested the efficacy of AAV1-LPL^{S447X} in LPL -/- cats, which demonstrate hypertriglyceridemia (plasma TG up to 250 mM vs. 0.12 mM in normal cats) and clinical symptoms including pancreatitis. Importantly, LPL -/- cats show no detectable LPL protein or activity. **METHOD:** Male LPL -/- cats (2-5/group) were injected IM with saline or AAV1-LPL^{S447X} (dose: $1x10^{11}$, $5x10^{11}$, or $1x10^{12}$ gc/kg), combined with weekly oral doses of cyclophosphamide (0, 6, or 12 mg/kg) to inhibit an immune response against human LPL. **RESULTS:** Administration of $5x10^{11}$-$1x10^{12}$ gc/kg resulted in the rapid and complete resolution of visible lipemia: plasma TG was reduced by >99% to normal levels (0.1-0.2 mM) within 4-6 days. A >90% reduction in plasma TG (to 0.8-2.1 mM) was accomplished with the lower dose of 1x10(11) gc/kg within 2 weeks. At $5x10^{11}$-$1x10^{12}$ gc/kg, efficacy was transient despite the use of cyclophosphamide: plasma TG returned to baseline within 2 weeks with the appearance of inhibitory anti-LPL antibodies in plasma and blunting of LPL expression. In contrast, the low dose combined with cyclophosphamide resulted in prolonged TG reduction (so far >8 weeks) without a discernable anti-LPL immune response. The efficacy at this low dose, however, was lost in the absence of immune suppression. **CONCLUSION:** AAV1-mediated delivery of LPL^{S447X} in muscle is an effective therapy to provide complete correction of hypertriglyceridemia associated with murine and feline LPL deficiency. Since human LPL deficiency is often characterised by loss of LPL activity but persisting LPL protein mass, the current study suggests that AAV1-LPL^{S447X} may be successful for the treatment of this disease in man.

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Body-wide delivery of a microdystrophin gene via intravascular administration of rAAV6 vectors for treatment of muscular dystrophy. *P. Gregorevic, M. Blankinship, J. Allen, L. Meuse, S. Abmayr, J. Han, J. Chamberlain.* Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center, Dept. of Neurology, University of Washington, Seattle, WA.

Severe neuromuscular disorders, such as Duchenne muscular dystrophy (DMD), lead to reduced quality of life and premature mortality. Historically, genetic interventions for these diseases have been limited by an inability to achieve widespread gene transfer to the affected tissues. Here we demonstrate a method that, for the first time, enables transduction of the vast majority of both the cardiac and skeletal musculature of adult mammals via a single intravenous administration of pseudotype 6 recombinant adeno-associated virus (rAAV6) vectors. As a means to enhance gene transfer, we have determined that IV coadministration of vascular endothelium growth factor (VEGF) with rAAV6 vectors can significantly increase vector accumulation and transgene expression in striated muscles. Having established in reporter gene studies that this technique enables tolerable, high-level transgene expression, we sought to evaluate the potential of this technique for delivering a therapeutic transgene to the musculature of the mdx dystrophic mouse, a model of DMD. Using this technique, we have observed that treated mdx mice exhibit expression of microdystrophin throughout the striated musculature in a manner that is sufficient to reduce pathological features of the dystrophic phenotype. Compared with untreated mice, the muscles of treated animals exhibit reduced susceptibility to contraction-induced injury, and reduced serum creatine kinase levels, reflecting a global reduction in muscle degeneration. These data are the first to demonstrate body-wide amelioration of symptoms associated with dystrophin deficiency in an adult animal following a genetic intervention. In subsequent studies we have established that these techniques can be adapted to express alternate transgenes for the treatment of other muscle diseases. We are currently undertaking studies to determine whether the existing techniques are effective in animals more similar in size and immunology to humans, and present relevant data summarizing our progress.

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Program Nr: 276 from the 2004 ASHG Annual Meeting

Complete, life-long correction of hyperbilirubinemia in the Gunn rat model of Crigler-Najjar syndrome type I.
G. Toietta[1, 2], V.P. Mane[1], M.J. Finegold[3], P. Ng[1], A.L. Beaudet[1], B. Lee[1, 4]. 1) Molec & Human Genetics, Baylor College Med, Houston, TX; 2) IDI-IRCCS, Rome, Italy; 3) Pathology, Baylor College Med, Houston, TX; 4) HHMI, Baylor College Med, Houston, TX.

Crigler-Najjar syndrome type I is a recessively inherited disorder characterized by severe unconjugated hyperbilirubinemia, due to a deficiency of the hepatic bilirubin uridine diphosphate-glucuronosyltransferase (B-UDPGT) activity. Current therapy is based on phototherapy to prevent kernicterus, but liver transplant is the only permanent form of correction; therefore, gene therapy may represent an alternative treatment. Until now, with various approaches, only partial long term or complete short term correction has been achieved. We evaluated a gene therapy approach in the Gunn rat model for the disease using helper dependent adenoviral vectors (HD-Ad) for liver-restricted expression of human B-UDPGT. Plasma total bilirubin levels were reduced from above 7.0 mg/dl to less than 1.5 mg/dl for more than 20 months after single systemic administration of $3x10^{12}$ viral particles/kg of HD-Ad expressing human B-UDPGT. There was no significant chronic elevation of serum enzymes to indicate hepatocyte injury. Transient, dose-related thrombocytopenia developed after viral injection at higher dose. The clinical relevance of the study is demonstrated by the potential to achieve lifetime complete normalization of hyperbilirubinemia in Gunn rats by single injection of HD-Ad. Moreover, partial but clinically relevant correction was achieved with a low dose injection making clinical application a possibility. This is the first study describing the use of HD-Ad for overexpression of a hepatic microsomal protein. It also is the longest correction using a viral delivery method achieved to date in this model. Considering the current burden of daily phototherapy and the risks of liver transplant, we believe Crigler-Najjar type I patients might be considered as candidates for HD-Ad mediated gene therapy. Nonetheless, future studies aimed at further minimizing the acute toxicity associated with systemic administration of Ad vectors will be needed before achieving intravascular Ad-mediated gene therapy in humans.

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Cytogenetic Evaluation for Precocious Sister Chromatid Separation in 86 Probands with Cornelia de Lange Syndrome. *M. Kaur[1], J. McCallum[1], D. Yaeger[1], N.B. Spinner[1, 2], I.D. Krantz[1]*. 1) Divisions of Human Genetics and Molecular Biology; 2) Clinical Laboratories, The Children's Hospital of Philadelphia, PA.

The Cornelia de Lange syndrome (CdLS) (OMIM# 122470) is a dominantly inherited multisystem developmental disorder consisting of characteristic facial features, hirsutism, abnormalities of the upper extremities ranging from subtle changes in the phalanges and metacarpal bones to oligodactyly and phocomelia, gastroesophageal dysfunction, growth retardation, and neurodevelopmental delay. Prevalence is estimated to be as high as 1 in 10, 000. Recently mutations in NIPBL were identified in sporadic and familial CdLS cases. To date mutations in this gene have been identified in over 45% of individuals with CdLS. NIPBL is the human homolog of the Drosophila Nipped-B gene. Although its function in mammalian systems has not been elucidated, Nipped-B has been shown to be an essential regulator of cut, Ultrabithorax, and Notch receptor signaling in Drosophila. Sequence homologs of Nipped-B in yeast (Scc2 and Mis4) are required for sister chromatid cohesion during mitosis, and a similar role was recently demonstrated for Nipped-B in Drosophila. In order to evaluate NIPBLs role in sister chromatid cohesion in humans, metaphase spreads on 86 probands (31 mutation positive and 55 mutation negative) with CdLS were evaluated for evidence of precocious sister chromatid separation (PSCS). We screened 20-30 metaphases from each proband and found evidence of PSCS in 18 of 86 (21%). Of these 18 probands with PSCS, 7 (39%) were mutation positive and 11 (61%) mutation negative. Of the 68 probands without evidence of PSCS, 24 (35%) were mutation positive and 44 (65%) were mutation negative. Fifty control slides were screened and 2 (4%) demonstrated evidence of PSCS. Both severe and mild CdLS phenotypes were seen in PSCS positive and negative groups. Missense, frameshift and nonsense mutations have been seen in both groups. Two of the patients with PSCS have the same missense mutation: an R2298H in exon 40 in a highly conserved amino acid residue. These studies indicate that NIPBL may play a role in sister chromatid cohesion in humans as has been reported for its homologs in Drosophila and yeast.

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Program Nr: 278 from the 2004 ASHG Annual Meeting

ATM and/or ATR Deficiency Disrupts the Chromatin of the X Chromosome. *J.V. Goldstine, Y. Ouyang, R. Gatti, Y. Marahrens.* Dept Human Genetics, Univ California, Los Angeles, Los Angeles, CA.

Checkpoint proteins maintain the integrity of genomic DNA by responding to DNA damage and preparing the cell for repair. Here we show that ATM deficiency compromises both euchromatic properties of the active X chromosome and heterochromatic properties of the inactive X chromosome. Replication timing is one feature that defines euchromatic genes from heterochromatic genes; in general highly heterochromatic genes replicate late in the cell cycle, while euchromatic genes replicate early. We demonstrate that in AT deficient cells, the active (euchromatic) X chromosome replicates later in the cell cycle than in normal cells. A change in the heterochromatic properties of the inactive X chromosome was also observed. Moreover, the ATM/ATR inhibitor 2-aminopurine also caused the inactive X chromosome to undergo abnormal gene reactivation as well as accumulate acetylated histones, a characteristic of euchromatin. Individually inhibiting ATM and/or ATR using siRNA compromised X-inactivation. The expression of dominant-negative constructs of ATM or ATR proteins similarly triggered reactivation of the inactive X chromosome. ATM and ATR, therefore, not only function in DNA damage checkpoints but also play fundamental and widespread roles in the maintenance of chromatin structure.

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Upregulation of a novel repressor, RUNX2-interacting protein, in patients with 8q22 rearrangements phenocopies cleidocranial dysplasia. *G. Zhou*[1], *P. Hermann*[1,2], *E. Munivez*[1,2], *R. Morello*[1], *Y.Q. Chen*[1,2], *Q.P. Zheng*[1], *M. Carette*[3], *H. Kingston*[3], *M. Tassabehji*[3], *B. Lee*[1,2]. 1) Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Howard Hughes Medical Institute; 3) Medical Genetics, St Mary's Hospital, manchester, UK.

RUNX2 is one of three vertebrate members of the runt family of transcription factors which have in common a 128 amino acid motif that mediates both DNA binding and protein-protein interaction. Runx2 null mice have complete absence of osteoblast and bone but an intact cartilagenous skeleton and loss of function mutations of RUNX2 result in the human skeletal disorder-cleidocranial dysplasia (CCD). To identify potential RUNX2-interacting proteins, we screened a human osteosarcoma cDNA library using a yeast two-hybrid approach with RUNX2 as bait and isolated a 500-bp partial cDNA which was termed RIP (Runx2-interacting protein). Northern blot analysis and in situ hybridization on mouse E15.5 embryos revealed its ubiquitous expression including osteoblasts. Sequence analysis of RIP revealed a multidomain structure characterized by several protein-protein interaction domains and a nuclear localization signal. GST pulldown experiment demonstrated that RUNX2 bound RIP with its RUNT domain. In transient transfection experiments RIP down-regulated the transactivation by RUNX2 in a dose-dependent manner in both 10T1/2 cells and ROS17/2 cells. Furthermore immunofluorescent staining showed that RIP mainly localized in the nucleus. FISH and radiation hybrid mapping studies showed that RIP localizes to chromosome 8q22 syntenic with a hypothesized second CCD-like locus. Interestingly RIP expression is upregulated in CCD-like patients with 8q22 rearrangements, supporting its function as a repressor of RUNX2. In these patients, we hypothesize that upregulation of a putative repressor of Runx2 would cause loss of Runx2 transactivation and hence a CCD phenocopy in vivo. To elucidate the potential effect of RIP on skeletogenesis, we are studying its action by generation RIP null mice and transgenic mice overexpressing RIP in osteoblasts.

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Program Nr: 280 from the 2004 ASHG Annual Meeting

Translocation breakpoint mapping in three 1p36 subjects with der(1)t(1;9)(p36;q34.3). *L.G. Shaffer*[1,2], *C.D. Glotzbach*[1,2], *M. Jarmuz*[1], *B.C. Ballif*[1], *M. Gajecka*[1]. 1) Health Research and Education Center, Washington State University, Spokane, WA; 2) Sacred Heart Medical Center, Spokane, WA.

Although terminal deletions of 1p36 are relatively common, unbalanced translocations of 1p are more rare, and little is known about their mechanisms of formation. Our previous molecular studies suggest that a substantial percentage of apparently terminal deletions of 1p36 are stabilized by the acquisition of telomeric sequences from other chromosome ends, forming derivative chromosomes. Of the 95 ascertained to date, 13 (14%) are derivative chromosomes. Here we present the mapping and cloning of the breakpoints in three subjects with der(1)t(1;9)(p36;q34.3) unbalanced translocations. Two subjects have *de novo* derivative chromosomes while the third subject and his sister have a maternally derived derivative chromosome 1. To determine the sizes of the 1p36 deletions we performed array CGH with cell lines derived from each of the three subjects using a contig of 1p36 consisting of 97 large-insert BAC clones spanning the most distal 10.5 Mb of 1p36 and 41 subtelomere clones from all chromosomes except the acrocentric short arms. These and additional metaphase FISH analyses showed that the 1p36 deletions varied from ~3.1 Mb to 5.8 Mb in size while the 9q partial trisomies varied from 2.3 Mb to 5.4 Mb. To identify the 1p36 breakpoints, we generated somatic cell hybrids from each subject containing the derivative chromosomes 1 segregated from the normal chromosomes 1. To refine further each breakpoint, DNA was extracted from these hybrids and examined by PCR and STS marker walking. The 1p36 breakpoints in two cases are in unique sequences. These results indicate that the breakpoint locations are variable in the subjects, with no common 1p deletion or 9q translocation breakpoint. These results support a break-induced replication model, as opposed to nonallelic homologous recombination, for forming these derivative chromosomes. However, other models, such as subtelomeric pairing with proximal recombination, can be considered as possible mechanisms for telomere capture events resulting in derivative chromosomes.

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Program Nr: 281 from the 2004 ASHG Annual Meeting

Identification of a novel gene implicated in cleft lip and palate, learning disability and retinal dystrophy and detection of a missense mutation in a patient with cleft lip and palate. *D. Trump[1,2], J. Boylan[1], K. Miles[2], A. Vieira[3], L.R. Willatt[2], N.T. Bech-Hansen[4], J.C. Murray[3], A.T. Moore[5].* 1) Academic Unit of Medical Genetics,St Mary's Hospital, University of Manchester, Manchester, United Kingdom; 2) Department of Medical Genetics, Addenbrookes Hospital, University of Cambridge, United Kingdom; 3) Department of Medical Genetics, Department of Pediatrics, University of Iowa, USA; 4) Department of Medical Genetics, Faculty of Medicine, University of Calgary, Canada; 5) Institute of Ophthalmology and Moorfields Eye Hospital, London, United Kingdom.

Through investigation of a de novo apparently balanced translocation we have identified a novel 15 exon gene implicated in cleft palate, learning disability and retinal dystrophy. The patient, an 8 year old girl, was found to have a de novo balanced reciprocal translocation with karyotype 46XX, t(1;8)(p32.3;q13.1). We investigated the breakpoints initially using fluorescent in situ hybridization (FISH) with bacterial artificial chromosomes clones (BACs). We found breakpoint spanning BACs for each of the breakpoints and used a combination of FISH mapping and Southern blotting to map the breakpoints to within 1Kb. We then used long range PCR to amplify a product across the breakpoints from each of the derived chromosomes. DNA Sequencing revealed the translocation at a molecular level: a balanced translocation on Der1 and a translocation associated with 24 bp duplication on Der8. The breakpoint on chromosome 1 fell within intron 2 of a predicted 15 exon gene. Multitissue northern blot analysis and RT-PCR revealed ubiquitous gene expression and we have found evidence of expression of 2 splice variants, one lacking exon 15 and the other lacking exon 14. The gene is predicted to encode an intracellular calcium binding protein containing an EF hand and may be involved in intracellular signalling. Mutation analysis has revealed a missense mutation in the calcium binding region in a patient with a cleft lip and palate which is not present in 100 control patients. Investigation in additional patients and functional analysis of this mutation is underway.

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Program Nr: 282 from the 2004 ASHG Annual Meeting

Cloning the breakpoints of an uncommon deletion in a Prader-Willi syndrome patient. *E. Thorland[1], J.W. Ireland[2], A. Adeyinka[1], L.K. Courteau[1], D.B. Dawson[1], D. Babovic-Vuksanovic[2], S.M. Jalal[1]*. 1) Laboratory Medicine/Pathology, Mayo Clinic, Rochester, MN; 2) Medical Genetics, Mayo Clinic, Rochester, MN.

Deletions of approximately 5 Mb are the most frequent cause of Prader-Willi syndrome (PWS), accounting for 70% of the cases. Since the deletions are thought to occur as LCR-mediated homologous recombination events, the proximal and distal breakpoints of the deletions occur at predictable loci and are consistent in the great majority of cases. However, a few uncommon deletion sizes have been reported. Methylation testing by Southern blot using the PW71 probe was consistent with PWS on a 34 year old female patient who was clinically diagnosed with PWS at one month of age. FISH analysis indicated that the D15S10 probe was present. However, the SNRPN probe was deleted, indicating that this patient harbored a rare small deletion. Microsatellite markers across the 15q11.2-12 region demonstrated heterozygosity for several loci, including D15S1021 and D15S1506 flanking the SNRPN gene indicating that the deletion could be no larger than 500 kb, which is the smallest deletion reported. To more precisely define the deletion, EBV-transformed lymphoblasts were used to create somatic cell hybrids containing each of the chromosome 15s. PCR primers were designed to PAR5, IPW and exon 1 of the SNRPN gene. PCR using DNA extracted from the somatic cell hybrids demonstrated that all 3 regions were deleted in the hybrid. Subsequent sets of PCR primers have now narrowed the breakpoints to within approximately 40 kb on either side of the deletion. Once the breakpoint has been further spanned with a single PCR product, this product will be sequenced to reveal the precise breakpoints of the deletion. This sequence data should provide insight into the general mechanisms involved in generating these types of deletions.

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Delineation of the critical region involved in Potocki-Shaffer syndrome [del(11)(p11.2p12)] and further characterization of the phenotype. *L. Potocki*[1,2], *K. Wakui*[1], *G. Gregato*[3], *B.C. Ballif*[3], *C.D. Glotzbach*[3], *K.A. Bailey*[3], *P.-S. Kuo*[4], *W.-C. Sue*[5], *L.J. Sheffield*[6], *M. Irons*[7], *E.G. Gomez*[8], *J.T. Hecht*[9], *L.G. Shaffer*[3]. 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Texas Children's Hospital, Houston; 3) Washington State University and Sacred Heart Medical Center, Spokane; 4) Obstetrics and Gynecology, National Cheng-Kung University Medical College, Taiwan; 5) Pediatrics, Taipei Municipal Women and Children's Hospital, Taiwan; 6) Genetic Health Services Victoria, Murdoch Childrens Research Institute, Paediatrics, University of Melbourne, Victoria, Australia; 7) Division of Genetics, Department of Medicine, Children's Hospital, Harvard Medical School, Boston, MA; 8) Area de Genetica. Centro de Desarrollo Infantil y Departamento de Pediatra Hospital Materno Infantil-Hospital Regional Universitario "Infanta Cristina", Badajoz, Spain; 9) Pediatrics, University of Texas Medical School, Houston.

Potocki-Shaffer syndrome (PSS) is a contiguous gene deletion syndrome that results from haploinsufficiency of at least two genes within the short arm of chromosome 11. Clinical features of PSS include developmental delay, mental retardation (MR), multiple exostoses (due to a deletion of *EXT2*), parietal foramina (due to a deletion of *ALX4*), craniofacial anomalies, and genital abnormalities in males. We constructed a natural panel of 11p11.2-p13 deletions using cell lines from ten affected individuals, FISH, microsatellite analyses, and array-based comparative genomic hybridization (CGH), and compared the deletion sizes and clinical features. The full spectrum of PSS manifests when deletions are 2.1 Mb, spanning from D11S1393 to D11S1385/D11S1319, and encompassing *EXT2* and *ALX4*. Based on comparative deletion mapping of seven individuals with the full PSS syndrome including MR and three PSS families with no MR, at least one gene related to MR is likely located between D11S554 and D11S1385/D11S1319. We discuss genotype/phenotype correlation, the possible molecular mechanism of deletion, and utility of microarray analysis by CGH in the diagnosis of PSS.

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Program Nr: 284 from the 2004 ASHG Annual Meeting

A new phenotypic map in Wolf-Hirschhorn syndrome: new insights for genetic counseling and candidate genes.

M. Zollino[1], M. Murdolo[1], I. Mancuso[1], R. Lecce[1], F. Faravelli[2], V. Pecile[3], G. Neri[1]. 1) Dept Medical Genetics, Univ Catt Sacro Cuore, Rome, Italy; 2) Dept of Human Genetics, Galliera Hospital of Genova, Genova; 3) Dept of Genetics, University of Trieste, Trieste.

Wolf-Hirschhorn sindrome(WHS) is a contiguous gene syndrome caused by partial 4p deletion, with critically deleted region (WHSCR-2) within 4p16.3. The minimal phenotype is defined by the association of mental retardation, severe growth delay, typical facial appearance and seizures. Depending on the basic genetic defect, additional clinical signs include microcephaly, cleft palate, hypospadias, coloboma, congenital heart diseases, brain anomalies and renal defects. We performed a clinical-genetic analysis in a total of 50 WHS patients and 4 non WHS patients with either an interstitial or a terminal deletion. Individual deletion size was assessed by FISH with a total of 90 molecular probes. Although silencing of genes by positional effects can be considered, relevant results are in order:1)seizures map in the 2 Mb terminal interval and appear to be multigenic;2)growth delay is restricted to an about 400 kb interstitial interval between loci D4S3327 and D4S168;3)facial dysmorphisms map in a relatively large interstitial region and are multigenic;4)distal boundaries for microcephaly, cleft palate, hypospadias, and CHD are also established. These observations provide new insights for genetic counseling and for the search of new candidate genes in WHS.

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Program Nr: 285 from the 2004 ASHG Annual Meeting

Cytogenetic Approaches to Finding Auditory Genes. *R.E. Williamson[1], C.C. Morton[1, 2]*. 1) Harvard Medical School, Boston, MA; 2) Brigham and Women's Hospital, Boston, MA.

Hearing loss is a common sensory disorder with an estimated incidence of 1 in 1000 human births. Approximately half of cases are attributed to environmental factors, while at least 50% are due to genetic causes. Genes with a role in the auditory system have been identified through both genetic linkage studies of families with heritable deafness and positional candidate gene approaches. Another method for gene discovery is to ascertain deaf individuals that carry balanced translocations and identify disrupted or disregulated genes at the site of chromosomal rearrangement. Here, FISH experiments were performed to map the breakpoint regions on each of two derivative chromosomes in a deaf individual. One case, designated DGAP056, has a translocation between chromosome 2 and chromosome 13 [t(2;13)(p24;q21)]. Breakpoints were assigned initially by GTG-banding and BAC probes for FISH experiments to map precisely the breakpoints were chosen based on proximity to the assigned region. Successive FISH experiments were then performed to identify a split BAC that hybridized to the normal chromosome as well as to both of the derivative chromosomes. Further refinement of the breakpoint was done using PCR products in FISH experiments and in Southern blot analysis. This translocation disrupts a hypothetical gene, *FLJ21820*, between exons 4 and 5. This gene is expressed in the cochlea and Northern blot analysis shows that it is more highly expressed in fetal tissues than in adult tissues. *In situ* experiments have demonstrated that *FLJ21820* is expressed in a very select number of cells in the cochlea further supporting its importance in the hearing pathway. Hearing studies of a knock-out mouse model will soon be underway to establish the role of *FLJ21820* in the auditory system.

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Localisation of a modifier of FRAXA repeat expansion susceptibility using linkage disequilibrium mapping. *S. Ennis[1], A. Murray[2], N.E. Morton[1], P.A. Jacobs[2]*. 1) Human Genetics Division,University of Southampton, Southampton General Hosp (Mp 808), Hampshire SO16 6YD, U.K; 2) Wessex Regional Genetics Laboratory, Salisbury District Hospital, Salisbury, Wiltshire SP2 8BJ, U.K.

Fragile X syndrome is the most common form of inherited mental retardation affecting ~1/5500 males. Purity and length of the CGG array influence stability but other *cis*-acting factors are postulated to exist. Although high-risk haplotypes have been identified using microsatellite markers, causal determinants of expansion remain elusive.We typed 30 single nucleotide polymorphisms (SNPs) across 650 kb of the fragile X region on a panel of 877 independent male chromosomes that span the range of FRAXA repeat sizes. We have produced a linkage disequilibrium (LD) map of the region which defines 5 discrete blocks of very high LD interspersed by regions of LD breakdown, probably due to increased recombination. Using information gleaned from the LD analysis, an association study using the ZMAP software (http://cedar.genetics.soton.ac.uk/public_html/) was conducted. The program uses allelic association to estimate the location of a causal mutation in case/control data. We have applied this method to our sample by dividing the 877 haplotypes into pseudo cases and controls by taking haplotypes with FRAXA alleles in excess of 40 repeats as cases and those with less than 41 repeats as controls. Within a composite likelihood framework, the program estimates association between each SNP and the pseudo affection status and the likelihood of causality of each SNP marker is estimated. These likelihood scores were converted to LOD scores and plotted against the kilobase (kb) map. As the FRAXA expansion mutation is already known to be the causal mutation, we postulate that the program identifies a region that contains a *cis* element which has an impact on repeat expansion susceptibility. Plotting the resultant LOD scores shows a highly significant association of a region 5' of FMR1 with the expanded phenotype. The tract encompassing the 95% confidence interval was identified and we have intensified our search in this region using long PCR and sequencing in order to identify the precise nature of the proposed modifier.

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