PLATFORM PRESENTATIONS IN CLINICAL GENETICS I

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Report of a familial OFD1 mutation among affected and unaffected relatives. *C Hess*[1,] *K Monaghan*[1], *J Roberson*[1]. [1]*Henry Ford Hospital, Detroit, MI.*

Type I Oral Facial Digital syndrome (OFD1) is an X-linked dominant disorder characterized by anomalies of the mouth (multiple, hypertrophic frenula; cleft lip and/or palate; and hamartomas of the tongue), face (asymmetry, milia, hypoplastic alar cartilage and hypertelorism), and digits (syndactyly, polydactyly, clinodactyly and brachydactyly). Mental retardation occurs in approximately 60% of affected individuals and the disorder has been associated with adult onset polycystic kidney disease. We report a young girl who was diagnosed with sporadic OFD1 shortly after birth. Molecular analysis of our proband, performed at the Telethon Institute of Genetics and Medicine (TIGM) in Italy, revealed a frameshift mutation (702insA) in the recently identified OFD1 gene (Xp22). A detailed clinical evaluation of the proband's mother was unremarkable, however, she was later identified to have the same frameshift mutation. In addition, prenatal testing revealed that her second daughter had also inherited this mutation. This child is currently 5 months old and clinically unaffected. Data received from TIGM indicates that 80 individuals with features of sporadic Oral Facial Digital syndrome have been identified as carriers of an OFD1 mutation. Although familial molecular studies have not been routinely performed, TIGM has reportedly identified at least one other family in which a deleterious OFD1 mutation is present in both affected and unaffected individuals. Therefore, prenatal analysis for OFD1 should be performed with caution, due to the incomplete penetrance of the mutated gene. Although the OFD1 gene has previously been shown to escape X-inactivation, X-inactivation studies are in progress for this family. If skewed X-inactivation is ruled out, it is possible that unknown modifying genes are responsible for the OFD1 phenotypic presentation. In addition, further information about the etiology of this condition may be learned from molecular studies on first degree relatives of apparently sporadic cases of OFD1.

Disclosure(s): None

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Beckwith-Wiedemann syndrome and isolated hemihyperplasia following ART: association with paternal UPD of 11p15. *C Clericuzio*[*1], *C Shuman*[2,3,4], *L Steele*[3,5], *J Milisa*[1], *P Ray*[3,4,5], *R Weksberg*[2,3,4,6]. [1]*Ped-Div Dysmorph/Clinical Gen, Univ New Mexico Sch Medicine, Albuquerque, NM,* [2]*Div Clin & Metab Genetics, Hosp Sick Children, Toronto,* [3]*Dept Mol & Med Genetics, Univ Toronto, Toronto,* [4]*Research Inst, Hosp Sick Children, Toronto,* [5]*Dept Lab Med, Hosp Sick Children, Toronto,* [6]*Dept of Paed, Hosp Sick Children, Toronto.*

Beckwith-Wiedemann syndrome (BWS) is a prototypical imprinting disorder, caused largely by epigenetic alterations of imprinted genes in the chromosomal region 11p15.5. Fifteen infants with BWS have been reported following conceptions with assisted reproductive technology (ART). Molecular genetic analysis of 7 of these cases has identified imprinting defects involving hypomethylation of KvDMR1. These data taken together with a report of 3 cases of ART-associated Angelman syndrome due to imprinting defects supports an association between ART and imprinting disorders. Here, we report 1 child with BWS and another with isolated hemihyperplasia (HH) who were conceived by ART (IVF) and who were found to have uniparental disomy (UPD) of chromosome 11p15. Pt 1: A 36 week gestation male twin was diagnosed with BWS and later developed a Wilms tumor. Genetic studies on foreskin fibroblasts from the patient and parental blood DNA showed somatic mosaicism for paternal UPD of 11p15. Pt 2: A term female twin was diagnosed with right HH at 6 mo, following presentation with Stage 3 hepatoblastoma. Genetic studies of the patient and paternal blood DNA showed somatic mosaicism for paternal UPD 11p15. The finding of UPD 11p15 in these 2 cases suggests that errors in post-zygotic recombination that lead to UPD may be associated with ART. The development of tumors in these cases is not surprising, as BWS with UPD 11p15 is known to have a significant cancer risk. Moreover, the clinical spectrum associated with UPD 11p15 is now believed to encompass HH in addition to BWS. Hence, ART may be associated not only with epigenetic imprinting errors, but may also influence the risk of somatic mitotic recombination.

Disclosure(s): None

PLATFORM PRESENTATIONS IN CLINICAL GENETICS I

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Not just skin deep: the association of livedo reticularis and recurrent strokes in childhood. *R Rogers*[1, 4, 5], G Mathias[2], R Cowley[3], A Morales[4]. [1]Greenwood Genetic Center, Greenwood, SC, [2]University of South Carolina School of Medicine, Columbia, SC, [3]Department of Radiology, Greenville Hospital System, Greenville, SC, [4]Department of Pediatrics, Greenville Hospital System, Greenville, SC, [5]Department of Obstetrics and Gynecology, Greenville Hospital System, Greenville, SC.*

We report two children with recurrent strokes beginning at age 2 years who also had congenital cutaneous vascular instability. Livedo reticularis in association with cerebrovascular accidents was first noted by Sneddon in 1965 and the combination has since been called Sneddon syndrome (OMIM # 182410). Numerous case studies have reported varying patterns of expression in an autosomal dominant pattern or sporadic occurrence. In most cases, livedo reticularis precedes the onset of cerebral vascular events. Livedo reticularis is the presence of a fishnet reticular pattern in unbroken circles. Pathogenesis of this disease is unknown. Some studies suggest that the lesions occurring in the medium-sized vessels affected are inflammatory in nature. Evidence supporting this hypothesis has included elevated erythrocyte sedimentation rates during acute events, complement consumption, immune complexes in circulation, and inflammation involving vessels from skin biopsy specimens. Other studies and case reports have found no evidence of inflammation involving digital artery biopsies or vessels of skin biopsy. Moyamoya ("cloud of smoke") syndrome is a narrowing of the carotid arteries that progresses to affect arteries in the circle of Willis and has frequently been discovered in association with Sneddon syndrome. Treatment is highly variable with unpredictable results and the treatments include vasodilators, beta blockers, methylprednisolone, anticoagulants, and platelet inhibitors. The prognosis of patients is highly dependent upon comorbid disorders and hypertension, in particular, is associated with progression of the cerebrovascular disease. Both patients presented with congenital cutis livedo reticularis. Their general health was good until they began having stroke-like episodes at age 2 years, when each child had the acute loss of function in one extremity. Cranial imaging studies showed multiple extensive infarcts and Moyamoya disease. They underwent vascular diversion surgeries with good response. The family histories were negative for recurrent strokes or stroke-like episodes, livedo reticularis, or developmental delay. Patient 1 was examined at age 38 months and had generalized livedo reticularis, developmental delay, and incoordination. At age 7 years patient 2 continued to have severe language disabilities, developmental delay, and hemiparesis. Our patient's findings seem to fit the description of Sneddon syndrome well but no cases of congenital livedo reticularis have been classified as Sneddon syndrome to date. The association of strokes and livedo reticularis has rarely been reported in the pediatric and genetic literature. Given the congenital nature of the cutaneous vascular abnormality in these patients and the subsequent involvement of the central nervous system, Sneddon syndrome may deserve consideration as a phakomatosis.

Disclosure(s): None

4

Fryns syndrome - report of ten cases. *A Slavotinek*[1], H Robinson[2], M Steele[3], G Schauer[4], G Machin[5], M Dasouki[6], K Ornvold[7], M Rueda-Pedraza[8], F Chiricosta[8], K Jasnosz[9], R Keller[10]. [1]Dept. Pediatrics, Division of Genetics, UCSF, San Francisco, CA, [2]Dept. Genetics, Children's Hospital Medical Center of Akron, Akron, OH, [3]Dept. Pathology and Laboratory Medicine, Children's Hospital Medical Center of Akron, Akron, OH, [4]Dept. Pathology, Children's Hospital and Clinics, Minneapolis, MN, [5]Kaiser Permanente Medical Center Oakland, Oakland, CA, [6]Section of Medical Genetics and Molecular Medicine, Children's Mercy Hospital, Kansas City, MO, [7]Dept. Pathology, Dartmouth Hitchcock Medical Center, Lebanon, NH, [8]Dept. Pathology, Walter Reed Army Medical Center, Washington, DC, [9]Dept. Pathology and Laboratory Medicine, Allegheny General Hospital, Pittsburgh, PA, [10]Dept. Pediatrics, Division of Neonatology, UCSF, San Francisco CA.*

Fryns syndrome (FS; OMIM 229850) is the commonest syndrome associated with congenital diaphragmatic hernia (CDH) and comprises CDH, pulmonary hypoplasia, brachytelephalangy and nail hypoplasia, craniofacial dysmorphism, orofacial clefting and malformations including neuronal heterotopias, cardiac septal defects and renal cysts. FS shows extreme variation in phenotypic expression and it is critical to recognize this diagnosis as the autosomal recessive inheritance of FS contrasts with the sporadic inheritance of most patients with CDH. We therefore report ten new cases of FS to aid in the diagnosis and availability of clinical information concerning this rare and striking malformation syndrome. Our cases were five males and five females and there were two sibships that both had an affected male and female. Chromosome analysis showed no abnormalities in all children in which it was performed (6/10). 8/10 of the babies were diagnosed by prenatal sonograms and AFP was elevated in three pregnancies. All children had CDH but four out of ten were reported to have normal fingers and nails; of these four children, two had sibs with brachytelephalangy and CDH and thus enabled the diagnosis of FS. There was no relationship between nail hypoplasia and gestational age. The commonest anomalies were pulmonary hypoplasia (9/10), craniofacial dysmorphism (7/10) with dysplastic ears (5/10) and a broad and flat nasal bridge (5/10), malrotation or non-fixation of the bowel (5/10) and orofacial clefting (4/10). Infrequent but previously described findings were hypoplasia of the olfactory nerve, hypoplasia of the optic nerve, Hirschsprung disease, duodenal and esophageal atresias, pancreatic hyperplasia and osteochondrodysplasia. A bifid epiglottis, coarctation of the aorta, nephromegaly and fusions between the liver, testis, kidney and adrenal gland were each described in unrelated children for the first time. Interestingly, one child was exposed to maternal synthroid and was the second documented case of FS associated with thyroid hormone ingestion in pregnancy. Only one child survived the neonatal period and lived to one year of age with supplemental oxygen. We conclude that marked variability and phenotypic heterogeneity complicate the diagnosis of FS and that nail hypoplasia may be less common than previously described. We therefore suggest that strong consideration be given to this syndrome in babies with CDH and additional anomalies consistent with FS even in the absence of nail hypoplasia.

Disclosure(s): None

PLATFORM PRESENTATIONS IN CLINICAL GENETICS I

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Long-term correction of sialidase deficiency in cells and mice by recombinant helper-dependent adenovirus-mediated gene transfer. *M Mitchell*[*1], Z Iqbal[1], S Pattison[1], F Graham[1,2], S Igdoura[1,2]. [1]Department of Biology, McMaster University, Hamilton, Ontario Canada, [2]Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario Canada.*

Sialidosis, an inherited primary deficiency of lysosomal sialidase, is an autosomal recessive lysosomal storage disease which results in liver, kidney and brain accumulations of sialoglycoconjugates. Currently there is no treatment for sialidosis, while there have been some moderate success reported for the treatment of several lysosomal storage diseases using enzyme replacement therapy or bone marrow transplantation. First generation (FG-Ad) as well helper-dependent adenoviruses (HD-Ad) have been extensively studied as potential gene therapy vehicles for the treatment of genetic diseases. To this end, a full-length mouse lysosomal sialidase gene and promoter was cloned into a FG-Ad vector and a HD-Ad vector and were tested in vitro and in vivo in sialidase-deficient cells and mice respectively. Following both FG-Ad and HD-Ad sialidase infections, lysosomal sialidase activity and mRNA levels in cultured deficient cells were dramatically increased. After an intravenous administration of 10 particles/mouse of the FG-Ad and HD-Ad sialidase vectors, mice were examined at one, two and six and 12 weeks post infection. Our in vivo results show that while the FG-Ad sialidase demonstrated a more robust increase in liver lysosomal sialidase activity within the first two weeks post infection, the HD-Ad demonstrated a much more sustained expression beyond three months post-infection. The HD-Ad sialidase also corrected sialidosis-associated phenotype in relation to body weight and liver pathology as well as blood and urine profiles. The in vitro and in vivo preclinical studies presented herein using helper-dependent adenovirus provide a strong rationale for gene therapy clinical trials for sialidosis type I.

Disclosure(s): None

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Update on phase I/II and phase II studies of recombinant human N-acetylgalactosamine 4-sulfatase (rhASB)) enzyme replacement therapy in patients with mucopolysaccharidosis VI (Maroteaux-Lamy syndrome). *PR Harmatz*[*1], C Whitley[2], R Steiner[3], B Plecko[4], P Kaplan[5], J Waterson[1], D Ketteridge[6], R Giugliani[7], I Schwartz[7], N Guffon[8], C Sa Miranda[9], L Keppen[10], E Teles[11], J Hopwood[6]. [1]Children's Hospital & Research Center at Oakland, Oakland, CA, [2]University of Minnesota Medical School, Minneapolis, MN, [3]Oregon Health Science University, Portland, OR, [4]U Kinderklinik Graz, Austria, [5]Children's Hospital of Philadelphia, Philadelphia, PA, [6]Women's and Children's Hospital, Adelaide, Australia, [7]Hospital de Clinicas de Porto Alegre, Brazil, [8]Hospital Edouard Herriot, Lyon, France, [9]Medica Jacinto de Magalhaes, Portugal, [10]University of South Dakota, Vermillion, SD, [11]Hospital de Sau Joao, Porto, Portugal.*

Mucopolysaccharidosis VI (MPS VI; Maroteaux-Lamy syndrome) is a lysosomal storage disease caused by a deficiency of the enzyme N-acetylgalactosamine-4-sulfatase (ASB), leading to a progressive disorder with multiple organ and tissue involvement. Weekly treatment with recombinant ASB (rhASB) has been studied in two clinical trials: Seven subjects participated in a randomized, double-blind, two-dose Phase I/II study (3 males; age 7-16 years), and 10 subjects participated in a Phase II, open-label, single dose study (3 males; age 6-22 years). Week 96 data for the Phase I/II study and week 48 data for the Phase II study are presented here. All subjects tolerated the weekly enzyme infusions well and 15 subjects continue on the treatment. There were 16 SAEs, 1 drug-related, in the Phase I/II study, and 7 SAEs, 6 unrelated to study drug and 1 possibly related to study drug, in the Phase II study. Although some rhASB antibody production has been noted, it has not substantially interfered with reduction in urinary glycosaminoglycan (GAG) excretion. Biochemical response was documented by sustained reduction in urinary excretion of GAG by more than 70%. Improvements in endurance and mobility have been noted: the 6-minute walk test yielded a mean increase of 96% in the Phase I/II study, and the 12-minute walk test yielded a mean increase 139% in the Phase II study; shoulder flexion increased by a mean of 14% in the Phase I/II study and improvement of 147% in the 3 minute stair climb was observed in the Phase II study. In conclusion, rhASB treatment has been well tolerated with positive clinical and biochemical responses. A report of continued response through weeks 144 and 72 for the Phase I/II and Phase II studies, respectively, will be provided. (Sponsored by BioMarin Pharmaceutical Inc., Novato, CA).

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PLATFORM PRESENTATIONS IN CLINICAL GENETICS I

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Open label study of rivastigmine tartrate in pediatric Down syndrome (preliminary data). *BG Crissman*[1], JH Heller[1], GA Spiridigliozzi[1], JA Sullivan[1], PS Kishnani[1]. [1]Duke University Medical Center, Durham, North Carolina.*

Down syndrome (DS), resulting from trisomy 21, is the most common genetic cause of mental retardation (MR), accounting for approximately 1/3 of cases of moderate to severe MR. At the current time there is no FDA approved treatment for the cognitive symptoms associated with DS. DS has features in common with Alzheimer disease (AD), with numerous studies confirming similarities between the neuropathological and neurochemical changes seen in both of these conditions. A marked reduction of cholinesterase acetyltransferase activity (ChAT), the biosynthetic enzyme for acetylcholine production, is seen in the cerebral cortex and nucleus basalis of Meynert in patients affected by AD. Based upon the cholinergic hypothesis that cholinergic neurons play an important role in memory, attention, language and other cognitive function, the similarities in AD and DS brain neuropathology and neurochemistry and the successful use of donepezil hydrochloride in our previous studies of adults with DS, we have initiated an open label exploratory study of the use of rivastigmine in children with DS. This study is designed as a twenty week, single-center, open label trial of 15 individuals with a diagnosis of DS between the ages of 10 to 18 years. Participants are seen for four visits, screening (week -4), baseline (week 0), low dose (week 8) and high dose (week 16). To be enrolled in the study, participants should have reliable caregivers, no contraindication for the use of cholinesterase inhibitors, and mild to moderate cognitive impairment (IQ 40-70 inclusive). Rivastigmine was administered at 1.5 mg liquid formulation (0.75 mg bid) following the baseline visit and slowly titrated to 4.5 mg per day. To date, seven participants (2F:5M, mean age 13 years) have completed the study and three additional subjects have completed 3 of four visits. The primary objectives of the study include establishing safety data, developing an appropriate neuropsychological and language battery for children with DS, and investigating the effects of rivastigmine on specific cognitive functions and language in this population. Subjects have gained an average of 0.45 kg over the course of the study. There have been no serious adverse events related to study drug to date. Adverse events possibly related to medication were transient and decreased with slower titration. Overall, subjects have shown improved expressive language performance with treatment and increased effect with higher dose. The preliminary data obtained from this study, though very limited, provides valuable information about safety, drug dosage and efficacy of rivastigmine in the pediatric DS population. A larger, double-blinded study of cholinergic agents in pediatric Down syndrome is important and warranted.

Disclosure(s): Novartis has sponsored research activities relevant to this presentation.

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Feasibility of human hematopoietic stem cell injection via celocentesis into the pre-immune baboon fetus. *J DeLeon*[1], I Galan[1], V Noble[1], J Gooch[1], J Santolaya-Forgas[1]. [1]Division Reproductive Genetics. Texas Tech University. Amarillo.TX.*

In-utero gene therapy using erythropoietic stem cells could prevent the development of severe metabolic and hematological diseases of genetic origin. In order to consider in-utero gene therapy with somatic stem cells, it is desirable to develop a technique that would allow the transfer of these cells to the pre-immune fetus to avoid rejection of the donor cells. It is also important to develop a transferring technique that causes no embryonic damage. In primates the extraembryonic organs are developed before the embryo proper. During the first weeks of pregnancy a wide extracelomic cavity arises in which the small 2-chambered embryo is suspended by the body stalk. Extracelomic fluid sampling and infusions into the extracelomic cavity could be potential methods for making very early prenatal diagnosis and for attempting stem cell treatment. Genetic studies have already been performed in extracoelomic cells obtained very early in pregnancy from women undergoing elective termination of pregnancy and from non-human primate models. The objective of this study was to test the hypothesis that the pregnancy would continue after the injection of human stem cells in the baboon's extracelomic space. MATERIAL AND METHODS: Nine celocentesis procedures were performed transabdominally in sedated timed-pregnant animals, at 40 days gestation, under ultrasound guidance and using a 21-gauge needle. After the extracelomic space was reached (avoiding the amniotic and yolk sacs) the stylet was removed and 0.5 mL of the extracelomic fluid aspirated using 1-mL syringe and then 10.000 to 250.000 human CD34+, Lin- cells, isolated from human umbilical cord blood and diluted in 1 mL of PBS, were injected into the extracelomic cavity. RESULTS:Four animals have delivered at term, 4 animals have aborted between 24-72 hours post procedure and 1 pregnancy is still ongoing CONCLUSION : Primate pregnancies can continue to term after injection of non-haploidentical donor cells into their celomic cavity. Our current aim is to study for the presence of microchimerism in the newborns.

Disclosure(s): None

PLATFORM PRESENTATIONS IN MOLECULAR GENETICS

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Maternal cell contamination studies: donor oocytes as a source of apparent discrepancy. *LS Rosenblum-Vos*[1], KJ Treat[1], RA Heim[1], AN Lamb[1]. [1]Genzyme Genetics, Molecular Diagnostics Laboratory, Westborough, MA.*

Maternal cell contamination (MCC) studies are important to ensure the integrity of prenatal specimens tested in the molecular diagnostic laboratory; assisted reproductive technology (ART) can lead to apparent discrepancy in these test results. We routinely perform MCC studies on prenatal specimens. In the past year we have investigated cases in which the MCC assay showed that the fetus had no alleles in common with the mother at multiple loci. Several illustrative examples will be presented. In each case we stopped the reporting of all prenatal specimens in process and all blood samples extracted at the same time as the maternal specimen. In addition, we initiated a comprehensive investigation of the discrepancy. This included A) Review of the test requisition form and associated materials for sources of error or clues pointing to donor oocyte use, such as referral from an ART center or maternal age of greater than 45 years. B) Rechecking specimen tubes to rule out labeling errors. C) Where possible, re-extraction of specimens and re-testing to confirm original results. D) Review of the MCC assay in entirety for the presence of additional discordant pairs, to rule out the possibility of a sample mix-up in assay. E) Contacting the client to ascertain donor oocyte status of the pregnancy. The investigation process was stopped and standard test reporting was resumed when a donor oocyte pregnancy was confirmed by the client. In our laboratory, apparent maternal/fetal discrepancies due to ART have led to a significant number of redundant and expensive investigations that resulted in reporting delays for hundreds of patients. Compliance with recent American College of Medical Genetics guidelines that MCC studies be performed on all prenatal specimen types, together with the increasing use of donor oocytes in ART, will uncover apparent maternal/fetal discrepancies more often. Accordingly, we are modifying our test requisition form to specifically request donor oocyte status. Our experience illustrates both the need for molecular diagnostic laboratories to have in place mechanisms for rapidly resolving these cases when donor oocyte information has not been provided and the importance of communicating donor oocyte status when molecular testing is ordered.

Disclosure(s): Genzyme Genetics provides travel expenses and sponsors research activities relevant to this presentation. Presenting author also has stock options with Genzyme Genetics relevant to this presentation.

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Molecular results of connexin26/30 testing as adjunct to newborn hearing screening. *W Grody*[1], M Telatar[1], A Martinez[1], M Fox[1], J Jen[1], B Crandall[1], N Shapiro[1], Y Sininger[1], CGS Palmer[1], LA Schimmenti[2]. [1]Univeristy of California Los Angeles, Los Angeles/California, [2]University of Minnesota, Minneapolis/Minnesota.*

Mutations in the connexin-26 (GJB2) gene are responsible for up to 50% of cases of congenital nonsyndromic hearing loss in some populations. We are studying the feasibility and impact of introducing testing for these mutations, along with a common deletion in the related gene connexin-30 (GJB6), into routine newborn hearing screening as currently practiced in California. Our hypothesis is that offering genetic testing early in the screening process will allow for more timely diagnosis and intervention, leading to improved long-term outcomes of hearing and speech. Here we describe our initial molecular genetic findings in 16 babies, drawn from an ethnically diverse population, who were tested in this program. Testing was offered at either of two time-points in the screening process: (1) after failure of inpatient newborn screening but before final diagnosis (pre-diagnosis; n=2), and (2) after the audiologic diagnosis of hearing loss (post-diagnosis; n=14). Families were eligible for entry if the child with (potential) hearing loss had no other known medical complications and was under 3 years of age. Following informed consent through parental genetic counseling, genomic DNA was collected from participating infants by buccal brush sampling and subjected to targeted analysis for the 3 most prevalent GJB2 mutations (35delG, 167delT, 235delC) and the 342-bp GJB6 deletion, followed by complete GJB2 gene sequencing if these tests were negative. Of the 16 babies, 3 were non-Hispanic Caucasian, 1 was Hispanic/Caucasian, 1 was Caucasian/Ashkenazi-Jewish, 9 were Hispanic, 1 was mixed Hispanic/African-American, and 1 was Korean. The two infants in the pre-diagnosis group subsequently passed outpatient hearing screening, and both of their DNA tests were negative except for the finding in both of a common GJB2 polymorphism, V27I. Of the 14 post-diagnosis infants, 3 (1 Hispanic and 2 Caucasian) were homozygous for the 35delG mutation, 1 (Caucasian/Jewish) was compound heterozygous for 35delG and 167delT, 1 (Caucasian/Hispanic) was compound heterozygous for 35delG and 311del14, and 1 (Korean) was homozygous for 235delC. Six (4 Hispanic, 1 Hispanic/African-American, 1 Caucasian) were negative. Two other Hispanic cases were most likely negative, though complete sequencing could not be completed due to repeated PCR failures on exon 1 of the GJB2 gene; one carried the V27I polymorphism. Thus, of 14 babies with hearing loss, 6 (42.9%) had their hearing loss explained by biallelic mutations in the connexin-26 gene. This high yield, which spanned several racial/ethnic groups, suggests that connexin DNA testing may be a useful adjunct to newborn hearing screening. Medical and psychosocial follow-up is now in progress to assess the impact on early audiologic intervention and parental attitudes toward this program.

Disclosure(s): None

PLATFORM PRESENTATIONS IN MOLECULAR GENETICS

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X-linked lethal infantile (XL)-SMA: new clinical information, variant phenotypes, and candidate disease gene studies. *M Ahearn*[*1], *D Dressman*[2], *K Yariz*[1], *E Estrella*[1], *H Basterrecha*[1], *A Meindl*[3], *F Palau*[4], *T Bech-Hasen*[5], *RD Clark*[6], *B Wirth*[7], *J Gerritsen*[5], *MM Barmada*[8], *EP Hoffman*[2], *LL Baumbach-Reardon*[1]. [1]*University of Miami School of Medicine, Miami, FL,* [2]*Children's National Medical Center, Washington, DC,* [3]*Ludwig Maximilians University, Munchen, Germany,* [4]*Unidad de Genetica, Valencia, Spain,* [5]*University of Calgary, Alberta, Canada,* [6]*Loma Linda University School of Medicine, Loma Linda, CA,* [7]*Institute of Human Genetics, University of Cologne, Cologne, Germany,* [8]*University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA.*

Genetically-distinct and variant forms of autosomal-recessive spinal muscular atrophy (SMA; MIM 253300) have recently been recognized. We have described an X-linked recessive form of infantile lethal motor neuron disease (MIM 30021), which closely resembles Werdnig-Hoffman disease, with additional features of early onset or congenital contractures and/or fractures, and maps to Xp11.3-Xq11.2. We have identified 16 unrelated families, mostly from North America and Central or Western Europe. All affected males display a severe disease course, similar to that of Type 1 SMA, with neonatal death in 75% of patients. Eleven of the sixteen families were collected for linkage studies. A series of two point and multipoint linkage analyzes were performed. Nine of these families demonstrated positive linkage to the Xp11.3-Xq11.2 region, with a cumulative maximum LOD score (Zmax = 8.71) at DXS1003 with $\theta = 0.00$. However, two of the families had an unremarkable family history of SMA except for two affected brothers (born to assymptomatic unrelated parents) with clinically identical phenotypes to XL-SMA. To date, DNA linkage studies of the X-chromosomes have revealed discordant inheritance in both sets of affected male sibs, suggesting that their disease phenotype is not X-linked. SMN gene testing has been normal in both cases. There are greater than 200 genes within the current XL-SMA critical region. We have implemented approaches for candidate gene selection and mutation screening. The first strategy is selection of genes based on the known or predicted function of the protein product. Our efforts are focused on genes in four main functional categories, which will be described. The second strategy involves hybridization of RNA samples from affected males to a proprietary DNA microarray containing approximately 150 genes from within the XL-SMA critical region. The initial hybridization and preliminary data, using only one affected male, has not demonstrated significant differentially expressed genes, however, these experiments are continuing. In conclusion, we have demonstrated strong evidence for linkage to a narrowed X chromosome region in multiple unrelated XL-SMA families. The XL-SMA phenotype is quite severe, with onset in utero, and often early gestational loss. We have recently recognized the existence of a disease phenocopy that appears to be unlinked to both the 5q11.2 and Xp11.3-Xq11.2 regions. Our data further suggests that XL-SMA may not be as rare as previously assumed and that patients testing negative for SMN mutations might instead be affected by XL-SMA. We expect that through continued narrowing of the XL-SMA candidate region, candidate gene screening, and newer microarray approaches, we will soon identify the causal gene for this intriguing disease.

Disclosure(s): None

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Extensive sequencing of the CFTR gene: lessons learned from the first 100 patients. *MJ McGinniss*[*1], *C Chen*[1], *JB Redman*[1], *GJ Putignani*[1], *N Dekov*[1], *JS Teigiser*[1], *A Buller*[1], *F Quan*[1], *Mei Peng*[2], *Charles M. Strom*[1], *Weimin Sun*[1]. [1]*Quest Diagnostics Nichols Institute, San Juan Capistrano, CA,* [2]*Harvard Partners Center for Genetics and Genomics, Cambridge, MA.*

Cystic fibrosis (CF) is the most common monogenic disease affecting Caucasians with an incidence of approximately 1:3,200 births. To assist in CF diagnosis, our laboratory offers a clinical test for extensive sequencing of the CFTR gene, as well as tests for either 1 or 2 specific exons. Samples were submitted from a diverse population of people aged from newborn to 60 years. The ethnicities of patients included: European Caucasian, African American, Ashkenazi and Sephardic Jewish, Hispanic, American Indian, and Middle Eastern. Most specimens were submitted by pulmonary clinics, medical geneticists, genetic counselors, and GI specialists. The two most common indications for testing were diagnosis of: 1) nonclassic CF (48%), including failure to thrive and 2) diagnosis of classic CF (29%). With few exceptions, all individuals had previously undergone CF mutation screening based on either the ACMG/ACOG-recommended screening panel of 25 mutations or an expanded CF mutation panel which included up to 87 mutations. The CF Complete™ sequencing assay was performed as previously described (Strom et al. Genet Med. 2003;5:9-14). In brief, the CFTR regions sequenced include the promoter, all exons and their splice junction sites, and regions of selected introns including i11 and i19. Two reviewers independently analyzed DNA sequences (10,132 bases) for mutations using ABI SeqScape® automated base-calling software. All sequence variants located in the coding region were reported at the nucleotide level; intronic and splice junction mutations as well as amino acid coding changes were also reported. Among 102 complete CFTR gene sequences, 46 had no mutations, 30 were positive for 1 mutation (most commonly delta F508), and 26 were positive for 2 mutations. We ascertained 8 novel, and potentially disease-associated, mutations including 3 deletions (1198del6, 1641AG>T, 2949del5) and 5 missense mutations (V358I, G451V, C491S, H949L, F1099L). Two of these novel mutations (1198del6 and V358I) were found in a patient with meconium ileus at birth, respiratory symptoms, and two abnormal sweat test results. CFTR mutations as a cause of disease was established or ruled out in studies of 13 atypical families. Extensive CFTR gene sequencing can detect rare mutations not detected in other screening and diagnostic tests, thus establishing a definitive diagnosis in symptomatic patients with previously negative results. This then enables carrier detection and prenatal diagnosis in additional family members. Finally, sequencing of selected regions of the CFTR gene also allows resolution of rare ambiguous results in a carrier screening program.

Disclosure(s): Quest Diagnostics Inc. is providing travel expenses and sponsors research activities relevant to this presentation. Presenting author is an employee and stockholder of Quest Diagnostics Inc.

PLATFORM PRESENTATIONS IN MOLECULAR GENETICS

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Optimal Tay-Sachs screening strategy in a panethnic population: DNA, enzyme analysis or both? *E Schlenker*[*1], S Puck[2], B Allitto[3], F Myrick[2], E Sugarman[3], L Berry[3]. [1]Genzyme Genetics, Philadelphia, PA, [2]Genzyme Genetics, Santa Fe, NM, [3]Genzyme Genetics, Westboro, MA.*

Purpose: To determine the optimal carrier screening strategy for Tay-Sachs disease in an ethnically diverse population. Materials and Methods: Between October 2001 and February 2003, 10,638 samples were referred for Tay-Sachs carrier screening by enzyme analysis on leukocytes and mutation analysis. The hexosaminidase A levels were determined by the heat inactivation technique; the 8 alleles were the common Ashkenazi Jewish mutations (+TATC1278, 1421+1G→C), common French Canadian mutations (7.6 kb deletion, IVS7+1G→A), a late-onset mutation (G269S), a common non-Jewish mutation (IVS9+1G→A) and pseudodeficiency alleles (R247W, R249W). Results: The population was diverse, with 53.7% of patients reported as Ashkenazi Jewish and 3.5% French Canadian. The remaining 42.8% were other or mixed ethnicity. Individuals of other ethnicity are often tested because their partners are Ashkenazi Jewish. Enzyme analysis revealed that 270 patients (2.5%) were carriers, 417 patients (3.9%) were in the indeterminate range and 9951 were non-carriers. DNA analysis of the non-carriers revealed that 9947 (99.96%) were negative. Two patients had true mutations (+TATC1278); two patients had pseudodeficiency alleles (R247W). Of the 417 patients with indeterminate enzyme levels, 50 (12%) were positive by DNA analysis: 41 true mutations and 9 pseudodeficiency alleles. These patients were Ashkenazi Jewish (n=28), Caucasian (n=11), Other (n=10) and French Canadian (n=1). Of the 270 carriers identified by enzyme assay, DNA analysis was positive in 245 samples (90.4%). True mutations were identified in 222 and pseudodeficiency alleles in 22 (9.0%). One Ashkenazi Jewish individual was a compound heterozygote for a true mutation and a pseudodeficiency allele. DNA analysis alone would have failed to detect 25 carriers (9.3%) identified by enzyme analysis. These patients were Caucasian (n=8), Ashkenazi Jewish (n=5), Other (n=4), French Canadian (n=3), African American (n=2), Hispanic (n=1), Sephardic Jewish (n=1), and Asian (n=1). Of 172 Ashkenazi Jewish carriers by enzyme assay, DNA analysis was positive in 167 patients (97.1%). One hundred sixty one patients (96.4%) had true mutations, while six (3.5%) had pseudodeficiency alleles. DNA analysis alone in the Ashkenazi Jewish population would have failed to detect 5 individuals identified as carriers by enzyme assay (2.9%). Conclusions: DNA analysis alone should not be used for Tay-Sachs carrier screening. The optimal screening strategy in a panethnic population includes both DNA and enzyme analysis. While enzyme analysis has a higher detection rate, DNA analysis can clarify indeterminate enzyme results as well as identify which individuals are at risk to have a child with classic Tay Sachs disease or adult-onset Tay-Sachs disease, or are carriers of benign pseudodeficiency alleles.

Disclosure(s): Presenter/all authors are employees of Genzyme Genetics, which provides support for all expenses and sponsors research activities relevant to this presentation. Presenter/all authors have investments with Genzyme Genetics.

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Analysis of the UBE3A and MECP2 genes in the molecular evaluation of patients with an Angelman syndrome-like phenotype. *P Fang*[*1], R Gin[2], AL Beaudet[1], BB Roa[1]. [1]Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX, [2]Littleton Pediatric Medical Center, Highland Ranch, CO.*

Angelman syndrome (AS) is characterized by mental retardation, seizures, absence of speech, motor dysfunction, and behavioral problems. The molecular mechanisms that cause AS include maternal deletion of 15q11-q13 in the majority of cases, as well as paternal uniparental disomy, imprinting defects, or mutations in the UBE3A gene. Current methods can detect ~85-90% of AS cases, including ~10% of cases due to UBE3A mutations. The differential diagnosis of AS includes Rett syndrome, which is an X-linked neurodevelopmental disorder that is caused by mutations in the MECP2 gene on Xq28. Our laboratory offers Angelman syndrome methylation analysis to detect maternal deletions, UPD and imprinting defects, in conjunction with sequence analysis of the UBE3A gene major coding region. Our laboratory has performed UBE3A sequencing on 445 probands referred for clinical testing over a four year period. We identified UBE3A mutations in 35/445 patients (~8%), including 28 truncating nonsense and frameshift mutations, 2 missense, 3 splicing, and 2 in-frame deletion mutations. Six of the 35 UBE3A positive cases were shown to be familial. Among the patients who tested negative for UBE3A mutations, 150 were also tested for mutations in the MECP2 gene for Rett syndrome. Eight female patients (~5%) tested positive for MECP2 mutations, including 3 nonsense, 3 missense, 1 frameshift, and 1 stop codon elongating mutation. These results underscore the clinical overlap between AS and Rett syndromes. We report on particularly interesting family with AS. The proband is a six year old female with a classic presentation for Angelman syndrome. Sequence analysis showed a novel UBE3A missense allele, 2283T>A (M566K) in the proband and her asymptomatic mother, but not in the proband's normal sibling. However, a different allelic nucleotide change, 2283T>G (M566R), was found in the proband's asymptomatic maternal grandfather and aunt. These unexpected results were confirmed by testing on repeat blood samples from key family members. The collective data suggest that the maternally inherited 2283T>A (M566K) allele is associated with AS in the proband. This report summarizes our molecular testing experience, which includes a highly interesting and unusual case of Angelman syndrome. Our collective results support the clinical overlap between Angelman and Rett syndromes, and underscore the clinical utility of molecular testing for the UBE3A and MECP2 genes in patients with associated clinical phenotypes.

Disclosure(s): None

PLATFORM PRESENTATIONS IN MOLECULAR GENETICS

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Significant genotype phenotype correlation for GLI3 mutations refutes prior assertions of a lack of correlation. *JJ Johnston*[1], J Turner[1], LG Biesecker[1]. [1]National Human Genome Research Institute, NIH, Bethesda, MD.*

Mutations in the zinc finger transcription factor GLI3, on chromosome 7p13, cause the Greig cephalopolysyndactyly (GCPS) and Pallister-Hall syndromes (PHS). GCPS and PHS are variable but distinct entities with numerous non-overlapping features. It has been hypothesized that GCPS is caused by functional haploinsufficiency of GLI3, while PHS is caused by the production of a truncated GLI3 protein that retains DNA binding activity. To test this hypothesis we have analyzed patients with features consistent with either PHS or GCPS for GLI3 mutations. Individuals diagnosed with GCPS were first analyzed for deletions of the GLI3 locus. Deleted individuals were not included in this study. GCPS patients who were not deleted and all other patients were subjected to sequencing and/or dHPLC analysis to identify point mutations in GLI3. Our patient group consisted of 61 probands, 32 probands with GCPS and 29 with PHS. The 32 GCPS families include 58 affected persons and the 29 PHS families include 54 affected persons. A total of 40 mutations were identified and five additional probands have sequence alterations that predict amino acid substitutions that may be causative. Of the 22 mutations identified in PHS probands, all predict a truncated protein on the C-terminal side of the zinc finger domain between amino acids 666 and 1152 in exons 13, 14 and the 5' end of exon 15. Mutations in GCPS probands were more varied than those in PHS probands and occurred over a greater extent of the gene. When mutations from prior reports were melded with the present results, a significant genotype-phenotype correlation was found for nonsense, frameshift, and splice site mutations. Mutations in roughly the first third of the gene (from amino acid 1-604) caused only GCPS, nearly all mutations in the second third of the gene (from amino acid 666-1161) caused PHS, and mutations in the last third of the gene caused only GCPS. The majority of truncating mutations (30/33) identified in GCPS patients occur within or 5' to the zinc finger domain or at the 3' end of exon 15. Five of our GCPS patients had sequence alterations that predict amino acid substitutions of unknown significance. The distribution of mutations in these two phenotypes is significantly associated with the phenotypes ($p<.01$). These data support the hypothesis of a distinct pathogenetic mechanism for GCPS and PHS.

Disclosure(s): None

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Frequency of the 3199del6 cystic fibrosis mutation among I148T carriers: results from a collaborative study. *K Monaghan*[1], W Highsmith[2], J Amos[3], V Pratt[4], B Roa[5], M Friez[6], L Pike-Buchanan[4], J Redman[7], CM Strom[7], AL Young[2], W Sun[7]. [1]Henry Ford Hospital, Detroit, MI, [2]Mayo Medical Laboratories, Rochester, MN, [3]Specialty Laboratories, Santa Monica, CA, [4]Quest Diagnostics Nichols Institute, Chantilly, VA, [5]Baylor College of Medicine, Houston, TX, [6]Greenwood Genetics Center, Greenwood, SC, [7]Quest Diagnostics Nichols Institute, San Juan Capistrano, CA.*

ACOG/ACMG recommends cystic fibrosis (CF) carrier screening for pregnant Caucasians or those considering pregnancy and that screening be made available to individuals in lower risk groups. A pan-ethnic mutation panel, including the most common 25 CF mutations with a frequency ≥0.1% in the general population, is recommended for CF carrier screening. This panel is expected to be modified as new information is learned regarding the phenotype associated with specific mutations and allele frequencies in various populations. One such mutation under consideration is I148T, currently included in the panel. I148T was described as a severe CF mutation in 1994 and accounts for 9% of French Canadian CF mutations. Following implementation of CF population-based carrier screening, two studies noted a >100-fold increase in the frequency of I148T among individuals undergoing carrier screening compared to patients with clinical CF. Further studies of CF patients revealed the 3199del6 CF mutation in-cis with I148T, a finding previously reported to the CF Consortium in 1998. 3199del6 in-cis with I148T is associated with pancreatic insufficient CF. In contrast, asymptomatic individuals with a severe CF mutation and I148T did not exhibit the 3199del6 mutation. Initial studies reported that approximately 1.8% of I148T heterozygotes identified by carrier screening also have the 3199del6 mutation. In our collaborative study of 662 I148T carriers, 0.9% also had 3199del6. Among subjects with a known indication for testing and/or ethnicity/race (excluding subjects tested because of a suspected or clinical diagnosis of CF or positive family history), only 0.3% of I148T carriers had 3199del6. We identified 8 individuals with I148T and second CF variant, 2 of whom also carried 3199del6. Two fetuses with echogenic bowel (I148T/ΔF508 and I148T/M82I), 2 males with infertility (I148T/S1235R and I148T/ΔF508), 1 fertile male (I148T/ΔF508), 1 CF patient (V520F/I148T and 3199del6), one woman with a family history of a CF mutation (D110H/I148T and 3199del6), and 1 I148T homozygote. Reflex testing for 3199del6 should be considered whenever I148T is identified. Such testing is of particular importance in any patient with features of CF or whenever one member of a couple carries a deleterious CF mutation and the other member carries I148T. Further studies are necessary to determine if I148T, in the absence of 3199del6, is associated with mild or atypical CF or male infertility.

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PLATFORM PRESENTATIONS IN CYTOGENETICS/PERINATAL GENETICS

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A population-based study of congenital heart defects and chromosome abnormalities: structural abnormalities other than del 22q11.2. *K May**[1], *L Botto*[2], *S Rasmussen*[2], *P Fernhoff*[1], *L O'Leary*[2], *R Campbell*[3]. [1]*Department of Human Genetics, Emory University, Atlanta, GA,* [2]*National Center for Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, GA,* [3]*Sibley Heart Center, Childrens Healthcare of Atlanta, Atlanta, GA.*

Chromosome abnormalities play a significant role in the etiology of congenital heart defects (CHD). Our recent population-based study of children born in the metropolitan Atlanta area during the years 1994 through 1999 indicated that a minimum of 1 in 7 infants with CHD have an abnormal chromosome pattern (Botto et al, 2002, Am J Hum Genet. 71S:211). Structurally abnormal chromosome patterns other than deletion 22q11.2 account for at least 2% of all cases of CHD. We now report our detailed analysis of 46 cases of deletions, duplications and rearrangements that were detected among 2,396 children with CHD who had chromosome testing. Children were ascertained through the Metropolitan Atlanta Congenital Defects Program (MACDP, a birth defects surveillance system with active case-ascertainment. Further details were obtained from Emory University cytogenetic records. A goal of this analysis was to evaluate the relative frequencies of specific chromosome abnormalities and types of associated heart defects. Ten areas of recurring segmental imbalances were found including deletions in 1p36; 4p, 4q, 5p, 9p, 18q,and 21q and duplications in 3q, 10q, and 17q. Derivative chromosomes accounted for 20 of the cases, and at least half of these were due to a familial rearrangement. As expected, most of the children had other congenital abnormalities apart from CHD. Over half (27/46 or 59%) of the children had CHDs that could be classified as minor (e.g., small muscular VSD, PFO, PDA). Major heart defects that involved obstructions of the left ventricular outflow tract, including aortic stenosis, coarctation and hypoplastic aortic arch, were present in 7 (15%) of the children. A strength of this study is that bias was minimized by the active case-ascertainment methods used by MACDP and by the population-based nature of the study cohort. These data have important implications for prenatal and postnatal diagnosis of CHD, both for clinical management and for determining recurrence risks. The specific areas of imbalance may provide additional important clues for locating genes that play a role in cardiac development.

Disclosure(s): None

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Refinement of the Kabuki syndrome critical region. *JM Milunsky**[1,2,3], *TA Maher*[1], *X Huang*[1]. [1]*Center for Human Genetics, Boston University School of Medicine, Boston, MA,* [2]*Department of Pediatrics, Boston University School of Medicine, Boston, MA,* [3]*Department of Genetics and Genomics, Boston University School of Medicine, Boston, MA.*

Kabuki syndrome (KS) is a well recognized multiple congenital anomaly/mental retardation syndrome. We recently documented an 8p22-8p23.1 duplication in 6 unrelated KS patients by comparative genomic hybridization (CGH) and BAC-FISH. The size of the duplication was delimited to approximately 3.5 Mb. No duplication was observed in their parents. We now have studied a total of 13 unrelated KS patients by CGH and additional BAC-FISH probes. 6/13 KS patients appear to have the approximately 3.5 Mb duplication. 7/13 KS patients have smaller overlapping duplications of various sizes within the candidate region ranging from 0.83 Mb to 3.4 Mb. All KS patients and their mothers have a heterozygous submicroscopic inversion at 8p23.1. The candidate region lies between two complex low-copy repeats (LCRs) similar to other genomic disorders. These LCRs likely mediate unequal crossing over and generate different size duplication products as we have demonstrated in our cohort. These studies further refine the KS critical region and narrow the minimum critical region to 0.68 Mb.

Disclosure(s): None

PLATFORM PRESENTATIONS IN CYTOGENETICS/PERINATAL GENETICS

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Autism and chromosome 15: evidence for atypical progression of autistic symptoms among children with a supernumerary isodicentric 15 chromosome. *BM Finucane*[*1], B Haas-Givler[1], EW Simon[1]. [1]Elwyn Training & Research Institute, Elwyn, PA USA.*

Numerous case reports have linked autism with abnormalities of the PWS/AS critical region of chromosome 15, particularly supernumerary isodicentric 15. In 1998, we reported a high incidence of autistic symptoms among children and young adults with isodicentric 15 (Rineer et al., 1998. Am J Med Genet 81:428-33.) Using the Gilliam Autism Rating Scale (GARS), we confirmed high autism scores in 20 of 29 patients studied. Because younger children with isodicentric 15 in our cohort were more sociable and less likely to be autistic than older children, we hypothesized at the time that onset of autistic symptoms, particularly social interaction deficits, was age-related in these patients. Such a progressive pattern of autistic symptoms is unusual, as sociability generally remains stable or improves with age in people with autism of other etiologies. We recently had the opportunity to complete a follow-up study of our original cohort. Of the 9 children with isodicentric 15 whose initial GARS scores were below the average range for autism, 1 was deceased and 1 was subsequently diagnosed with Angelman syndrome. The latter patient had undergone molecular studies which showed that her supernumerary 15 lacked the PWS/AS critical region; she did, however, have paternal uniparental disomy of her normal 15s. The 7 remaining children were all available for study and the GARS was readminstered. On retesting 6 years after the original study, all 7 showed a significant increase in the GARS Autism Quotient, specifically due to increasing deficits in social interaction. Six of the 7 previously non-autistic children scored well within the average range for autism on retesting. Although the remaining non-autistic child still scored below the average range on retesting, his scores had significantly increased since the previous study (ie. he had more autistic symptoms than before.) Our data support the hypothesis that there is an age-related increase in the severity of autistic symptoms among individuals with isodicentric 15, particularly in the area of social interaction. This is different than the usual course of autism, where overall improvement in social functioning is found with increasing age. We propose that individuals with isodicentric 15, as a group, demonstrate an autistic phenotype that has a specific developmental course with varying degrees of individual severity. The natural history of isodicentric 15 syndrome remains to be shown through additional longitudinal work and could have important implications for educational intervention.

Disclosure(s): None

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CGH array analysis enhances the detection of aneuploidies and submicroscopic imbalances in spontaneous miscarriages. *C Lese Martin*[*1], AJ Schaeffer[1], J Chung[1], K Heretis[2], A Wong[2], DH Ledbetter[2]. [1]Department of Human Genetics, University of Chicago, Chicago, IL, [2]Department of Human Genetics, Emory University, Atlanta, GA.*

Miscarriage is a condition that affects 10-15% of all clinically recognized pregnancies, most of which occur in the first trimester. Of these first trimester miscarriages, approximately 50% result from fetal chromosome abnormalities. G-banded chromosome analysis, the current standard of care, is used to determine if large-scale genetic imbalances are the cause for these pregnancy losses. This technique relies on the culture of cells derived from the fetus, which has many limitations, including a high rate of culture failure, maternal overgrowth of fetal cells and poor chromosome morphology. Comparative Genomic Hybridization (CGH) array analysis is a powerful new molecular cytogenetic technique that allows genome-wide analysis of DNA copy number. By hybridizing patient DNA and normal reference DNA to arrays of genomic clones, unbalanced gains or losses of genetic material across the genome can be detected. In this study, 41 product of conception (POC) samples, that were previously analyzed by G-banding, were tested using CGH arrays to determine not only if the array could identify all reported abnormalities, but also whether any previously undetected genomic imbalances would be discovered. The array methodology detected all abnormalities as reported by G-banding analysis and revealed new abnormalities in 4/41 (9.8%) cases. Of these, one trisomy 21 POC was also mosaic for trisomy 20, one had a duplication of the 10q telomere region, one had an interstitial deletion of chromosome 9p and the fourth had an interstitial duplication of the Prader-Willi/Angelman syndrome region on chromosome 15q, which if maternally inherited, has been implicated in autism. Metaphase and/or interphase Fluorescence In Situ Hybridization analysis was used to verify the CGH array findings. This retrospective study demonstrates that the DNA-based CGH array technology overcomes many of the limitations of routine cytogenetic analysis of POCs while enhancing the detection of fetal chromosome aberrations.

Disclosure(s): None

PLATFORM PRESENTATIONS IN CYTOGENETICS/PERINATAL GENETICS

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Subtelomere FISH analysis of 8,000 cases: a collaborative evaluation. *J Ravnan*[*1], J Tepperberg[2], P Papenhausen[2], A Lamb[1], D Eash[4], J Degner[4], D Ledbetter[3], C Martin[4]. [1]Genzyme Genetics, Santa Fe, NM, [2]Laboratory Corporation of America, Research Triangle Park, NC, [3]Department of Human Genetics, Emory University, Atlanta, GA, [4]Department of Human Genetics, University of Chicago, Chicago, IL.*

In recent years, subtelomere FISH analysis has increasingly been used as an adjunct to routine cytogenetic testing in order to detect small rearrangements at the ends of the chromosomes. Leslie Biesecker (AJMG, 107:273,2003) recently reviewed 14 studies including 1,718 subjects reported in the literature where subtelomere testing was performed. These studies showed an overall abnormality rate of 6%, with a range of 2% to 29% in individual studies. The large variance in frequency of abnormalities observed is most likely due to different criteria for inclusion in the various studies. This study presents the combined data from over 8,000 subtelomere FISH studies performed in three clinical cytogenetic laboratories. The laboratories did not limit the patient population referred for testing, though the most common clinical indication was developmental delay or mental retardation with or without dysmorphic features. In this study population, the detection rate for clinically significant subtelomere abnormalities was approximately 3% with an additional 0.5% detection of presumed familial variants. Approximately half of the clinically significant abnormalities identified consisted of terminal deletions, the majority of which were found to be de novo when parental studies were performed. The majority of the remaining cases were unbalanced translocations between two chromosomes or the two arms of the same chromosome. Parental FISH studies showed that about half of the unbalanced translocations were inherited from a parent carrying a balanced form of the rearrangement. Other abnormalities included tandem duplications, interstitial deletions of the control probe, and apparently balanced translocations. The most common clinically significant imbalances found, with 8 or more instances diagnosed of each, resulted in deletions of 1p, 22q, 4p, 9q, 8p, 2q, and 20p. Apparent familial variants, where a phenotypically normal parent carries the same unbalanced rearrangement as the proband, were detected in 0.5% of cases examined. The most common variants consist of deletion or duplication of 10q, deletion of 4q, and rearrangement of the X chromosome. The discovery of such unbalanced subtelomere rearrangements that have been inherited from a phenotypically normal parent complicates the clinical interpretation and highlights the critical nature of parental follow-up studies.

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Monosomy 1p36: deletions and rearrangements. *L Shaffer*[*1,2], G Gregato[1], W Yu[3], H Heilstedt[3], C Kashork[3], P Stankiewicz[3], S Yatsenko[3], B Ballif[1,3], KA Bailey[1,2], M Gajecka[1], CD Glotzbach[1,2]. [1]Health Research and Education Center, Washington State University Spokane, [2]Sacred Heart Medical Center, Spokane, WA, [3]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.*

We previously published a minimal tiling path covering the most distal 10.5 Mb of 1p36 and placed the breakpoints of 60 subjects with Monosomy 1p36 on this contig. Characterization of the breakpoints by FISH and comparative genomic hybridization applied to a 1p36 microarray (array CGH) identified terminal deletions, interstitial deletions, unbalanced translocations, and complex rearrangements. We compare the results of 30 newly ascertained subjects to the previously published cases. In the first 60 subjects, 57 were de novo. Of these, 72% had apparently terminal deletions, 7% had interstitial deletions, 12.3% had unbalanced derivative chromosomes, and 8.7% had complex rearrangements. Twenty-five of the 30 newly ascertained subjects have been fully characterized by array CGH and FISH. Of these, 72% have apparent terminal deletions, 4% have interstitial deletions, 12% have derivative chromosomes, and 12% have complex rearrangements. This distribution of abnormalities is not significantly different from the first 60 cases. Collectively, ~60% of de novo rearrangements are maternal in origin, as determined using highly informative microsatellite analysis. Although 72% of de novo rearrangements are apparently terminal deletions, surprisingly, 28% of rearrangements are not simple truncations but interstitial deletions, derivative chromosomes, or complex rearrangements. The breakpoints continue to be variable within the 10.5 Mb region, not clustering in a single location. It is unknown if the variety of abnormalities that are found in Monosomy 1p36 are representative of other terminal deletion syndromes. Finally, the use of array CGH allowed for the rapid characterization of the rearrangements and uncovered complexities not recognized at the resolution of the light microscope that would be too labor intensive to delineate through individual FISH experiments.

Disclosure(s): Presenter is owner of and has investments in Signature Genomic Laboratories.

PLATFORM PRESENTATIONS IN CYTOGENETICS/PERINATAL GENETICS

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Maternal serum integrated screening in clinical practice. *J Roberson*[1], S Diment[1], A Anderson-Miller[1], G Lambert-Messerlian[2], J Canick[2]. [1]Detroit, MI, [2]Providence, RI.*

The Henry Ford Hospital Maternal Serum Screening Program introduced maternal serum integrated screening in March 2003. As of December, approximately 30% of all samples received are for serum Integrated testing. Pregnancy associated plasma protein-A (PAPP-A) is drawn between 10-13 weeks and MSAFP, estriol, human chorionic gonadotropin (hCG) and inhibin-A are drawn between 14-22 weeks gestation. Down syndrome screen positive rate is set at 1:150 (second trimester) with an anticipated 3% screen positive rate. Fetal ultrasound dating is required prior to issuing a final report but can be obtained in either the first or second trimester. PAPP-A analysis was performed by Women and Infants Hospital in Rhode Island. 437 women enrolled but 345 completed serum Integrated tests have been reported. 92 enrollees resulted in PAPP-A single tests. 200 additional PAPP-A samples are pending the receipt of a second trimester quad test. Of the 345 completed serum Integrated reports, 23% were updated with scan dating after the preliminary report was issued. 53% of all scans were obtained between 5-13 weeks gestation, 44% were between 14-19 weeks gestation and 3.7% were after 19 weeks gestation. Two patients were uninterpretable and 47 could be interpreted as a quad test only based on the subsequent ultrasound dating. The Down syndrome screen positive rate for the 294 remaining serum Integrated patients was 3.1%. 1.4% had raised AFP, 0.3% were screen positive for trisomy 18. 3.5% were screen negative for Down syndrome only because the quad test was drawn at 14 weeks gestation. Review of the same 294 serum Integrated patients determined that 7.5% would have been screen positive for Down syndrome using the triple test and 5.4% would have been positive with the quad test using the standard 1:270 second trimester cut-off. During this same period, 92 PAPP-A samples were reported without an accompanying quad test. 46% were determined by ultrasound to be drawn before 10 weeks gestation, 2% were drawn after 13 weeks, 10% had amniocentesis instead of a quad test, 12% had fetal demise or miscarriage, 5% declined to have a quad test, 11% were not run due to incorrect gestational age by preexisting ultrasound, 8% were repeat samples that were not required, and 16% failed to return to their doctor after the PAPP-A was drawn. Maternal serum Integrated screening was well received by our referring physicians and patients. Based on the decreased false positive rate and the anticipated improved Down syndrome detection rate with this program, a major HMO and several other insurers in our area approved PAPP-A analysis as a covered benefit. The major advantage sited was the lower number of patients who were classified as screen positive for Down syndrome and thus required to consider invasive testing.

Disclosure(s): None

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Analysis of 3,208 CF prenatal diagnoses reveals that less than 2% of CFTR prenatal testing is performed for the sole indication of parental 5T allele(s). *VJ Weinblatt*[1], KJ Treat[2], EM Rohlfs[2], EA Sugarman[2]. [1]Genzyme Genetics, Philadelphia PA, [2]Genzyme Genetics, Westborough, MA.*

In response to reports of significant numbers of prenatal procedures being performed solely to identify 5T alleles, we surveyed our experience of prenatal diagnosis for CF over a three year time period. In our laboratory, poly T analysis is not part of the routine CF mutation panel, and must be ordered separately. Reflex testing for poly T alleles is performed in accordance with ACMG guidelines. Referral indications for prenatal CF mutation analysis in our laboratory were compared for an 18 month period both before and after the ACOG/ACMG/NIH statement regarding prenatal and preconception testing for cystic fibrosis. The pre-ACOG group comprised 1330 specimens received from April 2000 through September 2001; the post-ACOG group included 1878 specimens received from October 2001 through April 2003. Referral indications unrelated to poly T status accounted for 98.6% of analyses in the pre-ACOG group and 96.1% in the post-ACOG group. Of the 98.6% in the pre-ACOG group, 49.3% were referred for abnormal ultrasound findings, 38.6% for a family history of CF or a CF carrier, and 10.7% for prenatal screening with no known increased risk for CF. The corresponding post-ACOG experience was respectively, 30.7% for ultrasound anomalies, 35.3% for family history, and 30.2% for prenatal screening. Specimens referred for CF testing when one or both parents carried a 5T allele and neither parent had a CF mutation, made up 0.8% (10) of the pre-ACOG group and 1.8% (33) of the post-ACOG group. In all of the 10 pre-ACOG specimens, and the 33 post-ACOG specimens, parental 5T alleles were identified previously at another laboratory. These 43 cases make up only 1.3% of referral indications in our total prenatal CF testing cohort. The remaining 0.7% (pre-ACOG) and 2% (post-ACOG) of specimens were referred when one parent carried a CF mutation and the other, a 5T allele. Contrary to recent reports, these data indicate that prenatal diagnosis for 5T is rare.

Disclosure(s): Presenter/authors are employees of, receive travel support from and have investments with Genzyme Genetics. Genzyme Genetics sponsors research activities relevant to this presentation.

PLATFORM PRESENTATIONS IN GENETIC COUNSELING/EDUCATION AND PUBLIC HEALTH/ETHICAL AND LEGAL ISSUES

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Identifying cardiology patients who can benefit from genetic counseling through family history questionnaires. *HM MacLeod*[*1], *EM McNally*[1]. [1]*University of Chicago, Chicago, IL.*

An inherited susceptibility to heart disease contributes significantly to cardiovascular risk. As cardiovascular disease is the leading cause of death in the United States, causing 1 out of every 2.5 deaths in United States in 2000, family history can serve as an inexpensive public health screening tool for identifying people at risk for heart disease. Given the high heritable component to cardiovascular disease, we hypothesized that genetic counseling would provide information to clinicians and patients in a cardiology setting. We evaluated the family history of subjects with non-ischemic dilated cardiomyopathy. Pedigrees were completed by a genetic counselor on 172 probands. This analysis revealed that 31% of probands had a first-degree relative affected with a related heart condition. An additional 27% had a second degree relative with a related heart condition and/or a first degree relative with a possible diagnosis of a related heart problem. Based on pedigree analysis individuals were counseled on inheritance, screening recommendations and at-risk relatives were identified and provided appropriate screening recommendations. These results indicate there is a great need for genetic counseling services in cardiovascular disease. Previous studies have shown genetics-based questionnaires detect a higher proportion of patients at risk for a genetic disorder than is customarily documented in medical records. We now developed a questionnaire for patients seen at the University of Chicago's outpatient clinic to better quantify the need for cardiovascular genetic counseling at a tertiary care center. This questionnaire was developed by a cardiovascular genetic counselor and is based on clinical experience with patients with familial heart disease as well as previous family history tools used for adult-onset genetic diseases. The questionnaire addresses cardiomyopathies, arrhythmia syndromes, stroke, coronary artery disease, congenital heart disease and muscular dystrophy. We expect the questionnaire will identify patients who will benefit from the added knowledge genetic counseling can provide as well as serve as a public health tool for identifying relatives of individuals who can benefit from cardiac surveillance and preventative drug therapy.

Disclosure(s): None

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Parental attitudes toward connexin26/30 genetic testing in their infants with hearing loss. *A Martinez*[*1], *M Fox*[1], *B Crandall*[1], *N Shapiro*[1], *M Telatar*[1], *Y Sininger*[1], *W Grody*[1], *L Schimmenti*[2], *CGS Palmer*[1]. [1]*Univeristy of California Los Angeles, Los Angeles/California,* [2]*University of Minnesota, Minneapolis/Minnesota.*

Introduction: We have begun a longitudinal 4 year study in an ethnically diverse population to assess the timing and impact on parents of providing Connexin26/30 testing and counseling in the Early Hearing Detection and Intervention (EHDI) process. We present preliminary data on parents' knowledge, understanding of outcomes of genetic testing, and attitudes toward genetic testing. Methods: Families with a child with potential or diagnosed hearing loss, no other conditions and <3y, are enrolled into the prediagnosis group (failed newborn hearing screening, prior to final diagnosis) or the postdiagnosis group (diagnosed hearing loss). The option of genetic testing is considered in the context of genetic counseling in which etiologic heterogeneity of hearing loss, Connexin26-related hearing loss, possible outcomes of genetic testing, potential benefits, and limitations are discussed. Result disclosure occurs during a second genetic counseling session. Parents' attitudes toward genetic testing, risk perception, and knowledge, are assessed shortly after enrollment, and 1 month and 6 months post result disclosure. Results: Data on 12 mothers and 4 fathers (12 families) in the postdiagnosis group are available. Following the enrollment session the majority of parents understood concepts of heterogeneity and inheritance, and perceived benefits to genetic testing, including identifying the cause of the hearing loss. About a quarter of parents were concerned about potential harms of genetic testing, including emotionally handling genetic information or effects on health insurance. Most parents felt it was somewhat or very likely their baby had Connexin26-related hearing loss. 5 babies had biallelic Connexin26 mutations: 1m after result disclosure 100% of those parents indicated their child definitely had Connexin26-related hearing loss, that the test helped them understand why their child has hearing loss, and that the recurrence chance is 25%. Hearing loss of 7 babies was not explained by genetic testing: 1m after result disclosure, 75% of those parents indicated the cause of their baby's hearing loss was undetermined, 50% responded that it was not at all likely that their child had Connexin26-related hearing loss; 33% felt the test had helped them understand why their baby has hearing loss; and there was variability in their understanding of recurrence chance. Conclusions: This early experience indicates parental interest in genetic testing for their newborns/infants, and reasonable understanding of complex genetic information. However, data suggest that negative test results are difficult for parents to understand. Our results also suggest that although parents see benefits of genetic testing, it also raises some concerns. These results highlight the importance of including pre- and post-test genetic counseling if genetic testing is incorporated into the EHDI process.

Disclosure(s): None

PLATFORM PRESENTATIONS IN GENETIC COUNSELING/EDUCATION AND PUBLIC HEALTH/ETHICAL AND LEGAL ISSUES

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Bicuspid aortic valve detection and counseling: echocardiography vs. medical history. *KA Shooner*[*1], *L Cripe*[1], *LJ Martin*[1], *DW Benson*[1]. [1]*Cincinnati Children's Hospital, Cincinnati, Ohio.*

Bicuspid aortic valve (BAV) is the most common congenital cardiovascular malformation (CVM), with a population prevalence of approximately 1%. Although often regarded as a benign cardiovascular lesion early in life, BAV is associated with significant health risks, including aortic stenosis and aortic insufficiency which frequently require surgical treatment. BAV also exhibits familial clustering. Despite the potential clinical and familial significance of BAV, it may remain undiagnosed by standard examination. Counseling family members of individuals with BAV about risk for the appearance of new cases of BAV or other heart malformations is challenging, because some individuals may have unrecognized disease. Recognizing all affected family members is essential. The aim of this study was to measure the detection of heart malformations in first-degree relatives of people with BAV using family history alone compared to following a systematic echocardiographic screening protocol in first-degree relatives. We recruited 50 probands with BAV through a regional outpatient pediatric cardiology clinic. We recorded 3-generation pedigrees, obtained echocardiographic records on probands and any family members already known to have BAV (n=62), and performed echocardiograms on the first-degree relatives of each of these affected individuals (n=229). Considering only the family history data, the prevalence of BAV plus other CVMs in our population was 27.1% (n=291). The prevalence of BAV alone was 21.3% (n=291). Echocardiographic screening proceeded as follows: when a new affected individual was identified, all of that person's first-degree relatives were subsequently evaluated. This "sequential sampling" method revealed 18 additional individuals with heart lesions, including 12 individuals with BAV. In 24 out of the 50 families (48%), two or more individuals were affected with some kind of CVM. Assuming a prevalence of 1% for BAV and 2% for BAV plus CVM in the general population, the relative risk for a first-degree relative of someone with BAV to have any CVM (including BAV) was 13.1285 (95% CI 3.113 – 55.358), while the relative risk of having BAV alone was 13.41 (95% CI 1.78 – 100.97). This indicates that first-degree relatives of people with BAV are approximately 13.41 times more likely than the general population to have BAV. Due to both the substantial number of CVMs revealed by an echocardiographic screening protocol and an increased risk 13.41 times that of the general population for first-degree relatives of having BAV, echocardiographic screening of first-degree relatives of people with BAV, in addition to family history, is recommended.

Disclosure(s): None

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Public health response to a direct-to-consumer marketing campaign for genetic testing for breast and ovarian cancer susceptibility. *MF Myers*[*1], *C Jorgensen*[2], *J Litch*[3], *K Sidibe*[2], *L Bradley*[1]. [1]*Office of Genomics and Disease Prevention, Office of the Director, CDC,* [2]*Division of Cancer Prevention and Control, National Center on Cancer Prevention and Control, CDC,* [3]*Epidemiolgy Office, Washington State Department of Health.*

From September, 2002 to February, 2003, the major United States provider of genetic testing for breast and ovarian cancer susceptibility (BRCA1/2 testing) conducted a pilot DTC advertising campaign that targeted women aged 25-54 and their health care providers in two cities, Atlanta, GA and Denver, CO. This multi-media campaign had a stated objective of creating awareness among women 25-54 with a personal or family history of breast and ovarian cancer, and was the first promotion of an established genetic test directly to consumers. In an attempt to assess the public health impact of the campaign, surveys of women and their physicians were conducted in the two pilot cities and two comparison cities, Raleigh-Durham, NC and Seattle, WA. The objectives of the surveys were to: 1) compare knowledge and attitudes about genetic testing for susceptibility to breast and ovarian cancer in pilot and comparison cities among participants; 2) investigate demographic and other factors that may influence knowledge and attitudes about this testing among participants; and 3) determine physicians' perceptions of patient demand for information about this testing. Working groups consisting of content experts, public health officials and epidemiologists from the four states, and staff of the Office of Genomics & Disease Prevention and the Division of Cancer Prevention and Control at CDC developed the survey instruments. Between April 21 and May 20, 2003, the 51-question consumer telephone survey was conducted with randomly selected women aged 25-54 in each of the pilot and comparison cities. A 35-question printed provider survey with a $50 incentive was sent out on May 1, 2003 to a proportional sample of 1,600 physicians selected from the AMA Physician Masterfile from four specialties (family practice, internal medicine, obstetrics/gynecology and oncology) in the pilot and comparison cities. A total of 1,635 phone surveys were completed with consumers (overall participation rate 45%; Atlanta 56%; Denver 42%; Raleigh-Durham 39%; Seattle 43%); 1,054 surveys were received from eligible physicians (overall participation rate 66%; Atlanta 62%; Denver 70%; Raleigh-Durham 68%; Seattle 65%). The average age of consumers was 40 years, and the majority was white (79%), married (68%), and had higher than a 12th grade education (75%). Thirteen percent of consumers reported a first degree relative with breast or ovarian cancer. Providers predominately were male (65%), had been in practice more than 10 years (58%), and saw fewer than 100 patients per week (65%). We will report findings regarding awareness about the DTC campaign and knowledge about genetic testing for breast and ovarian cancer susceptibility among participants in the pilot and comparison cities.

Disclosure(s): None

PLATFORM PRESENTATIONS IN GENETIC COUNSELING/EDUCATION AND PUBLIC HEALTH/ETHICAL AND LEGAL ISSUES

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Integration of Internet-based genetic databases into medical school curriculum. *DJ Waggoner*[*1], C Lese Martin[1]. [1]University of Chicago, Chicago, Illinois.*

Medical genetics is one of the most rapidly advancing fields of medicine, and genetic testing is now integral to all aspects of biomedical science. In recognition of the growing role of molecular, cytogenetics, and medical genetics in clinical medicine, the Information and Education Committee of the American Society of Human Genetics (ASHG) has developed recommendations for medical school curricula including the use of computer based resources. Despite these recommendations there are few published examples of medical school curricula that address these objectives. One publication in 2001 from Washington University School of Medicine outlined a module where students were taught these skills using clinical cases to access information from GenBank, Online Mendelian Inheritance in Man (OMIM), and PubMed databases at the National Center for Biotechnology Information (NCBI). Here we report a similar approach, which was used to design a two part educational module for the Pritzker School of Medicine at the University of Chicago. Part one of the module is presented during a one hour session in the first year Medical Genetics class. The students are divided into small groups and meet in a computer training classroom where each student has access to a computer terminal with an internet connection. Part two is presented in a 1.5 hour session at the beginning of the required Pediatric rotation in the third year of medical school in a similar classroom. Part one was designed to introduce students to the databases available at the NCBI Web site. Using a problem based format students are instructed on the use of BLAST, Entrez, PubMed, OMIM, and Locus Link. Students are given a DNA sequence and then answer a series of questions about this sequence regarding the gene it encodes, naming mutations at the DNA and protein level, researching the pathogenicity of the mutations, finding homologous genes in humans and other species, and identifying associated diseases. Part two was designed to introduce students to databases available on the Web, which could be used to assist patient evaluations, diagnosis, and genetic counseling. Problem sets regarding clinical scenarios are used to instruct students on the use of OMIM to identify diseases and direct evaluations, GeneTests to find laboratory facilities for testing purposes, and NORD for providing information regarding specific diseases and their support groups. The sessions were well received by the students and highly evaluated. The specifics of the design and implementation of these sessions will be presented. In addition, plans for assessing whether these sessions impacted the medical practice of the students and their residents will be discussed.

Disclosure(s): None

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The use of objective structured clinical exams (OSCES) to assess genetic counseling and communication skills of pediatric residents. *S Barrett*[*1], A Shein[1], A Hilfer[1], L Altshule[1]r, E Kachur[1], G Kupchik[1]. [1]Maimonides Medical Center Brooklyn, New York.*

Genetic counseling is a complex communication challenge that requires knowledge about medical genetics, understanding of individual and family information-processing and coping styles, awareness of the impact of culture and community, and the ability to communicate in emotionally charged situations. Performance-based teaching methods utilizing simulated patient encounters, such as Objective Structured Clinical Exams (OSCEs), offer a powerful teaching tool to address these issues. During the exercise, trainees rotate though multiple stations in a timed fashion, and face actors trained to portray standardized clinical tasks. OSCEs provide trainees with an opportunity to practice difficult patient encounters and to receive immediate feedback on their performance. Using criterion-based rating forms, such methods can also reveal gaps in knowledge and skill, and indicate areas for remediation. As part of a larger training program on cultural competence and communication for second year pediatrics residents, we have developed two standardized patient encounters that highlight some of the communication challenges in genetic counseling. The first scenario highlights the difficult issues confronting Orthodox Jewish parents of a newborn with Down Syndrome. The second scenario requires the resident to discuss autopsy consent with the Pakistani parents of a newborn with multiple anomalies facing imminent death. We will report data from resident performance in these stations and present trainee, faculty and standardized patient evaluations. The use of OSCEs in teaching genetic counseling skills and cultural awareness to pediatric residents, as well as the strengths and short-comings of the residents' ability to effectively communicate within the context of these scenarios will be addressed.

Disclosure(s): None

PLATFORM PRESENTATIONS IN GENETIC COUNSELING/EDUCATION AND PUBLIC HEALTH/ETHICAL AND LEGAL ISSUES

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Genetic counselor training programs in the United States, capacities and needs: a report of the National Society of Genetic Counselors (NSGC). *RL Bennett*[1], D Allain[2], D Baker[3], N Callanan[4], B LeRoy[5], AP Walker[6]. [1]Division of Medical Genetics, University of Washington, Seattle, WA, USA, [2]Children's Hospital of Wisconsin, Milwaukee, WI, [3]Genetic Alliance, Washington, DC, [4]Genetic Counseling Program, University of NC at Greensboro, Greensboro, NC, USA, [5]Institute of Human Genetics, U of Minnesota, Minneapolis, MN, [6]Division of Human Genetics, Department of Pediatrics, U of CA, Irvine, CA.*

Masters-level genetic counselors comprise the largest genetics specialty workforce; the American Board of Genetic Counseling certified over 400 genetic counselors in 2002 alone. Counselors have responded to changing demands by entering new clinical areas (42% reported doing cancer counseling in 2002 vs. only 10% in 1994) and adapting to increased patient load (51% saw more patients in 2002 than 2000). But last spring only about 200 students graduated from genetic counseling training programs. Although the genetic counseling workforce expands by about 10% annually, additional genetic counselors will be needed to meet the demands of genomic medicine. We summarize the current status of masters-level genetic counseling training programs and propose mechanisms for increasing the numbers, diversity and quality of genetic counselors trained in the U.S. Data were collected by surveying the 25 U.S. genetic counseling program directors and interviewing members of the ABGC Board, the NSGC Industry Special Interest Group and the Association of Genetic Counseling Program Directors. Additional data came from NSGC's Executive Office and Professional Status Surveys and the HRSA-funded Genetic Counselor Workforce Study. Programs now admit eight students per year on average, but there are at least two well-qualified applicants for every genetic counseling training position. It currently costs about $30,000 per year to train a genetic counseling student (range $25,000-$50,000). This does not include most physical resources or in-kind contributions of ancillary faculty and clinical supervisors. Major factors limiting capacity of current training programs are quality field placement sites and faculty to teach and provide research and clinical supervision. Proposed mechanisms to increase the capacity of existing training programs include travel and living stipends for out-of-area clinical rotations; additional faculty (genetic counselor, clinical geneticist); training grants for students ($25,000/student/year diversity scholarships, and $3,000/student/year for thesis projects, books and professional conference attendance). Time-limited matching start-up funds would encourage development of new programs in underserved areas. It is estimated that $553,000 would be needed to graduate eight students per year. This includes salary support for 1 FTE genetic counselor program director, 1 FTE MD or PhD geneticist, and 1 FTE program administrator; stipends of $2,600 each for 32 supervisors; $20,000 for resources; and $100,000 for diversity scholarships. By providing funding opportunities for existing genetic counseling programs to expand their faculty and clinical training opportunities, and matching start-up funds for new programs, the genetic counseling workforce could easily double in as little as five years. Creative methods of supporting small training programs in underserved and under-populated areas (such as using telecommunication for coursework and student interaction) could also increase professional diversity and patient access.

Disclosure(s): None

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"Duty" to recontact participants in a population based genetic database: the NUgene experience. *K Ormond*[1,2], M Smith[1], A Cirino[1], R Chisholm[1], W Wolf[1]. [1]Center for Genetic Medicine, Northwestern University, Chicago IL, [2]Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago IL.*

Genetic databases are generally created with the long-term goal of establishing genotype-phenotype correlations, and are explicitly NOT intended for participant benefit through the personal receipt of genetic information. In fact, most well-known genetic databases are set up to preclude the recontact of participants, both to protect confidentiality and because any genetic discoveries will likely have unclear implications in the near future. However, in general medical practice, recent years have brought an increasing sense of "rights" toward personal medical information; the question remains whether this "right" extends to control medical information obtained through research. If it does, study participants would need to be recontacted to reveal experimental genetic test results, or at least their availability. We will discuss our experience with the NUgene study, a longitudinal genetic database at Northwestern University created to assess the genetic components of common diseases. In summer 2001, prior to the start of NUgene recruitment, a planning committee met for over one year to discuss the project's format, including ethical aspects. The project's advisory committee felt strongly that recontact of study participants was not warranted. However, because of the broad and longitudinal nature of the project, the IRB requested a modified consent process for recontacting subjects. This consent allowed participants to opt for recontact under either of the following circumstances: (1) if more information was required for a future study or to participate in future research and (2) if "clinically significant results" were discovered through research examination. During the first year of the study, 808 participants were enrolled in NUgene. 92% opted for recontact regarding more information or future research and 96% opted for recontact for "medically significant" findings. A parallel ELSI study of NUgene participants examined informed consent, including recontact options. Of 200 surveyed participants, most had a good understanding (93% correct) that the purpose of the study was to benefit future patients, but they displayed a poorer understanding of whether they would learn specific personal health information from the study (62.6% correct). In-depth interviews with 109 participants suggested that approximately 1/3 of study participants expected to receive results and an additional 1/3 hoped to receive results. Respondents were also relatively open with regards to how they would prefer to be recontacted (e.g. mail, phone), but rarely provided reasons for their preferences. Such findings raise the issue of how participants interpret the option to be recontacted. We will discuss our experience in the context of available ethical and scientific literature, and raise additional questions for future research. A portion of this study was funded by DOE #DE-FG02-02ER634737.

Disclosure(s): None

PLATFORM PRESENTATIONS IN CLINICAL GENETICS II

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Distribution of dystrophin gene mutations in 58 unselected dystrophinopathy patients using improved molecular diagnostic techniques. *K Dent*[*1], D Dunn[2], A von Niederhausern[2], A Aoyagi[2], B Duval[2], L Kerr[2], G O'Neil[2], S White[3], JT den Dunnen[3], RB Weiss[2], KM Flanigan[1,2,4]. [1]University of Utah, Department of Pediatrics, Salt Lake City, UT, [2]University of Utah, Department of Human Genetics, Salt Lake City, UT, [3]Leiden University Medical Center, Human and Clinical Genetics, Leiden, The Netherlands, [4]University of Utah, Departments of Neurology and Pathology, Salt Lake City, UT.*

Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are the most common inherited disorders of muscle. Both are caused by mutations in the dystrophin gene (MIM 300377) located at Xp21. Mutation analysis of dystrophin is complicated because it is large, consisting of 79 exons and 8 promoters spread over 2.2 million base pairs of genomic DNA. Deletions in dystrophin account for the underlying etiology in approximately 55% of BMD and 65% of DMD patients. Point mutations are found in 30% of patients with duplications accounting for the remainder. Clinical genetic testing has typically relied on a multiplex PCR technique examining deletion “hotspots” with a 98% detection rate. Testing for point mutations has been available only on a research basis. Recent advances in molecular technology including the SCAIP (Flanigan et al., AJHG 2002) and MAPH (den Dunnen et al. AJHG 2002) methodologies allow improved characterization of dystrophin mutations using rapid direct sequence analysis and quantitative recovery of genomic DNA, respectively. We tested for dystrophin mutations in 58 probands with BMD or DMD followed in the Utah Muscular Dystrophy Association clinic. The diagnosis was determined by the presence of clinical features consistent with DMD or BMD, along with either 1) absent or altered dystrophin expression as determined by immunohistochemical, immunofluorescent, or immunoblot analysis or 2) a family history consistent with X-linked inheritance. The sample consisted of 35 individuals with DMD, 21 with BMD, and 2 carriers, one of whom was symptomatic. Using SCAIP methodology, deletions were found in 36 individuals or 62% (23 DMD and 13 BMD); premature stop mutations in 8 or 14% (4 DMD, 3 BMD, and 1 carrier); and missense mutations were identified in 3 patients (5%) (1 DMD and 2 BMD). A single frameshift deletion was identified in one DMD case (1.5%), and a frameshift insertion was identified in the manifesting carrier (1.5%). Testing via MAPH identified 4 duplication mutations (7%), 3 DMD and 1 BMD. No disease causing mutation was identified in five individuals (9%). In summary, mutations in dystrophin were detected in more than 90% of cases using improved methods of detecting point mutations and duplications of the dystrophin gene. The increased availability of testing and rate of mutation detection will have an immediate impact on genetic counseling for dystrophinopathies.

Disclosure(s): None

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Parkin mutation analysis in clinic patients with early-onset Parkinson's disease. *H Payami*[*4], P Poorkaj[1], J Nutt[2], J Schellenberg[3], D James[1], T Bird[3],. [1]Dept of Medicine, Univ of Washington, Seattle, WA, [2]Dept of Neurology, OHSU, Portland, OR, [3]GRECC, VA, Seattle, WA, [4]Genomics Inst., NYS Dept of Health Wadsworth Center, Albany, NY.*

Once believed to be non-genetic, Parkinson's disease (PD) has proved to be a heterogeneous disorder with a significant genetic component. In the past seven years, more than ten loci have been linked to PD, four of which (a-syn, UCHL1, parkin and DJ-1) have been identified and mutations characterized. parkin is the most common genetic cause of PD. parkin mutations are found predominantly in early-onset PD (at or before age 40) and are presumed to be recessive. It has been proposed that parkin testing should be a part of clinical work up for young onset PD. The purpose of this study was to assess parkin mutation frequency in a clinic setting, correlate genotype and phenotype, and evaluate the current justification for clinical parkin testing. Patients were selected from a movement disorder clinic based on diagnosis of PD (N=442) and early-onset(39/442). parkin was genotyped by sequence and dosage analysis for all exons. Relatives and controls were screened for mutations. Mutations were found in 7/39 patients and 0/96 controls. Two were compound heterozygous; 5 were heterozygous. Mutations included deletions in E2, E3, E8, duplications in E2-4, E9, and P437L. 70% of mutations were detected by dosage analysis. A novel substitution (R402W) was found in one patient and one control. The phenotype of parkin-positive patients was remarkably similar to patients without parkin mutations. In conclusion, parkin mutations are common in early-onset PD patients (18%). parkin cases cannot be distinguished on the basis of clinical features and require genetic testing. parkin testing is critical for research, but its wide application for diagnosis and counseling is premature. parkin mutations are presumed to be recessive, yet 70% of parkin cases were heterozygous. It is unclear if heterozygous mutations are pathogenic. wide application of parkin-based diagnosis and counseling is premature and requires a better understanding of the mode of inheritance, penetrance and carrier frequencies.

Disclosure(s): None

PLATFORM PRESENTATIONS IN CLINICAL GENETICS II

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Behavior and sleep disturbances in the 17p112 microduplication and microdeletion syndromes: comparisons and contrasts between human and mouse. *L Potocki*[1,6], K Walz[1,6], D Treadwell-Deering[2,5,6,7], K Krull[2,5,6,7], D Glaze[3,5,6,7], R Paylor[1,4,6], JR Lupski[1,5,6,7]. [1]Department of Molecular and Human Genetics, [2]Department of Psychiatry and Behavioral Sciences, [3]Department of Neurology, [4]Department of Neurosciences, [5]Department of Pediatrics, [6]Baylor College of Medicine, [7]Texas Children's Hospital, Houston.*

Nonallelic homologous recombination within region-specific low-copy repeats is known to give rise to DNA rearrangements associated with many genetic disorders. Smith-Magenis syndrome (SMS) is a well characterized multiple congenital anomalies syndrome due to a heterozygous deletion within 17p11.2. The neurobehavioral abnormalities of SMS have been well described and include maladaptive, aggressive, and self-injurious behavior and significant sleep disturbances associated with an abnormal circadian rhythm of melatonin. The homologous recombination reciprocal of the SMS deletion—dup(17)(p11.2p11.2) is a unique multiple congenital anomalies syndrome; however, this syndrome is not as often recognized as this duplication is difficult to detect by routine cytogenetic analysis and thus many of these patients are not ascertained. Neurocognitive and behavioral phenotypes, and objective measures of sleep (polysolmnography) were assessed in 6 duplication patients through a multidisciplinary clinical protocol in the General Clinical Research Center at Texas Children's Hospital. These patients were found to have neurocognitive impairment, dysmorphic craniofacial features, hypotonia, failure to thrive, oropharyngeal dysphasia, congenital cardiovascular disease, obstructive and central sleep apnea, and behavioral abnormalities including autistic behavior, hyperactivity and self-injurious behavior in some patients. Human chromosome 17p11.2 is syntenic to the 32-34 cM region of murine chromosome 11. We previously constructed mouse models corresponding to the SMS microdeletion and the 17p11.2 microduplication using chromosome engineering. The mouse models (heterozygous Dup mutant (N=34), and heterozygous Del mutant (N=29)) were subjected to a battery of behavioral assays to determine locomotor activity, anxiety, startle, conditioned fear, and further assessed for circadian activity in controlled light/dark conditions. Interestingly, the deletion and duplication animals recapitulate some of the neurobehavioral features seen in the human counterparts; such as hyperactivity, increased anxiety, and poor growth in the duplication animals, in contrast to circadian disturbances and tendency toward decreased activity and obesity in the deletion animals. Circadian abnormalities were not seen in the duplication animals. Measures of sleep apnea were not assessed. Self-injurious behavior was not observed in the murine models of either SMS or dup17p11.2. These data further characterize the duplication 17p11.2 syndrome in human and support the employment of animal models for assessing the effects of gene dosage in microdeletion and microduplication syndromes. Systematic clinical evaluation of several more dup17p11.2 patients will be necessary to determine the features most characteristic of this microduplication syndrome.

Disclosure(s): None

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Evaluating children with developmental delay: quality and cost of diagnostic work-ups. *EV Bawle*[1], LN Teed[1], M Majkowski[2], RL Thomas[3]. [1]Children's Hospital of Michigan, Division of Genetics and Metabolism, Detroit, Michigan, [2]Children's Hospital of Michigan, Division of Neurology, Detroit, Michigan, [3]Children's Hospital of Michigan, Children's Research Center of Michigan, Detroit, Michigan.*

Introduction: Determining the cause of developmental delay (DD) or mental retardation (MR) in a child is often difficult given the range of possible etiologies. The American College of Medical Genetics and the American Academy of Neurology have similar practice guidelines for evaluating individuals with DD/MR. Few studies to date have addressed the incorporation of these guidelines into clinical practice or the cost of these evaluations. The goals of this study were to: 1) evaluate the diagnostic work-ups being performed, 2) determine their average costs and 3) ascertain whether better quality evaluations cost less and improve etiological determination. Methods: A retrospective chart review was conducted of new patient referrals to neurology or genetics for evaluation of DD/MR in 2002. Charts were selected using eight ICD-9 codes commonly assigned for children with DD/MR. One hundred and twenty-two charts met inclusion criteria. The first clinical evaluation was scored on an eleven-point scale. One point was awarded for each of the following criteria: documentation of prenatal, birth, developmental and family histories; the presence/absence of focal neurological symptoms, dysmorphology, major birth defects, micro/macrocephaly, and growth abnormalities; a specific differential diagnosis; and recommendations for sequential testing. Cases were divided into two groups based on score with a higher score indicating a more complete evaluation. Cases placed in the targeted group (n=59) had a score of 10 or 11. Cases scoring 4-9 were placed in the generalized group (n=63). Test recommendations were recorded and costs totaled. The determined etiology was noted if one was reached. Results: There were no differences between groups in mean age, gender or inclusion of two criteria - major birth defects and growth abnormalities. Using Fisher's Exact chi-squared test, there were significant differences between groups ($p<0.05$) in the frequency with which the other nine evaluation criteria were included. The greatest differences were seen in the inclusion of a prenatal history, dysmorphology, a specific differential diagnosis and recommendation for sequential test ordering. Using a two-tailed student's t-test, there was a significant difference in average total cost, with an average of $1159 (SD=$1347) in the targeted group compared to $3239 (SD=$1777) in the generalized group. The mean difference was $2080 (95%CI=$1511-$2648; standard error=$287; $p<0.001$). Etiologies were determined in 35.6% (21/59) of targeted group cases and 28.6% (18/63) of generalized group cases. This difference was not statistically significant. Conclusions: Less than half of the cases in our study had a targeted evaluation that included all components recommended by established guidelines. Targeted evaluations did not improve etiological determination but did significantly decrease cost. Guidelines should be reinforced to improve the cost-effectiveness of these diagnostic evaluations.

Disclosure(s): None

PLATFORM PRESENTATIONS IN CLINICAL GENETICS II

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Haplo-insuffiency of GFT2I implicated in mental retardation in Williams syndrome. *CA Morris*[*7], CB Mervis[1], ML Rowe[1], JS Fricke[2], HH Hobart[3], RG Gregg[4], C Rios[3], KK Kimberley[3], AE Toland[5], NI Stone[6]. [1]Dept Psychological and Brain Sciences, U Louisville, Louisville, KY, [2]Dept. Pediatrics, U of Nevada School of Medicine, Las Vegas, NV, [3]Molecular Cytogenetics Laboratory, Dept Pediatrics, Div Genetics, U of Nevada School of Medicine, Las Vegas, NV, [4]Dept Biochemistry and Molecular Biology and Center for Genetics and Molecular Medicine, U Lousiville, Louisville, KY, [5]Comprehensive Cancer Center, UCSF, San Francisco, CA, [6]Dept. Pharmacology, Johns Hopkins U, Baltimore, MD, [7]Dept Pediatrics, Div Genetics, U of Nevada School of Medicine, Las Vegas, NV.*

Williams syndrome (WS)(MIM:194050) is caused by a 1.5 Mb deletion of chromosome 7q11.23 that includes the elastin gene (ELN). Most individuals with WS have common breakpoints (classic deletion); the deletion includes FKBP6 on the centromeric end, and GTF2I on the telomeric end. Although most families with autosomal donimant supravalvar aortic stenosis (SVAS) (MIM: 185500) have intragenic ELN mutations, we have identified five SVAS families with normal intelligence and the WS cognitive profile that have overlapping deletions in the WS region. None of the familial deletions includes GTF2I, implicating the gene in the mental retardation or reduced IQ associated with WS. We have evaluated two individuals with WS who have short deletions in the WS region; data from these individuals support this hypothesis. Using both FISH probes and polymorphic markers that span the WS region,we evaluated 256 individuals with WS. Only two were found to have deletions shorter than the classic deletion. Both individuals have normal height, characteristic WS facial features, hoarse voice, SVAS, inguinal hernias, colon diverticulae, hyperreflexia of the lower extremities, joint contractures, and fit the WS cognitive profile. One deletion includes ELN through GTF2I; that individual has a full-scale IQ of 60 and lives in a group home. The other deletion includes FKBP6 through CYLN2; that individual has a full-scale IQ of 94 and lives independently. This person also scores significantly above the mean for WS for verbal short-term memory, verbal working memory, vocabulary, and visuospatial construction (block design, drawing). Comparison of the phenotype with the deletion length suggests that the critical region for WS includes ELN and the genes telomeric to it. Individuals with short deletions in the WS region, both reported by us and others, do not have mental retardation if they do not have a GTF2I deletion. Those individuals with short deletions that include GTF2I do have mental retardation. Studies of GTF2I in individuals with nonspecific mental retardation will be necessary to determine if this gene is a significant contributor to mental retardation in the non-WS population.

Disclosure(s): None

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The natural history of Freeman-Sheldon syndrome. *D Stevenson*[*1], J Carey[1], J Palumbos[1], A Rutherford[1], M Bamshad[1,2]. [1]University of Utah, Department of Pediatrics, Salt Lake City/Utah, [2]University of Utah, Department of Human Genetics, Salt Lake City/Utah.*

Objective: Approximately 1 in every 3000 children is born with multiple congenital contractures (MCC) that cause substantial morbidity. The most common causes of MCC include amyoplasia and the distal arthrogryposis (DA) syndromes. All of the 10 recognized DA disorders are notable for contractures of the hands and feet, but Freeman-Sheldon syndrome (FSS) is distinguished further by severe contractures of the facial muscles. However, the expressivity of FSS is broad, and consequently, it can be difficult to discriminate from other distal arthrogryposes. Our objective was to define the clinical characteristics and natural history typical of children and adults with FSS. This information will facilitate the development of more accurate diagnostic criteria, improve the predictive power of anticipatory guidance, and guide strategies to identify the molecular basis of FSS. Design: Phenotypic information was collected from more than 100 patients who were referred with the diagnosis of FSS. Data from questionnaires and family interviews were collected and analyzed for differences in demographics, clinical features, development, and treatment/outcomes. Results: only 22 patients met our diagnostic criteria for FSS. All had contractures of the face and the wrists, hands, ankles, or feet. The FSS phenotype changed through adolescence and adulthood but remained quite characteristic. Other notable findings included scoliosis (20/21), dental crowding (13/13), strabismus (10/20), hearing loss (7/22), and cryptorchidism (5/9). Children with FSS walked at an average at 17.9 months of age, and devices to assist ambulation were required in 13/16 patients. Eighteen of 20 individuals underwent an average of 11.8 surgical procedures. Problems with anesthesia/surgery were encountered in 10/17. Feeding was problematic in all children with FSS, with 9/17 requiring a special nipple, 8/18 requiring nasogastric tube feedings, and 3/20 requiring a gastric tube placement. All of the children with FSS were cognitively normal. Conclusions: Most children are diagnosed with FSS erroneously. However, consistent application of existing criteria distinguishes FSS from other DA conditions that have similar phenotypes. Patients with FSS require significant interventions (functional nutrition, orthopedic, surgical, and rehabilitative therapies) that are not required by most children with other DA disorders. Most children with FSS who receive these interventions do reasonably well and eventually function independently as adults.

Disclosure(s): None

PLATFORM PRESENTATIONS IN CANCER GENETICS

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New clinical insights into BRCA1/BRCA2 missense mutations in African-American breast cancer patients. *LL Baumbach-Reardon*[*1], *L Gayol*[1], *A Monterio*[2], *ME Ahearn*[1], *T Donenberg*[3], *JF Arena*[3]. [1]*Dept. Pediatrics/Div Genetics, Univ. Miami School of Medicine, Miami, FL,* [2]*Strang Cancer Prevention Center & Dept. of Cell and Dev. Biol., Weill Medical College of Cornell Univ. New York, NY,* [3]*FOBCC, Univ. Miami School of Medicine, Miami, FL.*

Recent evidence from our laboratory as well as others suggests that specific variants in BRCA1 and BRCA2 are associated with increased risk of breast cancer in women of African-American (AA) ancestry. Of particular note is the increasing number of missense mutations/unclassified variants in African-Americans, particularly in BRCA2. We are completing our investigations of the association of these variants with disease risk in a cohort of 35 AA probands and their families. The current study has a two-part design: collection of prevalence data for all novel variants, and assessment of functional effects for selected BRCA1 mutations using a transcriptional assay. No functional assay for BRCA2 is available, although we are pursuing newer comparative genomic approaches to biological predictions for BRCA2 missense substitutions. BRCA1 missense mutations were found in 14% (5/35) of the families, while 32% (13/35) of the probands had BRCA2 missense mutations. Several of the BRCA1 variants were novel. We have determined that BRCA1 variant, W1718C, which was detected in one family segregating with breast cancer, exhibits a proven loss-of-function phenotype in transcriptional assays. Another novel mutation, T1700A, also found in one high-risk family, also demonstrated a proven loss-of-function. Both of these mutations were not found in the presence of any other disease-causing mutation, nor in 100 AA control chromosomes. Regarding BRCA2, 5/13 (38%) missense mutations were novel, four of these were found in one family each (T77T, E425E, D1923A, V2820V) with two (E425E, V2820V) being the only variant detected. Their respective frequencies in ethnic-specific controls is either zero or very low (<3%). One variant, S2414S, was detected in two unrelated probands with breast cancer at less than 40 years of age. It is of interest that two missense mutations, H2116R and I2490T, although previously reported as recurrent unclassified variants in Caucasian breast cancer patients, were rare in this cohort, and not represented in ethnic-specific controls. Completion of this study should reveal important new clinical information regarding the association of BRCA1/BRCA2 missense mutations with breast and ovarian cancer risk in AA women.

Disclosure(s): None

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Should BRCA1/2 testing always be performed on an affected family member prior to testing the healthy at-risk patient? *GL Wiesner*[*1,2], *H Beaird*[3], *M Singer*[3]. [1]*Center for Human Genetics,* [2]*Department of Genetics,* [3]*Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland OH.*

Background: Genetic testing for breast and ovarian cancer susceptibility is currently provided to at-risk women in order to estimate the lifetime chances for cancer and to develop an individualized management plan. However, most Centers that provide genetic testing for high-risk women suggest that a family member affected with either breast or ovarian cancer should be initially offered BRCA1/2 mutational analysis to identify whether a recognizable mutation is segregating in the family. After such a mutation is found, testing is then offered to the healthy, at-risk female relatives. Methods: In order to investigate whether women given such a recommendation are able to have a family member undergo testing, we performed a retrospective abstraction on 212 medical files of women who were evaluated for predisposition to breast or ovarian cancer from 1995 through 2000 in a fee-for-service tertiary care specialty clinic. Results: In general, the 212 women were young, with 62.7% under the age of 50. Approximately half of the women had a personal history of breast and/or ovarian cancer and over 60% had a first-degree family member with a history of breast and/or ovarian cancer. 28% indicated they had Ashkenazi Jewish heritage. Following the initial counseling session, 180 (85%) women were offered BRCA testing. Among these patients, 115 (64%) women were offered testing directly either because of a personal family history of breast or ovarian cancer and/or the patient was aware of a known mutation in another family member. 65 (36%) were offered testing of a relative perceived to have a higher chance to carry a BRCA mutation. Ashkenazi Jewish ethnicity was strongly associated with an offer of direct testing. Of the 115 women who were offered direct testing, 75 tests were eventually performed, of which 32% were positive for BRCA mutations. An offer of indirect testing was made to a family member in 65 cases, with only 10 relatives accepting the offer. Of the relatives tested, 50% received positive test results for BRCA mutations. In all instances, the patient accepted testing after their relative tested positive. Of these patients, 60.0% also tested positive for BRCA mutation. Conclusions: Requiring at-risk women to contact family members for the initial phase of genetic testing for BRCA1/2 may limit the uptake of testing in women who seek clinical genetic counseling and testing for breast and ovarian cancer susceptibility.

Disclosure(s): None

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Common functional genetic polymorphisms interact to predict breast cancer risk. *J Mulvihill*[*1], D Ralph[2], C Aston[3], A West[2], D Branam[4], S Manjeshwar[2,4], B Gramling[2], D DeFreese[2], AE Whelan[2], LF Thompson[4], MA Craft[5], DS Mitchell[5], CD Shimasaki[2], ER Jupe[2,4,6,7]. [1]Department of Pediatrics, University of Oklahoma Health Sciences Center, Oklahoma City, OK, [2]InterGenetics, Inc. Oklahoma City, OK, , [3]Program in Arthritis and Immunology, Oklahoma Medical Research Foundation, Oklahoma City, OK, [4]Program in Immunobiology and Cancer, Oklahoma Medical Research Foundation, Oklahoma City, OK, [5]Breast Imaging of Oklahoma, Edmond, OK, [6]Department of Surgery, University of Oklahoma Health Sciences Center, Oklahoma City, OK, [7]Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.*

The proportion of breast cancer in the general population attributable to germline mutations in highly penetrant genes (e.g., BRCA1/2) is small because of the low frequency of these mutations. Common, but weakly penetrant, functional genetic polymorphisms are likely to account for most of the genetic risk for breast cancer in the general population. Current polygenic risk models assume, perhaps erroneously, that the effects of the component genes act independently. Potential gene-gene interactions among ten genes, with known or predicted functional consequences in development of breast carcinoma, were examined in 1050 cases and 1845 controls of Caucasian descent. Association of breast cancer risk with variation in single genes and two- and three- gene combinations was analyzed for two age groups: 53 years and under, and over 53 years. The odds ratio (OR) for a genotype was calculated and compared to a null distribution of ORs generated for this genotype by randomizing (10,000 times in these analyses) the case-control status of the individuals in the sample to give an empirical estimate for the p-value for the observed OR. Re-sampling was performed to give an empirical estimate of the 95% confidence interval and a likely more stable estimate of the OR for the genotype, particularly for the less common genotypes. In these analyses the population of individuals was resampled 10,000 times with each repetition composed of 80% of the controls and 80% of the cases selected at random from the whole sample. Over 100 genotypes met stringent criteria for significance (alpha]=1/10,000) with ORs ranging from 0.20 to 5.2, a 26-fold range Single gene analyses were largely uninformative, whereas, the majority of the significant ORs were two- and three-gene combinations. Comparing observed ORs to ORs predicted by an independent gene model showed that about 25% of the significant multigenic combinations differed markedly from the predicted value. Thus, combinations of genes interact to affect risk for breast cancer in a manner that is not predictable by combining the effects of the individual component genes. While independent genes are a valid starting point in a polygenic model, identifying and incorporating modification of risks associated with gene-gene interactions will improve accuracy of the model and should be taken into consideration when building polygenic models for breast cancer risk. In addition, further exploration of the biologic basis for these multigenic interactions might reveal etiologic or therapeutic insights into breast cancer and other cancers.

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Indication for genetics referral versus outcome of cancer genetic risk assessment: are community practitioners ready for cancer genetic testing? *C Farrell*[*1], M Lyman[1]. [1]Roswell Park Cancer Inst, Cancer Genetics Dept, Clinical Genetics, Buffalo, NY.*

As an increasing number of cancer-associated genes are discovered, and the ability for genetic testing becomes part of daily media, publications and marketing efforts, practitioners are expected to integrate such into practice. It is critical to assess understanding of genetic information and the implementation of cancer genetic testing into community practice. We report an ongoing study of the initial indication for referral for cancer genetic services versus the actual outcome of cancer genetic consultation and risk assessment in new patients seen (74 persons from January – October 2003) in a clinical cancer genetics service. Among new patients, 51% (38) arrived through external physician referral, 47% (18) being from GYNs and 29% (11) from PCPs. Of new patients, 41% (30) had personal histories of cancer, with the majority being breast cancer (67%, 20). Other cancers among referred individuals/families included colorectal, ovarian, endometrial, thyroid, renal, prostate, stomach and melanoma/skin cancer. In surveying patients' primary concerns for pursuing cancer genetic risk assessment, 82% (45) indicated concern for personal cancer risk, and 76% (42) indicated concern for family risk. Patients also indicated interest in genetic testing (71%, 39), risk reduction options (67%, 37), and screening recommendations (60%, 33). While many patients were referred for risk assessment based on family history of cancer in general, many were referred for specific genetic testing -- most often BRCA1 and BRCA2. While referral to "Genetics" was appropriate in these cases, it was not uncommon that the referral included request for a specific genetic test/s (e.g., prescription for BRCA1 and BRCA2 gene testing) which was not always appropriate. Among the new patients seen by the clinical genetics service, 73% were assessed as hereditary breast and ovarian cancer (HBOC) in the differential diagnosis. However, 24% (18) of these patients also warranted inclusion of a different cancer genetic syndrome in the differential diagnosis, such as Cowden syndrome and/or HNPCC. These syndromes were not considered by the referring physician although in some cases they became the primary differential. There was lack of awareness for hereditary cancer syndromes beyond BRCA1 and BRCA2. The results of this ongoing study support a need for: (1) directed education of community practitioners concerning assessment of cancer genetic risk, as well as use and interpretation of related genetic testing, to promote best health care management and professional practice, (2) establishment of guidelines for screening individuals to identify those at increased cancer risk (not just "classic" criteria), and (3) genetics professionals to provide resources, improve liaisons and collaborate with non-genetics practitioners. Case examples will be used to illustrate recurring patient and professional problematic themes.

Disclosure(s): None

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Family history knowledge in families at high risk for a BRCA1 mutation. *B Baty*[1], K Smith[1], L Bloor[1], A Kinney[1]. [1]University of Utah Health Sciences Center.*

Confirmation of cancer cases among family members is important for accurate risk assessment, yet prior studies have demonstrated that cancer pedigrees are often inaccurate. While some studies suggest that family history knowledge varies by gender, age and diagnosis, there are few studies that have examined variables associated with knowledge of family history. We examined factors associated with family history knowledge in two ethnically diverse kindreds with identified BRCA1 mutations. The Family Health Study (FHS) enrolled individuals in a single African American kindred with 30 cases of breast and/or ovarian cancer (BC/OC) and the Utah BRCA1 Study (UBS) enrolled individuals in a single Caucasian kindred with 62 cases of BC/OC. Baseline questionnaires were administered to study participants before genetic counseling and testing. We present data from the baseline questionnaires of 94 individuals (68% female) in the FHS and 408 individuals (60% female) in the UBS. Individuals were asked to identify all of their relatives with cancer and indicate the type of cancer. Information was also collected on demographics, state anxiety, family adaptability and cohesion (Faces II), concern about cancer, prior knowledge of high-risk status, and perceived risk of having BC/OC or a gene for BC/OC. Family members reported an average of 4.2 relatives with BC/OC in the FHS and 1.6 relatives with BC/OC in the UBS. Fifty percent of FHS participants, but only 5% of UBS participants, reported 4 or more relatives with BC/OC. Multiple linear regression analyses showed that knowledge of being in a high risk family ($p<0.01$), a personal history of cancer ($p<0.05$), and female gender ($p=0.05$) increased family history knowledge in the FHS. Also in this kindred, increased age ($p=0.08$) and increased anxiety ($p=0.08$) showed marginally significant positive associations with knowledge of family history. Knowledge of family history was not associated with education, family adaptability and cohesion, cancer worry, or perceived risk of being positive for BRCA1. In the UBS, greater knowledge of relatives with BC/OC was associated with a knowledge of being in a high risk family ($p<0.001$), perceived risk of having a gene for BC/OC ($p<0.001$), a personal history of cancer ($p=0.01$), greater concern about BC ($p<0.05$) and OC ($p=0.01$), perceived risk of developing BC ($p=0.02$) and OC ($p=0.001$), and more education ($p<0.05$). No associations were observed for gender, age, anxiety or family cohesion and adaptability. Individuals who knew or suspected they are in a high-risk family reported an average of 1.08 more relatives with BC/OC. Similarities and differences between the two kindreds will be discussed. Further research is needed to determine whether the different pattern of associations we observed is related to social network characteristics (e.g., family communication and family support).

Disclosure(s): None

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Targeted plasma cell FISH analysis but not unselected white blood cells detects residual disease in multiple myeloma. *ML Slovak*1, V Bedell[1], K Pagel[1], KL Chang[1], G Somlo[1]. [1]City of Hope National Medical Center, Duarte, CA.*

BACKGROUND: Fluorescence in situ hybridization (FISH) enables the rapid detection and quantification of nonrandom karyotypic abnormalities in the low proliferating lymphoid disorders by evaluating the non-proliferating neoplastic cells in interphase. In multiple myeloma, untargeted FISH usually provides meaningful results at disease presentation and when a minimum of 10% plasma cells are present. However, untargeted FISH for minimal residual disease (MRD) testing correlates poorly with pathology, underscoring the need for a more sensitive MRD FISH assay. METHODS: Using FISH to detect IgH rearrangements and deletions of 13q or 17p, we investigated untargeted white blood cell nuclei or targeted plasma cell nuclei from bone marrow aspirates or stem cell aliquots collected from 20 multiple myeloma patients on various therapies and at various stages in their treatment. 200 nuclei were scored for each probe in the untargeted analysis; only plasma cells (1 to 167 cells) were scored in the targeted analysis using a Bioview Duet image analyzer. Results were correlated with classical cytogenetics (CG), pathology, cell morphology and clinical features. RESULTS: The results of untargeted and targeted FISH were concordant in 11 cases (55%) but discrepant in 9 of 20 cases (45%). The discrepant cases showed IgH rearrangements (7 cases), del(13q)/-13 (5 cases), and 17p-/TP53 (2 cases) by targeted analysis only. Five cases showed two abnormalities; one case showed all three abnormalities. The targeted FISH results were in complete agreement with residual disease observed by pathology. The clinical impact of these findings is underscored by three cases: 1) A smoldering myeloma case with loss of the Y chromosome as the sole karyotypic aberration by CG showed both IgH rearrangement and monosomy 13 in 8 and 22 plasma cells, respectively, whereas the untargeted (area) scans of 240 cells were within normal limits; 2) Seven of 10 plasma cells detected among 7295 white cells in a stem cell aliquot collected for possible autologous transplant showed an IgH rearrangement; 3) One del(13q) case was found to have normal pathology and normal disomy 13 in the plasma cells, with a del(13q) in myeloid precursor cells only, indicating evolving myelodysplasia not residual multiple myeloma. CONCLUSION: Targeted FISH adds valuable diagnostic, prognostic and MRD data in multiple myeloma. It can also differentiate between del(13q) MDS versus del(13q) MM. We suggest the use of targeted FISH analysis in clinical trials if risk stratifications and MRD testing take into consideration IgH, 13q and 17p status.

Disclosure(s): None

PLATFORM PRESENTATIONS IN BIOCHEMICAL GENETICS

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Reduction of lysosomal storage in brain and meninges following intrathecal adminsitration of iduronidase in canine mucopolysaccharidosis I (MPSI). *E Kakkis*[*1,2], M Passage[1], P Belichenko[3], M McEntee[4], S Le[1], C Vogler[5], R Esquivel[1], W Mobley[3]. [1]Harbor-UCLA REI, Torrance, CA, [2]BioMarin Pharmaceutical Inc., USA, Novato, CA, [3]Stanford University, Stanford, CA, [4]Univ. Tenn. College of Vet. Med., Knoxville, TN, [5]St. Louis University, St. Louis, MO.*

Intrathecal (IT) administration of recombinant human a-L-iduronidase (rhIDU) was studied as an alternative approach to treating MPS I brain disease since intravenous (IV) rhIDU did not treat the brain in canine MPS I. A 4-week treatment study of weekly IT injections of 1.0 mg rhIDU in 4 MPS I canines demonstrated mean 21-fold normal IDU in brain and a mean 300-fold normal IDU in the meninges. Deep brain specimens had an 11-fold normal level whereas those on the surface reached 37 fold normal, indicating that the enzyme does penetrate the brain as observed in prior rat research. Tissue glycosaminoglycan (GAG) levels normalized in the brains of 4 MPS I canines relative to similar aged normal dogs and significantly below that of 12 canines treated with IV enzyme only. Histology showed improvements in perithelial, microglial, glial cell and meningeal storage. Lamellar vesicles in neurons changed to smaller dense granules. A lymphocytic/plasmacytic infiltrate was observed in treated dogs. Monthly injections of the same dose (4 doses over 3 months) also normalized brain GAG and achieved enzyme levels as high as weekly dosing. The data suggest that small amounts of rhIDU via the IT route can reduce GAG storage in the brain and meninges in canine MPS I and could be investigated as adjunctive therapy to IV enzyme therapy in human MPS I.

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Enzyme replacement therapy (ERT) for infantile onset Pompe disease: long term follow-up results. *P Kishnani*[*1], M Nicolino[2], T Voit[3], C Tsai[4], G Herman[5], J Waterson[6], R Rogers[7], J Levine[8], A Amalfitano[1], J Charrow[9], G Tiller[10], B Schaefer[11], E Kolodny[12], D Corzo[13], YT Chen[1]. [1]Duke University Medical Center, Durham, NC, [2]Hopital de Brousse, Lyon, France, [3]University Hospital, Essen, Germany, [4]Children's Hospital, Denver, CO, [5]Children's Hospital, Columbus, OH, [6]Children's Hospital, Oakland, CA, [7]Greenwood Genetics Center, Greenville, SC, [8]The Children's Hospital, Boston, MA, [9]Children's Memorial Hospital, Chicago, IL, [10]Vanderbilt University School of Medicine, Nashville, TN, [11]University of Nebraska Medical Center, Omaha, NE, [12]New York University School of Medicine, New York, NY, [13]Genzyme Corporation, Cambridge, MA.*

Background. Pompe disease is an autosomal recessive disorder caused by a deficiency of acid alpha-glucosidase (GAA). The infantile form presents in the first few months of life with rapidly progressive hypotonia, generalized muscle weakness and hypertrophic cardiomyopathy. Death usually occurs by one year of age. **Methods.** Two clinical trials explored the safety and efficacy of ERT with CHO-cell derived rhGAA in patients with infantile onset Pompe disease (cardiomegaly, cardiomyopathy, hypotonia, and muscle weakness by 6 months of age). Three patients in the first trial initially received rhGAA at 5 mg/kg IV twice weekly. Eight patients in the second trial received rhGAA at 10 mg/kg IV weekly. Currently, patients receive 10 mg/kg IV weekly or 20 mg/kg IV every two weeks. Clinical efficacy end-points in both trials included survival, ventilator-free survival, decrease in left ventricular mass index (LVMI) measured by echocardiography, and changes in motor development scores. Safety follow up included reporting of adverse events, monitoring of anti-rhGAA antibody titers and hearing evaluations, among others. **Results.** Four patients are alive (mean age 37.5 months; range 30-52 months); mean duration of ERT is 34.3 months (range 27-50 months). All four surviving patients have demonstrated marked decrease in LVMI, do not require the use of cardiac medications, remain ventilator-free, are ambulatory (without the use of walking aids), and fed by mouth. In the four surviving patients hearing has remained normal. Seven patients are deceased. Deaths were attributed to disease progression and unrelated to rhGAA. Mean age of death was 30 months (range 18-50 months); mean duration of ERT was 23.5 months (range 3.8-46 months). This subgroup of patients had also shown decrease in LVMI, although skeletal muscle response was variable. All patients treated with rhGAA developed anti-rhGAA antibodies. A downward trend in anti-rhGAA antibody titers has been seen in the four surviving patients. All patients experienced infusion-associated reactions. The majority of the infusion-associated reactions were mild and managed symptomatically. **Conclusions.** ERT in infantile onset Pompe disease appears to be safe and well tolerated. ERT-treated patients have demonstrated prolonged survival and marked improvement in cardiomyopathy, although skeletal muscle response has been variable. ERT has thus changed the natural history of infantile onset Pompe disease. Long-term follow-up of patients is required to further evaluate the safety and efficacy of rhGAA. Additional studies are needed to identify predictors of skeletal muscle response to treatment.

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PLATFORM PRESENTATIONS IN BIOCHEMICAL GENETICS

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Development, characterization, and treatment of a hypomorphic Smith-Lemli-Opitz syndrome mouse model. *F Porter*[1], C Wassif[1], L Kratz[2], R Kelly[2], L Correa-Cerro[1]. [1]Heritable Disorders Branch, NICHD, NIH. Bethesda, MD, [2]Kennedy Krieger Institute, Baltimore, MD.*

Smith-Lemli-Opitz syndrome (SLOS) is a multiple malformation/mental retardation syndrome due to an inborn error of cholesterol synthesis. Mutation of the 7-dehydrocholesterol reductase gene (DHCR7) results in increased 7-dehydrocholesterol (7DHC), and typically decreased cholesterol levels. An unanswered question in SLOS is: Are the impaired mental function and behavioral problems due to fixed developmental problems, functional defects due to effects of abnormal sterol composition, or a combination of these factors? Whereas fixed developmental problems cannot be treated, behavioral and mental manifestations of SLOS due to function defects should be amendable to correction of the sterol defect. Dietary cholesterol supplementation is currently used to treat SLOS patients; however, its efficacy to directly affect central nervous system sterol levels is precluded by the blood-brain barrier. Simvastatin therapy to decrease 7DHC levels has been proposed. Simvastatin is an HMG-CoA reductase inhibitor that crosses the blood-brain barrier. Both in vitro work and a small clinical trial suggest that simvastatin can both decrease 7DHC and paradoxically increase cholesterol levels. The later effect is likely due to increased expression of a mutant allele with residual DHCR7 activity. Mice homozygous for a null disruption (delta) of Dhcr7 die soon after birth, thus to investigate therapeutic interventions for SLOS, we have produced a hypomorphic SLOS mouse model by “knocking-in” a T93M mutation. T93M is the most common human missense mutation. T93M/T93M mutant mice are phenotypically normal except for mild ventricular dilatation. T93M/delta mice have mild ventricular dilatation and 2-3 toe syndactyly. 2-3 toe syndactyly is the most common physical finding in SLOS patients. Sterol profiles of liver, cortex, midbrain, and kidney in both one day old and six week old mice showed elevated 7DHC. As expected, 7DHC levels were higher in the T93M/delta compared to T93M/T93M mice. To experimentally evaluate dietary cholesterol supplementation we compared T93M/delta mice on a cholesterol-supplemented diet versus a cholesterol-deficient diet. After five months, no survival or pathological differences were found. Sterol analysis of tissue showed biochemical improvement in some peripheral tissues. As expected, cortex sterol levels were not altered. Initial neuromuscular testing indicated that cholesterol supplementation might improve neuromuscular status. Treatment of T93M/delta mice with simvastatin for three weeks significantly decreased 7DHC levels in some peripheral tissues. Notably, simvastatin therapy also decreased dehydrocholesterol levels in cortex. Effects of simvastatin therapy on DHCR7 expression, and combined simvastatin/cholesterol therapy are being evaluated. Correcting the sterol abnormality in the central nervous system may positively affect behavioral and developmental problems found in SLOS patients.

Disclosure(s): None

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Maternal liver diseases (MLD) in the pregnancies of infants with the spectrum of fatty acid oxidation defects (FAOD) compared to matched population controls. *MK Fearing*[1,2,3], HL Levy[1,3], LE Wilkins-Haug[1,4], C Larson[5], VE Shih[1,2]. [1]Harvard Medical School, [2]Massachusetts General Hospital Amino Acid Laboratory, [3]Children's Hospital Boston, [4]Division of Maternal Fetal Medicine; Brigham and Women's Hospital, [5]New England Newborn Screening Program/University of Massachusetts, Boston.*

BACKGROUND: Infant fatty acid oxidation defects (FAOD) are rare conditions, occurring in 1:12,000 births. Increasingly, fetal long chain FAODs are associated with rare maternal pregnancy complications, including acute fatty liver of pregnancy (AFLP) and hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome. MLD in the general population has a low prevalence rate of only 0.1-1.5% for AFLP and 0.6-1.0% for HELLP syndrome. Given the paucity of these conditions, elucidating the true epidemiological relationship is difficult. The lack of literature comparing the entire spectrum of FAOD and pregnancy outcomes compared to the population led us to perform the following study. METHODS: 50 case infants with fatty acid oxidation defects (FAOD) were identified in the New England region, either clinically or by expanded panel MS/MS newborn screening. For each affected infant, 25 controls were selected for each case, matched by date of birth and hospital setting, generating a total of 1300 infant-mother pairs. The pairs were phenotyped for antenatal, intrapartum and neonatal characteristics. The pairs were analyzed using a conditional logistic regression model. RESULTS: Case and control infants analyzed were similar with respect to mean gestational age = 38.2 (SD± 2.1) weeks, and 37.8 (SD±3.6) weeks, mean birth weight = 3264 (SD± 577) grams and 3308 (SD± 446) grams; and maternal age 30.2 (SD± 5) years and 28.4 (SD±6) years for the FAOD and control infants respectively. In the antenatal period, MLD was noted in 16% of all FAOD pregnancies (equally represented in long versus short-medium chain defects) compared to 0.88% in the general population (OR=20.4 ; 95% CI = 7.8-53.2). Isolated pre-eclampsia without hepatic involvement was not significantly different between the case (6%) and control pregnancies (6.1%). Post-natal results included elevated rates of neonatal jaundice [FAOD 36%, control 8% (OR 6.25; CI= 3.42-11.4)]. Subgroup analysis of all FAOD infants revealed 32% had long chain defects and 68% had a medium or short chain defects, without a significant difference in demographic characteristics. CONCLUSIONS: MLD is significantly higher across the entire spectrum of FAOD demonstrating an 18.1 fold increase in the pregnancies of FAOD neonates compared to our control population. Notably, the prevalence is equally high in the pregnancies of infants with short and medium chain defects and not isolated to those infants with long chain FAOD, implicating the entire spectrum of the acylcarnitine intermediates.

Disclosure(s): None

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Molecular findings and genotype/phenotype correlation in 57 MCAD deficient patients identified by newborn screening. *S McCandless*[*1], J Muenzer[2,3], D Millington[4], D Koeberl[4], B Andresen[5], N Gregersen[5], D Frazier[2]. [1]Department of Genetics, Case Western Reserve University, Cleveland, OH, [2]Department of Pediatrics, University North Carolina, Chapel Hill, NC, [3]Department of Genetics, University of North Carolina, Chapel Hill, NC, [4]Dept of Pediatrics, Duke University, Durham, NC, [5]Faculty of Health Sciences, Arhus Univ, Arhus, Denmark.*

Through October 2003, North Carolina has identified 57 individuals with medium chain acyl-CoA dehydrogenase (MCAD) deficiency from just over 700,000 MS/MS newborn screening (NBS) samples. Of the 57, 36 (63%) were homozygous for the common G985A mutation. One of these was an African-American infant, the rest were European-Americans. Fourteen individuals had one copy of the common mutation (2 African-American, 1 mixed Hispanic and European-American). Of four with no copies of the common mutation (2 African-American, 2 Hispanic), 2 are pending analysis, one had two other mutations, and one had no recognizable mutation found after sequencing the coding portion of the gene. He had classic biochemical findings in the newborn period (plasma AC8 and AC8/AC10 ratio both markedly elevated and diagnostic acylglycines in the urine), but has since been lost to follow-up. The second mutation was identified in eleven individuals who were compound heterozygotes with the common mutation. Several of the 9 mutations identified in twelve patients (11 with one copy of 985G>A) have not been previously described in patients presenting symptomatically. Four had a single mutation (199T>C) that appears to produce a thermolabile form of the enzyme. One mutation was seen in two unrelated patients (233T>C) and another was seen in two sibs (928G>A); six other mutations occurred just once. Based on initial screening, confirmatory plasma acylcarnitine results, and clinical findings, the 199T>C mutation is suspected to be clinically mild. In general, individuals without two copies of the common mutation had lower NBS and plasma C8 and C8/C10 ratios, with non-overlapping 95% confidence intervals, than did children homozygous for the common mutation. When individuals compound for the common mutation and 199T>C were removed from the analysis, the differences in NBS results were no longer significant, but the plasma confirmatory test result differences retained statistical significance. No patient lacking the common mutation had an hypoglycemic episode or significant metabolic decompensation. There appeared to be fewer hospital admissions among individuals not homozygous for the common mutation. In summary, the frequency of homozygosity for the common 985G>A MCAD mutation in children identified by newborn screening was lower than reported in children identified symptomatically (63% in this study compared to 75 – 85%). A relatively common mutation (199T>C) appeared to be less strongly associated with accumulation of abnormal metabolites and has not been reported in a patient with hypoglycemia or significant metabolic decompensation. There is insufficient data to comment on the clinical effect of the other 8 mutations identified. While suggestive, the current data do not support altering clinical management based on the genotype.

Disclosure(s): None

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Clinical spectrum, morbidity, and mortality in 113 patients with mitochondrial disease. *F Scaglia*[*1], J Towbin[1,2], J Belmont[1,2], W Craigen[1,2], E Smith[2], S Fernbach[1], L Wong[3], H Vogel[4]. [1]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, [2]Department of Pediatrics, Baylor College of Medicine, Houston, Texas, [3]Institute for Molecular and Human Genetics, Georgetown University Medical Center, Washington DC, [4]Department of Pathology, Stanford University Medical Center, Stanford, California.*

Mitochondrial disorders are clinical entities associated with abnormalities of oxidative phosphorylation. Organs such as the brain, heart and skeletal muscle are highly energy dependent and vulnerable to defects in energy metabolism. The natural history of this heterogeneous group of disorders remains largely unknown. The aim of this study was to determine the frequency of major clinical manifestations in children with mitochondrial disease, and elucidate the clinical course and rates of survival depending on the different presenting features. We have identified 113 patients with mitochondrial disease by using the modified Walker criteria. 102 patients (90%) underwent a muscle biopsy as part of their diagnostic work-up. A significant respiratory chain defect was found in 71% of the patients. In this cohort, we found that complex I (32%), and combined complex I, III, and IV deficiencies (26%) were the most common causes of respiratory chain (RC) defects, followed by complex III (19%), complex IV (16%), and complex II deficiencies (7%). Mitochondrial(mt)DNA abnormalities were found in 11% of the patients. A substantial fraction of patients with RC defects (42%) exhibited cardiac disease, diagnosed by echo-Doppler, however the majority of the patients (58%) had predominant neuromuscular manifestations. No correlation between the type of RC defect and the clinical presentation was noted. The mean age of diagnosis was 33 months in the cardiac group and 44 months in the non-cardiac group ($P<0.005$). In the group with cardiac manifestations, 55% of the patients exhibited hypertrophic cardiomyopathy, 32% exhibited dilated cardiomyopathy, and 13% left ventricular noncompaction. By Kaplan-Meyer analysis, when survival was measured in both groups at the age of 200 months, patients with cardiac manifestations had an 18% survival. This outcome was significantly different ($P<0.0001$) than the outcome of patients with neuromuscular features who had a 90% survival at the same age. This study gives strong support to the view that in patients with RC defects, cardiomyopathy is more common than previously thought and that in comparison to patients with predominant neuromuscular presentation, patients with cardiomyopathy follow a different and more severe clinical course. Cardiac function in mitochondrial patients deteriorates rapidly regardless of the associated RC defect, suggesting that early aggressive supportive treatment might increase survival in those with cardiac dysfunction. As expected, mtDNA mutations and deletions were found in a minority of patients, underlying the fact that most of the mitochondrial disorders of childhood follow a Mendelian pattern of inheritance.

Disclosure(s): None

BIOCHEMICAL GENETICS POSTERS

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Expanded newborn screening with tandem mass spectrometry in Oregon and Idaho: one year and counting. *S Copeland*[*1], J Tuerck[1], D Koeller[1], M Skeels[2], C Hermerath[2], K Gibson[1], C Harding[1]. [1]Oregon Health and Science University, [2]Oregon State Public Health Laboratory.*

Background: Newborn screening for metabolic disease started with PKU in 1962. Since then numerous studies have defined risk factors and modified treatment modalities based on information gathered over the lifetime of prospectively followed PKU children. With the development of tandem mass spectrometry it is now possible to identify over 30 metabolic disorders from a single blood spot. Altogether newborn screening can now detect 34 different diseases, including testing for hemoglobinopathies, endocrine disorders and other metabolic testing. However, data regarding individual outcome for many of these disorders remains incomplete. With many of these metabolic defects there are not enough cases that have been managed prospectively prior to metabolic complications, to ascertain if intervention will make a difference. Methods: In October 2002, Oregon and Idaho started expanded newborn screening with tandem mass-spectrometry for 30 different disorders. The samples are obtained on a filter paper card from a heelstick in the same manner that samples were previously obtained for PKU testing. The blood on the spot is extracted with alcohol and the mass spectrometers measure weight electronically and display results in the form of a mass spectrum. A mass spectrum is a graph that shows each specific molecule by weight and how much of each molecule is present. Results: In twelve months 23 patients were diagnosed in Oregon and Idaho with organic acidurias, urea cycle defects or fatty acid oxidation defects via tandem mass spectrometry. With 64,632 infants screened, the incidence of fatty acid oxidation defects, organic acidurias and urea cycle defects together was about 1 in 2800 live births. Oregon is one of a handful of states that has a mandatory second newborn screening, and in Idaho it is voluntary, at about 2 weeks of age. Of 23 patients identified via tandem mass spectrometry, 3 were normal on the first screen but abnormal on the second. The disorders identified by the second screen have been carnitine transport disorders and arginase deficiency. Conclusions: The rate of metabolic disease as detected by tandem mass-spectrometry is much higher than anticipated. Other programs have calculated incidences for these same disorders ranging from 1/4400 live births in Australia to 1/8000 live births in New England. This may be a statistical anomaly limited to the first year of screening, but it may be that with the inclusion of a second screen the detection rate is increased and the true disease incidence is higher than previously calculated.

Disclosure(s): None

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Natural history of infantile onset Pompe disease (IOPD): results from a retrospective chart review study. *D Corzo*[*1], W Hwu[2], H Mandel[3], M Nicolino[4], F Yong[1], P Kishnani[5]. [1]Genzyme Corporation, Cambridge, MA, [2]NationalTaiwan University Hospital, Taiwan, Taipei, [3]Rambam Medical Center, Haifa, Israel, [4]Hopital de Brousse, Lyon, France, [5]Duke University Medical Center, Durham, NC.*

Background. Pompe disease is an autosomal recessive disorder caused by a deficiency of acid alpha glucosidase (GAA). Reports on the natural history of IOPD are based on data from studies conducted in a relatively small number of patients. To fully characterize the disease, a retrospective chart review was conducted. **Methods.** This is a multinational, multicenter, historical cohort study. Inclusion required of onset of symptoms by 12 months of age and documentation of GAA deficieny or GAA mutation(s). Data collected from medical charts included demographics, family history, progression of signs and symptoms of the disease, diagnostic and other ancillary evaluations, and treatment modalities. **Results.** 300 cases were screened; 168 cases from 33 study sites in 9 countries met all eligibility criteria. 93 cases (55%) were born after 1995 and 75 cases (45%) were born before 1995. Median age +/- SD (months) at presentation of first symptoms was 2.0 +/- 2.5 (n=166); at diagnosis, 4.7 +/- 8.8 (n=165); at first ventilator use, 5.9 +/- 6.3 (n=165), and at death, 8.7 +/- 1.1 (n=163). By 12 months of age mortality was 78%. By 18 months of age mortality reached 88%. The most common sign/symptoms of the disease were cardiomegaly (92%, n=154), hypotonia (88%, n=148), cardiomyopathy (88%, n=147), respiratory distress (78%, n=131), failure to thrive (53%, n=89), congestive heart failure (50%, n=84), and pneumonia (45%, n=76). At least 20% of patients (n=49%) were ventilated. 90% (n=151) were treated with one or more medications, 77% (n=130) with nutritional support, and 93 (55%) with other supportive therapies. **Conclusions.** This is the largest retrospective case review study performed to date in IOPD. Results from this study support earlier literature reports with regard to the fatal course of the disease and the rapidity of the disease progression. In spite of widespread use of different therapeutic modalities mortality has changed little across the decades. Although a small group of patients survived past 12 months of age, IOPD is still a rapidly progressive and fatal disease, with the majority of patients dying before 12 months of age.

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BIOCHEMICAL GENETICS POSTERS

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A Fabry disease (FD) homozygote in the Nova Scotia kindred with a classic hemizygote phenotype. *S Dyack*[1,4], S Thanamayooran[2,4], K Lemoine[2], C Riddell[3,4], C Simms[2], M West[2,4]. [1]Dept. of Pediatrics, IWK Health Centre, Halifax, NS, Canada, [2]Dept. of Medicine, QEII Health Sciences Centre, Halifax, NS, Canada, [3]Dept. of Laboratory Medicine, IWK Health Centre, Halifax, NS, Canada, [4]Dalhousie University, Halifax, NS, Canada.*

Nova Scotia has a large FD kindred of uniform genotype (A143P) and classic phenotype. All kindred members are descended from a founder born in 1799 on Tancook Island, Nova Scotia and most live within 166 km of this site. We report here a homozygous FD female with a classic hemizygous phenotype. This 53-year-old woman was the offspring of a marriage not known to be consanguineous. Her father had FD with peripheral neuropathy and died of a cardiac event in his 40's. Her mother at age 72 was not clinically affected. Her brother however, was also affected with FD, indicating that her mother was a FD heterozygote. She had painful limb dysesthesia with fever and in hot weather by six years of age and was treated with diphenylhydantoin and later with carbamazepine. At age 48 she had recurrent paresthesias of her left face and arm. She had slight muscle wasting in her hands, decreased temperature sensation, decreased proprioception in her feet, and absent ankle reflexes. A CT scan of her brain was normal, however, a MRI was not performed. Opthalmologic exam revealed cornea verticillata and tortuous eye vessels. She had angiokeratomas of her skin. She was normotensive, had a grade 1/6 systolic murmur and an echocardiogram revealed concentric LVH with normal LV function. She had normal renal function. Her serum creatinine was 72 umol/l and 24 hr urine protein was 792 mg/d. Her urinalysis revealed: pH5, 1+ albumin; many lipid droplets, a few granular and fatty casts, and Maltese crosses under polarized light. She had occasional diarrhea. Laboratory testing confirmed the diagnosis of FD, with an α-galactosidase A plasma level of <1 nmol/h/ml and a leukocyte α-galactosidase A of 2 nmol/h/mg.protein (normal 13-38). Gb3 levels were not available. Molecular testing revealed homozygosity for the common Nova Scotia A143P mutation. In conclusion, we present an unusual case of a female with Fabry disease who is homozygous for the A143P mutation. She has a classic hemizygote phenotype characterized by peripheral neuropathy, cardiomyopathy and possible cerebrovascular disease. This case is unusual as this is an X-linked condition, and female homozygotes are rare. Because this kindred is in a small isolated community, there is a high frequency of the A143P mutation. As a result, her parents both carried this mutation, despite not knowing they were consanguineous. Enzyme replacement therapy with α-galactosidase A is likely to benefit this patient given her classic Fabry disease phenotype.

Disclosure(s): Presenting author has participated in a Genzyme-sponsored Natural History of Fabry Disease study and is involved with Genzyme's Fabry Registry.

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Weight based reference ranges for 17-hydroxyprogesterone in newborns. *DE Freer*[1], KC Donahue[1]. [1]Pediatrix Screening, Bridgeville, PA.*

Blood spots from over 60,000 consecutive newborns were assayed by sandwich immunoassay for 17 hydroxyprogesterone in a newborn screening program. Infants were grouped by weight in order to determine a weight-based reference range using non-parametric analysis. Since elevated 17 hydroxyprogesterone in a blood spot may indicate possible Congenital Adrenal Hyperplasia, it is instructive to determine expected values. The data presented below suggest that infant size must be considered when evaluating newborn blood spot results for 17 hydroxyprogesterone. This is becoming a more significant issue as the occurrence of premature births increases. Range of 17 OH Progesterone (observed 2 SD range)by Weight:

1.) 0.50 - 1.0 kg, range of 3-140 ng/nl (561 total samples)
2.) 1.01 – 1.5 kg, range of 3–105 ng/ml (1272 total samples)
3.) 1.51 - 2.0 kg, range of 2.5 - 109 ng/ml (1130 total samples)
4.) 2.01 -02.5 kg, range of 1.3 - 53 ng/ml (2713 total samples)
5.) 2.51 - 3.0 kg, range of 0.8 - 43 ng/ml (8754 total samples)
6.) >3.0 kg, range of 2.0 - 30 ng/ml (46044 total samples)

Disclosure(s): None

BIOCHEMICAL GENETICS POSTERS

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Deafness, onychodystrophy, osteodystrophy, mental retardation (DOOR syndrome) type I : another inborn error of energy metabolism. *J Hoffman*[1], R Deberardinis[1], E Zackai[2], P Kaplan[1]. [1]Children's Hospital of Philadelphia, PA, Division of Child Development, Rehabilitation, and Metabolic Diseases, Section of Metabolic Disease, [2]Division of Human Genetics and Molecular Biology, Section of Clinical Genetics.*

There is an increasing body of data that associates abnormalities in oxidative phosphorylation in utero with malformations and dysmorphic features. We report an infant with DOOR syndrome type I with further evidence that this is an inborn error of energy metabolism. A two month-old female from a non-consanguineous family in the Dominican Republic had unremitting seizures, absence of all finger- and toenails, depressed and slightly broad nasal bridge, long philtrum, and low-set ears. Radiologic exam showed absence or hypoplasia of all distal phalanges, described as brachydactyly type B. Echocardiogram revealed a secundum atrial septal defect and pulmonary valve stenosis. Hearing evaluation detected sensorineural deafness. Biochemical evaluation showed significantly elevated levels of urinary α keto glutarate (αKG): 8469 mg/g creatinine (normal 50-650), plasma acetylcarnitine was twice normal, propionylcarnitine was three times normal, plasma lactate: 4.3 mmol (normal <2mM), lactate/ pyruvate ratio 16 (normal), with intermittent elevations of plasma alanine and fumarate. Skin fibroblasts had normal activity of α KG dehydrogenase, pyruvate dehydrogenase, pyruvate carboxylase, phosphoenolpyruvate carboxykinase, and the electron transfer chain (ETC). The patient had a normal 46, XX karyotype, normal telomere analysis, and no detectable deletion by FISH in the 7p14-13 region (location of α KG dehydrogenase E1). The infant is treated with a "mitochondrial cocktail" of co-factors and anti-oxidant substances that have decreased her lactic acidemia but not improved her neurologic status; her seizures are partially controlled with anti-epileptic medications. The constellation of deafness, onchodystrophy, osteodystrophy, mental retardation and seizures with elevated urinary α KG has been described as both DOOR syndrome type I and Eronen Syndrome. Most cases have been seen in consanguineous families, and therefore autosomal recessive inheritance is most probable. Several DOOR type I patients have had defects in the E1 complex of the α KG dehydrogenase complex. Several children with similar dysmorphic findings, most specifically of the digits, have been found to have abnormal activity of ETC complex I or IV. The increased level of mitochondrial metabolites in this dysmorphic infant is additional evidence that abnormalities in oxidative phosphorylation in utero may influence developmental pathways of multiple systems, causing malformations and dysmorphism. DOOR syndrome in our patient is most likely due to mutations in a gene in a yet unidentified part of the mitochondrial pathway.

Disclosure(s): None

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Rhabdomyolysis in the military: two cases of late onset VLCAD deficiency in military men. *J Hoffman*[1], R Steiner[2], L Paradise[3], A Strauss[4], L Ding[4], M Deardorff[1], P Kaplan[1]. [1]Children's Hospital of Philadelphia,PA, Department of Pediatrics, Section of Metabolic Disease, [2]Departments of Pediatrics and Molecular and Medical Genetics, Oregon Health & Science University, [3]Department of Pediatrics, Oregon Health & Sciences University, [4]Vanderbilt Children's Hospital, Nashville, TN.*

There are many causes of rhabdomyolysis; the most common are muscle injury, inherited disorders of glycogen or fat metabolism, malignant hyperthermia, and muscular dystrophies. Very long chain acyl co-A dehydrogenase (VLCAD) deficiency, an inborn error of fat metabolism, is commonly thought of as a disease of infancy or early childhood with hypoglycemia and muscle weakness. However, several cases of later onset VLCAD have been reported. This report of 2 affected young men broadens the spectrum of the disorder, and demonstrates the fine line between vigorous, athletic men in good health versus severe decompensation. Patient 1 presented in the 3rd decade with multiple episodes of rhabdomyolysis, myoglobinuria, renal and cardiac dysfunction, from which he recovered. He was a drill sergeant in the Coast Guard, and tolerated the majority of his rigorous training and daily activities. The patient's acylcarnitine profile revealed elevations in C14:2, C14:1, and C12, consistent with VLCAD deficiency. Molecular analysis of the VLCAD gene revealed three mutations. The first was a previously described change in exon 3, G128A, resulting in an alteration from glycine to aspartic acid, which may be a rare normal variant. The second was a previously described deletion in exon 9 bp 830-2 of AGA resulting in an in frame deletion of lysine 238. The third was a unique change in exon 9 bp A790G, changing lysine 224 to glutamic acid. Family analysis is underway. Patient 2 presented in the 3rd decade with severe leg cramping and myoglobinuria. He required airlift rescue after these symptoms occurred during a vigorous mountain climb. He was an active member of the Marines and had completed basic training without difficulty. His acylcarnitine profile revealed elevations in C14:2, C14:1 and C14, consistent with VLCAD deficiency. Molecular analysis of the VLCAD gene revealed two missense mutations. The first, in exon 8, G694A, changed alanine 192 to threonine, which is unique. The second is G1388A, altering glycine 423 to glutamic acid. Due to the late presentation of disease in both of these athletic men, it is likely that their mutations result in protein with decreased function, rather than absence of protein. Despite the late presentation of disease in both of these fit, athletic young men, it is important to recognize the serious nature of late onset VLCAD deficiency. Possible outcomes such as renal and cardiac failure may be fatal if lifestyle is not modified.

Disclosure(s): None

BIOCHEMICAL GENETICS POSTERS

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Presentation and novel management of a patient with holocarboxylase synthetase deficiency. *S Josefsberg*[1], JA Thomas*[1], K Amos[1], J Van-Hove[1]. [1]Clinical Genetics and Metabolism, The Children's Hospital, Denver CO.*

We report a patient with holocarboxylase synthetase (HLCS) deficiency. HLCS catalyzes the biotinylation of the four human biotin dependent carboxylases. HLCS deficiency leads to multiple carboxylase deficiency, which is fatal in the absence of prompt diagnosis and treatment. The patient, a term female, was born to a 28-year-old G2, P2 mother by elective cesarean due to breech position after an uncomplicated pregnancy. Birth weight was 2580 g (25-50th%). Apgars were 8 and 9 (1 and 5 minutes respectively). Shortly after birth, the child presented acutely with significant respiratory distress and was noted to have taut, shiny skin. Chest x-ray showed a ground-glass picture with air bronchograms, compatible with respiratory distress syndrome secondary to surfactant deficiency. Laboratory evaluation demonstrated significant lactic acidosis. A head CT revealed bilateral ventricular enlargement. Results of an acylcarnitine profile and urine organic acid analysis showed increased propionil carnitine and c-5 hydroxy acylcarnitine, suggested the diagnosis of multiple carboxylase deficiency. The diagnosis was later confirmed by demonstrating low enzyme activity of propionyl-CoA carboxylase (PCC), 3-methylcrotonyl-CoA carboxylase (MCC), and pyruvate carboxylase (PC) in lymphocytes. Studies for HLCS enzyme activity and biotin responsiveness are pending. The child's initial treatment included high dose biotin (100 mg/d) and carnitine (300 mg/kg/day) as well as intravenous glucose infusion. The respiratory distress responded to surfactant therapy. We postulate that the surfactant deficiency could be related to the deficiency of acyl-CoA carboxylase. Glycine was added due to increased 3-hydroxyisovaleric evident on urine organic acid analysis. Aspartate, theoretically to increase production of oxaloacetate, was also supplemented. Citrate was utilized briefly to aid in the treatment of the lactic acidosis. The child was also begun on a branched-chain restricted diet to decrease precursors to MCC and PC. The child responded well to treatment with improvement of the acidosis and resolution of the respiratory distress. Interestingly, the skin, initially shiny and taut, quickly blistered and desquamated and later improved to appear normal. The child is currently treated with biotin, carnitine, and aspartate supplementation and a branched-chain restricted diet. Glycine was discontinued due to normalization of urine organic acids. She is presently growing well and developing appropriately for age.

Disclosure(s): None

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A method to induce immune tolerance and improve treatment with enzyme replacement therapy. *E Kakkis*[1,2], T Lester[1], R Yang[1], C Tanaka[1], V Anand[1], J Lemontt[2], M Passage[1]. [1]Dept. of Peds, Harbor-UCLA REI, Torrance, CA, [2]BioMarin Pharmaceutical Inc., USA.*

Significant immune responses can alter the safety and efficacy of protein therapies. We have developed a method to reduce the clinically significant antigen-specific immune response to a model antigen, recombinant human a-L-iduronidase (rhIDU) during treatment of canine mucopolysaccharidosis I (MPS I). The method uses an initial 60-day regimen of cyclosporin A (CsA) and azathioprine (Aza) to suppress an immune response initially while loading the host with weekly low dose intravenous infusions of rhIDU. Using the regimen, eight canines had a 20 fold lower antibody titer after 12 weekly infusions of rhIDU (the last 6 weeks after without CsA+Aza) and low titers did not increase with further rhIDU infusions up to 6 months and full therapeutic rhIDU doses. Studies demonstrated that one key factor determining success was a high serum trough level of CsA of preferably >400 ng/ml. Studies of the tolerizing antigen demonstrated that the high-affinity, mannose 6-phosphorylated (M6P) enzymes, rhIDU and a-glucosidase, can act as toleragens, whereas the protein ovalbumin could not. High affinity M6P markers appear essential since dephosphorylated rhIDU also did not allow induction of the tolerant state. Immune tolerant MPS I canines showed improved kidney enzyme levels, reduced glycosaminoglycan storage and improved microscopic appearance compared to non-tolerant canines. The immune tolerance method could be an important approach to reducing the effects of the immune response in the treatment of genetic deficiency disorders.

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Polysomnogram findings in patients with infantile Pompe disease. *P Kishnani*[1]*, J Koepke[1], J Mackey[1], S DeArmey[1], J Harris[1], R Kravitz[2]. [1]Division of Medical Genetics, Duke University, Durham, NC, [2]Division of Pediatric Pulmonary Disease, Duke University, Durham,NC.*

Pompe disease is caused by a deficiency of acid α-glucosidase. The infantile form presents in the first few months of life with hypotonia, generalized muscle weakness, and hypertrophic cardiomyopathy. This form is fatal, most patients succumb by age 1 year to cardiac and/or respiratory failure. Supportive care might prolong the child's lifespan by a few months, but is not associated with long term survival. Based on data from a natural history study of 168 patients with infantile Pompe disease, the mean age at first ventilator use was 7.0 ± 6.3 months (n= 49). Currently there is no FDA approved medical treatment for Pompe disease. Assessment of nocturnal ventilation is not routinely performed in these patients. Previous Phase II clinical trials using Chinese Hamster Ovary cell derived recombinant human acid alpha glucosidase (rhGAA) enzyme replacement therapy (ERT) have been conducted; results are encouraging. Given the potential for a treatment, optimal respiratory management is essential to improve outcome. To date, no prospective study has systematically examined the natural course of sleep disordered breathing in infantile Pompe disease. We present data obtained from 4 patients (mean age 7 months) enrolled in 2 ongoing open label studies of rhGAA every 2 weeks. At baseline, the 2 older patients demonstrated some degree of hypoventilation at ages 9 and 8 months, respectively. This pathology would not have been suspected based on history.

Patient	Age	Snoring	Apnea	AHI*	EtCO2 (awake)	EtCO2 (NREM)	EtCO2 (REM)
A	9 mo	No	No	2.6	47	51	NA
B	8 mo	No	No	0	46	46	45
C	6 mo	Unsure	Mild	0	30	36	36
D	5 mo	No	No	0	36	38	39

*apnea-hypopnea index (AHI)

Patient A presented with an elevated EtCO2 and obstructive sleep apnea which responded to Bi-level therapy. Patient B had milder hypoventilation which worsened and required Bi-level therapy. Compared to historical norms both would have required tracheostomy and mechanical ventilation by their second polysomnogram.

Patient	Age	Support	AHI	EtCO2 (awake)	EtCO2 (NREM)	EtCO2 (REM)
A-1	9 mo	1/8 lpm O2	2.6	47	51	NA
A-2	13 mo	1/8 lpm O2+BI*+	0.2	47	48	51
B-1	8 mo	RA	0	46	46	45
B-2	11 mo	1/8 lpm O2	0.2	49	54	61

*Bi-level

We report hypoventilation as a complication of infantile onset Pompe disease which presents before symptoms develop and is responsive to Bi-level therapy. In our series, hypoventilation was not seen prior to age 6 months. In 2 patients in whom F/U data is available, the expected worsening of sleep disordered breathing requiring ventilator use when compared to historic controls was not seen. With the advent of ERT as a treatment for Pompe disease, obtaining a polysomnogram at the time of diagnosis may prove useful in assessing and treating sleep disordered breathing before symptoms are present. More data, including longitudinal studies, are warranted to confirm these findings.

Disclosure(s): Genzyme has provided honoraria and/or travel support for participation in this presentation.

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Long-term follow-up of early and well-treated nephropathic cystinosis patients. *R Kleta*[1,2]*, WS Varade[3], I Bernardini[1], M Ueda[1], C Phornphutkul[1], D Krasnewich[1], WA Gahl[1,2]. [1]Section on Human Biochemical Genetics, MGB, NHGRI, NIH (DHHS), Bethesda, MD, [2]Office of Rare Diseases, Intramural Program, Office of the Director, NIH (DHHS), Bethesda, MD, [3]Department of Pediatrics, Golisano Children's Hospital at Strong, University of Rochester Medical Center, Rochester, NY.*

Cystinosis is an autosomal recessive inborn error of metabolism with intralysosomal cystine accumulation when untreated leads to glomerular kidney failure within the first decade of life and other nonrenal complications. The gene CTNS, mutated in cystinosis, codes for the protein cystinosin, which contains 367 amino acids and transports cystine out of lysosomes. A free thiol drug, cysteamine, depletes lysosomes of cystine, retards glomerular deterioration, and enhances somatic growth if begun in the first 2 years of life. Cysteamine is given orally every 6 hours at doses of 1.3-1.95 g/m2 of free base or 60-90 mg/kg per day. Here we report the clinical outcomes of two siblings treated with cysteamine from the ages of 20 months (Pt. #1) and 2 weeks (Pt. #2). The siblings were compound heterozygous for the common 57 kb deletion involving CTNS and for a missense mutation in exon 12, c1015G>A (1354G>A), G339R. Both siblings complied well with oral cysteamine therapy. The leucocyte cystine value in Pt. #1 was 10.3 nmol half-cystine/mg protein (normal, < 0.2) at diagnosis; under treatment with cysteamine (35 mg/kg day at age 14 years), it was 0.5. Pt. #2 had a leucocyte cystine of 9.7 nmol half-cystine/mg protein at diagnosis and 0.3 on cysteamine (60 mg/kg day at age 8 years). Leucocyte cystines, checked yearly have reflected compliance with cysteamine therapy. Neither child received growth hormone. Pt. #1 is now 164 cm (25th percentile); Pt. #2 is 144 cm (90th percentile). The glomerular filtration rates, based upon 24 hour urine collections, are 78 and 105 ml/min 1.73m2, respectively. The patients' urines show signs of renal Fanconi syndrome, but both children have normal thyroid function. These cases illustrate the excellent clinical outcome possible for nephropathic cystinosis patients treated early and diligently. Early diagnosis is critical.

Disclosure(s): None

BIOCHEMICAL GENETICS POSTERS

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Intermediate phenotype between type II and type III Gaucher disease. *V Krishnamurthy*[1], M McDonald[1], R Hanna[1], J Mackay[1], P Kishnani[1]. [1]Duke University Medical Center,NC.*

Gaucher Disease (GD) is caused by a deficiency of the lysosomal enzyme glucocerebrosidase resulting in glucocerebroside accumulation in the reticuloendothelial system. Neuronopathic GD, classically divided into two subtypes, can have a continuum of phenotypes, thus defying categorization.Patients with GD type 2 have a rapidly progressive neurologic course and a dismal prognosis, the average age of death is age 9 months. In contrast patients with GD type 3 have more slowly progressive neurologic disease with variable age of presentation ranging from infancy to adulthood in rare instances. We report 2 patients with GD who presented after age 1 year with progressive neurological disease, and survival beyond 2 years. These patients did not fulfill the classical definitions of either Type 2 or 3 GD but rather resembled an intermediate phenotype recently described by Goker-Alpan (Goker-Alpan et. al, 2003). Patient 1 was diagnosed at age 16 months This Hispanic male had hepatosplenomegaly, failure to thrive,diffuse-interstitial lung disease, and horizontal supranuclear gaze palsy at presentation. Brainstem auditory evoked potentials were normal. The patient had pooling of secretions-, videofluoroscopic swallowing studies showed an infantile suck and swallow pattern.EEG and MRI brain were normal The patient was initially treated with IV Cerezyme® at 60 U/kg and subsequently increased to 120 U/kg every 2 weeks, based on rapid disease progression. At age 33 months the patient continues to have supranuclear gaze palsy, choking episodes and has developed an unsteady gait. He remains seizure free. Patient 2 presented at age 13 months with developmental delay, bilateral esotropia and supranuclear gaze palsy, choking episodes, dysphagia, and hypertonia of the lower extremities. This African American female demonstrated normal brainstem auditory evoked potentials. Video fluoroscopic swallowing studies showed oral motor delay with risk for aspiration. The patient was treated with 60 U/kg Cerezyme®. At age 30 months the patient has severe neurological disease (discordant gaze, unsteady gait, repeated episodes of aspiration pneumonia). She remains seizure free. Genetics: Both patients are homozygous for the L444P mutation known to be associated with Type 3 disease.Both, have a phenotype intermediate to Type 2 and 3. Conclusion: This intermediate phenotype highlights that neuronopathic GD is a continuous spectrum. It also stresses the importance of clinical evaluation as a predictor of outcome. These patients serve as an example to the challenges of distinguishing between Type 2 and Type 3, counseling issues, prognosis and therapeutic options for patients with neuronopathic presentation of GD.

Disclosure(s): None

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First trimester levels of maternal serum hCG and inhibin A in pregnancies affected by fetal trisomy 18: a faster trial study. *G Lambert-Messerlian*[1], J Canick[1], F Malone[2], F Porter[3], D Nyberg[4], C Comstock[5], G Saade[6], R Berkowitz[7], S Gross[8], L Dugoff[9], S Craigo[10], I Timor[11], S Carr[1], H Wolfe[12], M D'Alton[2]. [1]Women and Infants Hospital and Brown Medical School, Providence, RI, [2]Columbia-Presbyterian Medical Center, New York, NY, [3]LDS Hospital, Salt Lake City, UT, [4]Swedish Medical Center, Seattle, WA, [5]William Beaumont Hospital, Royal Oak, MI, [6]University of Texas Medical Branch, Galveston, TX, [7]Mt. Sinai Medical Center, New York, NY, [8]Montefiore Medical Center, Bronx, NY, [9]University of Colorado Health Science Center, Denver, CO, [10]New England Medical Center, Boston, MA, [11]New York University Medical Center, New York, NY, [12]University of North Carolina, Chapel Hill, NC.*

Background Second trimester levels of three maternal serum markers used in Down syndrome screening, alpha-fetoprotein (AFP), unconjugated estriol (uE3), and total human chorionic gonadotropin (hCG), all tend to be low in pregnancies affected by trisomy 18. That pattern serves as the basis of very effective risk assessment for trisomy 18. In contrast, levels of inhibin A, a fourth Down syndrome screening marker used in the second trimester, are not significantly altered in pregnancies with trisomy 18. During the late first trimester, levels of two markers effective in screening for Down syndrome, pregnancy associated plasma protein-A (PAPP-A) and free beta-hCG, have been found to low in trisomy 18 pregnancies. It is not yet known whether first trimester levels of other maternal serum markers are also low in trisomy 18 pregnancies. Objective The goal of the current study was to determine whether maternal serum levels of hCG and inhibin A, measured in the late first trimester, are abnormal in pregnancies affected by trisomy 18. Methods Fourteen cases of trisomy 18 pregnancy were identified as part of the NIH-funded, multi-center FASTER (First and Second Trimester Evaluation of Risk) Trial that compared first and second trimester Down syndrome screening tests in the same women. Each case sample was matched to five control samples for same collection center, completed week of gestation at sample collection (11-13 weeks), and duration of freezer storage (+/- 3 months). Samples were thawed and assayed for hCG (Immulite, Diagnostic Products Corp., Los Angeles, CA) and inhibin A (Active ELISA, Diagnostic Systems Laboratories, Webster, TX) without knowledge of sample identity. Gestation-specific median levels of hCG and inhibin A in control pregnancies were calculated and all results were then normalized to the multiple of the median (MoM). Results First trimester maternal serum levels of hCG were markedly reduced (median = 0.30 MoM) in cases of trisomy 18, with a trend toward a greater reduction later rather than earlier in the window of testing (median at 11 weeks = 0.36 MoM; at 12 weeks = 0.24 MoM; at 13 weeks = 0.23 MoM). First trimester levels of inhibin A in cases of trisomy 18 were more modestly reduced (median = 0.77 MoM). The observed univariate detection rate at a fixed 5% false positive rate was 93% for hCG and 43% for inhibin A. Conclusion If they are being used in Down syndrome screening in the late first trimester, hCG and perhaps inhibin A can be used in the assessment of risk of trisomy 18 pregnancy.

Disclosure(s): Presenting author is a paid consultant to Diagnostic Systems Labs, which has sponsored research activities relevant to this presentation.

BIOCHEMICAL GENETICS POSTERS

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Dimeric inhibin-A as a marker for Down syndrome in the first trimester. *G Lambert-Messerlian*[*1], *G Palomaki*[2], *G Knight*[2], *L Neveux*[2], *J Canick*[1], *J Haddow*[2]. [1]*Women and Infants Hospital and Brown University Medical School, Providence, RI,* [2]*Foundation for Blood Research, Scarborough, ME.*

Background Maternal serum dimeric inhibin-A is an important addition to second trimester prenatal screening, resulting in significantly improved Down syndrome test performance. A substantial effort has been made to move screening to the late first trimester, using maternal serum levels of pregnancy-associated plasma protein-A (PAPP-A) and free beta (or total) hCG in combination with ultrasound measurement of nuchal translucency (NT). Objective The aim of the current study was to examine the performance of dimeric inhibin-A as a first trimester marker of Down syndrome. Methods Dimeric inhibin-A was measured using an ELISA (Diagnostic Systems Laboratories, Webster, Texas) in serum samples from 51 cases of Down syndrome and 241 matched control pregnancies (9 to 14 weeks gestation) collected as part of an NIH-funded prospective, non-intervention trial (Haddow JE et al., N Engl J Med 1998;338:955-61). Inter- and intra-assay coefficients of variation were less than 15%, and the assay sensitivity was 10 pg/mL. Measurements were done in Rhode Island without knowledge of karyotype. Results Among control pregnancies, maternal serum dimeric inhibin-A decreased by 12% per week. All results were converted to multiples of the median (MoM) and subsequently adjusted for maternal weight. The overall median dimeric inhibin-A value in cases was 1.52 MoM with 20% of cases (10/51) over the 95th centile of controls (2.55 MoM). The performance varied by gestational age with more pronounced separation occurring as gestational age increased. Conclusions Given that the performance of all first trimester markers (including NT measurement) appear to be dependent on gestational age, screening algorithms need to be appropriately designed. Overall, measurements of dimeric inhibin A improve Down syndrome screening in the first trimester.

Disclosure(s): Presenting author is a paid consultant to Diagnostic Systems Labs, which has sponsored research activities relevant to this presentation.

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False-positives in plasma ammonia measurement in a tertiary care pediatric hospital and their clinical impact. *B Maranda*[*1], *M Lambert*[1], *J Cousineau*[2]. [1]*Genetics Service, Hopital Ste-Justine, Universite de Montreal, Montreal, Canada,* [2]*Biochemistry department, Hopital Ste-Justine, Montreal, Canada.*

Plasma ammonia measurement is critical for the diagnosis and management of several inborn errors of metabolism, especially urea cycle defects. Its determination is subject to several sources of error both pre-analytical (collection, handling and storage of samples) and analytical. False-positive results might be harmful to patients by generating additional blood sampling and analyses, longer hospitalization and special diet. We wanted to evaluate the prevalence and clinical impact of false-positive results for plasma ammonia in a pediatric tertiary care hospital. All data from the Clinical Biochemistry Laboratory were scrutinized over a 28-month period. 1980 ammonia measurements were obtained from 479 patients. Ammonia concentration ranged from 5 to 1863 µmol/L (median; 57 µmol/L). Median values were significantly different between neonates (less than 1 month) and older patients, with a median of 65 and 50 µmol/L respectively ($p<0,001$). Abnormal results were found in 42% blood samples from 151 patients, which represents 32% of patients tested. Hospitalized patients accounted for 81% of the abnormal values and 82% were from non-neonates or older patients. To identify false-positive results we used the following algorithm. Each cases that presented at least one abnormal result was revised unless it was from a patient known for an inborn error of metabolism (10 subjects) or we had only elevated results available on that subject (63 subjects; median ammonia concentration: 81 µmol/L). Using this algorithm, 78 patient files were identified (52%). These files will be reviewed to determine if a medical treatment or condition could account for the initial elevation of ammonia and its subsequent normalization. Cases without satisfactory explanation for high plasma ammonia will be considered as false positive results. Clinical impact of these false-positives will be ascertained by chart review: supplemental blood draw and subsequent analyses, prolonged hospital stay, follow-up visits and special diet. Additional data will be presented at the conference.

Disclosure(s): None

BIOCHEMICAL GENETICS POSTERS

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Pompe disease in Rio de Janeiro – Brazil. *M Oliveira*[1], A Bernstein[1], C Santos[1], P Correia[2], J Llerena[3]. [1]Laboratory of Inborn Erros of Metabolism (LABEIM), Department of Biochemistry, Institute of Chemistry,UFRJ, [2]Hospital Geral de Bonsucesso, Rio de Janeiro, Brazil, [3]Institute Fernandes Figueira (IFF), Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.*

Pompe disease (Glycogen storage disease type II) is an autosomal recessive disorder of glycogen metabolism, resulting from deficient activity of the lysosomal acid alfa-glucosidase. The classic Pompe disease is the most frequently reported in infancy. It presents within the first months of life with cardiomegaly, hypotonia and rapidly progressive weakness. This deficiency results in loss of functional tissue in heart and skeletal muscle. Feeding difficulties, respiratory distress and motor delay are the presenting symptoms in this period. The characteristic increased heart size, due to the presence of a hypertrophic cardiomyopathy often raises suspicion of the diagnosis. This work reports clinical and biochemical findings of 9 cases of Pompe disease in children from Rio de Janeiro and was performed in collaboration between two research institutions - IFF and LABEIM. The patients urine samples were evaluated by screening tests for lysosomal storage disease (LSD) and thin layer chromatography for oligosaccharides and sialyloligosaccharides. In 8 patients cardiomyopathy was the foremost feature and in 7, it was associated with hypotonia. All patients showed symptoms during the first 6 months and 5 of them, in the neonatal period. Their condition rapidly worsened and all died of intractable heart failure. Although urinary oligosaccharide analysis are not sufficiently specific to establish the diagnosis, all the patients described in this work presented with abnormal pattern, suggesting a glycogenosis. Enzyme analysis in fibroblasts of 5 patients demonstrated reduced alfa-glucosidase activity. The clinical findings of all these children and the biochemical results are consistent with the diagnosis of Pompe disease. Although cardiomyopathy in the newborn period is considered a rare presentation of LSD, it should not be overlooked in Pompe disease, as observed in this study. Proper genetic counseling and prenatal diagnosis were offered to the families. In near future new patients will be able to be treated by enzyme replacement therapy.

Disclosure(s): None

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Familial presentations of short-chain l-3-hydroxyacyl CoA dehydrogenase (SCHAD) deficiency. *S Palmer*[1], J Heim-Hall[2], R Taylor[2], T Hayes[3], R Boriack[4], M Bennett[4]. [1]The University of Oklahoma Health Sciences Center, Oklahoma City, [2]The University of Texas Health Science Center at San Antonio, [3]Methodist Children's Hospital of South Texas, San Antonio, [4]The University of Texas Southwestern, Dallas.*

Short-Chain L-3-Hydroxyacyl CoA Dehydrogenase (SCHAD) Deficiency is a severe defect of short-chain fatty acid oxidation. A small number of isolated cases with early childhood lethality have been reported. Two sib pairs are presented in this report. FAMILY 1: A 4 mo. old Mexican male with a 3-day history of viral symptoms and poor oral feedings expired of arrhythmia and multiple organ failure within an hour of ICU transport. Autopsy revealed cardiomegaly and hepatic steatosis. SCHAD analysis of liver was 4% of control, while cardiac muscle activity was 75% of control. The family did not return for education and counseling of these results. Several years later a 40-day-old brother presented in a terminal state with cardiovascular shock and an almost identical history, clinical course, and autopsy result as his sibling. FAMILY 2: A 5 month-old Caucasian male born to consanguineous parents presented with a 12-hour history of mild respiratory and GI symptoms with decreased caloric intake. Due to ill appearance he was hospitalized but deteriorated with fatal arrhythmia within 30 hours. Autopsy revealed cardiomegaly and hepatic steatosis. He was presumed to have MCAD deficiency based on histology and history; the parents did not recall being referred to a metabolic geneticist, or receiving any information concerning precautions or treatment of future cases. Several years later a 5 month-old sister was evaluated for an 8-hour history of mild viral symptoms and poor oral intake. A mild arrhythmia progressed to a fatal rhythm within 12 hours. Autopsy revealed dilated heart and hepatic steatosis. Liver SCHAD activity was 14% of normal, while heart, skeletal muscle, and fibroblasts showed 58%, 32%, and 66% activity of normal, respectively. Fibroblast fatty acid oxidation flux using palmitic and myrisitic acids was normal, indicating normal long- and medium-chain enzyme activities. DISCUSSION: These 2 families showed very similar phenotypes, especially within each sib pair. These features can be shared by other fatty acid oxidation disorders, but those presentations may be more variable, even within families. The first family was indigent and itinerant with very poor access to medical care. The second family was middle-class with allied health training, and ready access to tertiary medical care, which still did not prevent the fatalities. Due to their rapidly fatal presentations, and the parents' lack (or perceived lack) of disease education, no opportunity for prevention or early intervention of these crises was available. It is therefore unknown if the usual precautions and treatment for fatty acid oxidation disorders could have altered the course of the second child in each family. The tissue-specific SCHAD activity variation and normal fibroblast oxidation studies would be expected in liver-specific SCHAD deficiency.

Disclosure(s): None

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Malonic aciduria presenting as dilated cardiomyopathy in the neonatal period. *L Sapiegiene*[*1], *C Jones*[1], *F Cetta*[2], *M Weiss*[1], *C Sajous*[1]. [1]*Loyola Medical Center,* [2]*Mayo Clinic.*

We present a patient with neonatal cardiomyopathy. He was later diagnosed with Malonic aciduria. R.L was a full term baby boy was born via vaginal delivery. He required resuscitation in the delivery room which included chest compressions. Apgar scores were 4 and 8, at 1 and 5 minutes respectively. An echocardiogram revealed moderate to severe left ventricular dysfunction with regional wall motion anomalies, and dysplastic tricuspid valve with regurgitation. A metabolic workup was initiated. Many of the analytes were found to be abnormal. The Carnitine total: 1.7 uM/dL (normal range 2.6-8.1), free carnitine: 1.2 uM/dL (normal range 2.3-7.0), carnitine esters: 0.5 uM/dL (normal range 0.0-1.9). Urine organic acids indicated marked elevation of malonic acid, with modest elevation of methylmalonic acid. The repeat testing of urine organic acids at another reference laboratory revealed the same results. The baby was diagnosed with malonic aciduria. Carnitine supplementation was begun. Additional carbohydrates (polycose)were added to the diet to prevent hypoglycemia. The infant was discharged on day fifteen of life in stable condition. The infants cardiomyopathy resolved within 6 months. At 8 months he presented to the emergency room with a febrile seizure. His development was assessed at 12 months of age using the Revised Developmental Screening Inventory-1980. He was noted to have global delays in adaptive, gross motor, fine motor, language and personal/social skills. He functioned at 6-8 months level in all categories. Neonatal cardiomyopathy has frequently been described in infants of diabetic mothers. Other etiologies include infection, rhythm disturbances, and metabolic diseases. Malonic aciduria is a rare metabolic disorder. Most babies present with acidosis or seizures at a few months of age. Cardiomyopathy has been described with this disorder but usually later in infancy. Malonic aciduria is a very rare metabolic disorder and almost never presents in neonatal period. The majority of patients with malonic aciduria come to medical attention after an acute illness with development of coma. Other clinical features include developmental delay, vomiting, seizures, ketoacidosis, hypoglycemia and cardiomyopathy. This is the first case of an infant presenting with cardiomyopathy in the neonatal period as the initial sign of Malonic aciduria. The infant has had a seizure as well as developmental delay without any clinical indication of hyoglycemia or acidosis.

Disclosure(s): None

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Mental development in patients with infantile onset Pompe disease (IOPD) treated with enzyme replacement therapy (ERT). *A Skrinar*[*1], *D Corzo*[1], *P Kishnani*[2]. [1]*Genzyme Corporation, Cambridge, MA,* [2]*Duke University Medical Center, Durham, NC.*

Background. Pompe disease is an autosomal recessive disorder caused by a deficiency of the lysosomal enzyme acid alpha glucosidase (GAA). Assessment of mental development is an important component of evaluating the response to ERT in IOPD. **Methods.** An open-label study of eight patients with IOPD is ongoing. A summary of clinical efficacy results is presented. Preliminary results of mental development evaluations conducted using the Bayley Scale of Infant Development, 2nd edition (BSID-II) are discussed. **Results.** Mean duration of treatment for all patients is 20.4 months (range 3.8-30.5 months). Three patients are currently alive (at 35.1, 31.8, and 30.3 months of age, respectively). The three surviving patients have demonstrated marked improvement in cardiomyopathy, require no ventilatory assistance, are currently ambulatory, and fed by mouth. Five patients are deceased. Deaths were attributed to disease progression and were unrelated to rhGAA. Mean age at death was 23 months (range 14.7-33.8 months). At baseline, mean chronological age for all patients was 6.0 months (range 2.5-14.6 months) with a mean BSID-II mental performance age equivalent of 4.5 months (range <1-14 months). After a mean duration of treatment of 15.2 months (range 3.2-25.2 months), the mean chronological age was 20.1 months (range 8.2-29.5 months) with a mean BSID-II mental performance age equivalent of 15.6 months (range 7-23 months). Improvement in raw scores from baseline was observed in all patients indicating the continued acquisition of cognitive, language and personal/social development skills. The changes BSID-II raw scores corresponded to increases in mental performance age equivalents. In spite of these gains, normalized standard scores of mental performance (BSID-II mental development index, MDI) indicate that IOPD patients were not functioning at the same level of age-matched normally developing peers. **Conclusions.** In patients with IOPD treated with rhGAA, an increase in BSID-II raw scores corresponded to gains in mental performance age equivalents. This finding indicates a continuous acquisition of new skills, although the improvement does not place these patients at the same functional level of age-matched normally developing children. The increasing complexity of items involving integrated oral-motor and fine-motor skills required for the administration of BSID-II to older children may contribute to the lag in MDI scores between normally developing children and patients with IOPD treated with ERT over time. There is a limited value in comparing IOPD patients and normally developing children with MDI scores alone. However, progress in the acquisition of new skills can be effectively monitored by evaluating changes in raw scores and mental performance age equivalents.

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BIOCHEMICAL GENETICS POSTERS

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Isolated sulfite oxidase deficiency – report of a case in a newborn and review of the literature. *W Tan*[*1], *F Eichler*[2], *S Hoda*[3], *M Lee*[4], *P Grant*[5], *K Krishnamoorthy*[2], *V Shih*[3]. [1]*Harvard Medical School Genetics Training Program, Boston, MA,* [2]*Pediatric Neurology Unit, Massachusetts General Hospital, Boston, MA,* [3]*Department of Neurology, Massachusetts General Hospital, Boston, MA,* [4]*Harvard Combined Medicine/Pediatrics Program, Harvard Medical School, Boston, MA,* [5]*Division of Neuroradiology, Massachusetts General Hospital, Boston, MA.*

Sulfite oxidase is an enzyme that catalyzes the oxidation of toxic sulfites to non-toxic sulfates in the final step of sulfur amino acids catabolism. Clinically, it can be difficult to distinguish isolated sulfite oxidase deficiency [ISOD], a rare disorder with only twenty reported cases, from the commoner molybdenum cofactor deficiency. In both disorders, patients often present with intractable seizures, usually in the immediate neonatal period, alterations in muscle tone and severe neurodevelopmental delay. We report a full-term newborn baby boy who presented with respiratory distress and poor feeding soon after birth. A high-pitched cry and episodes of opisthotonic posturing were noted. On his third day of life, he developed generalized tonic-clonic seizures that evolved into refractory sub-clinical seizures after treatment with phenobarbital and fosphenytoin. Laboratory investigations were significant for the presence of urinary sulfites, elevation of urinary thiosulfate and sulfocysteine, low plasma homocysteine levels, absence of plasma cystine and normal serum uric acid, all of which were consistent with isolated sulfite oxidase deficiency. Of note, this is the only disorder in which plasma homocysteine levels are depressed. An EEG showed diffuse bilateral epileptiform discharges on a background of burst suppression pattern. Subsequent magnetic resonance imaging of his brain with spectroscopy (MRI/S) showed changes that resembled those seen in perinatal hypoxic-ischemic encephalopathy. He was started on a low methionine and low cysteine diet using a mixture of methionine and cysteine-free formula with regular infant formula. As sulfites have previously been shown to destroy thiamine in animal studies, he is receiving thiamine supplementation. In addition, he is receiving dextromethorphan to help antagonize sulfocysteine toxicity at the NMDA receptor. At four months of age, he is hypertonic with opisthotonus, and has severe developmental delay. However, he is still able to feed orally. He is maintaining his linear growth, but his head circumference is falling off the growth chart. He has not had any convulsions since two weeks of life. We reviewed the clinical and biochemical features in all nineteen cases of isolated sulfite oxidase deficiency published in English. Clinical improvement with dietary therapy was seen in only two patients, both of whom presented after the age of six months. Among the six cases in which mutations in the SUOX gene were found, mutations leading to a prematurely truncated protein appeared to result in more severe phenotypes with early deaths than those mutations resulting in amino acid substitutions.

Disclosure(s): None

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Evidence of somatic mosaicism in the maternal grandfather of a male with Hunter syndrome (MPSII). *T Wood*[*1], *K Wilman*[2], *J Thompson*[3], *J Jones*[1], *M Friez*[1]. [1]*Greenwood Genetic Center, Greenwood SC,* [2]*Capitola, CA,* [3]*Department of Pediatrics, University of Alabama at Birmingham, Birmingham, AL.*

Hunter syndrome (Mucopolysaccharidosis type II) is an X-linked lysosomal storage disease. Patients have a profound deficiency of iduronate 2-sulfatase (IDS) which results in the accumulation of heparan sulfate and dermatan sulfate in various tissues. Severely affected children are noted to have neurological degeneration, dysotosis multiplex, hepatosplenomegaly, hearing loss, and joint contractures. While enzyme studies are normally used to diagnosis the condition, carrier status in at risk females is best determined by molecular analysis. The IDS gene is located at Xq28 and molecular studies have demonstrated that the majority of the gene changes are point mutations within exons while a minority are large deletions, insertions or intragenic rearrangements with a pseudogene, IDS-2, located 20kb telomeric to IDS. We report on a family in which the affected male was found to have a complete deficiency of iduronate-2-sulfatase enzyme activity and contained an S132W mutation. This amino acid change has been previously identified in a Hunter syndrome patient from a separate family. Subsequent carrier testing in the mother and maternal aunt demonstrated both were carriers of S132W. Analysis of the maternal grandparents showed the maternal grandmother to be normal. However, the maternal grandfather was found to carry both the normal and mutant S132W allele. A repeat analysis showed similar results and cytogenetic studies showed a normal XY karyotype. Haplotype analysis showed that the X chromosome in the affected male was inherited from his maternal grandfather. Two maternal great aunts and two maternal great uncles were found to be negative for the mutation. A haplotype was shared between one maternal great uncle and the maternal grandfather suggesting the maternal grandfather did not inherit the S132W change. Iduronate-2-sulfatase enzyme studies in plasma from the maternal grandfather demonstrated low normal activity. Further microsatellite studies did not show evidence of chimerism. Relative to his family, the maternal grandfather is short and has mild coarsing of his face. His intelligence and mobility are normal. We feel this is the first reported case of somatic mosaicism in a maternal grandfather for a pathogenic change in IDS. Whether this change occurred early in somatic development or represents a reversion of the mutant allele back to normal is difficult to ascertain. These results may provide a mechanism for the increased frequency of carrier mothers as would be expected for an often lethal X linked condition. Similarly, lack of serious somatic and neurological features in this individual provides further evidence that early treatment of Hunter patients can facilitate a normal phenotype.

Disclosure(s): None

CLINICAL GENETICS POSTERS

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Two rare VHL mutations presenting only as bilateral pheochromocytoma in children.

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Pheochromocytoma is a rare tumor in children, and bilateral disease is particularly unusual. This adrenal tumor usually is sporadic, but can be the manifestation of a cancer susceptibility syndrome, such as von Hippel-Lindau disease (VHL), Multiple Endocrine Neoplasia type 2, or familial paraganglioma syndrome. We present 2 unrelated cases of bilateral pheochromocytoma in children, each associated with a germline mutation in VHL. Manifestations of VHL include hemangioblastomas of the retina and CNS, renal cysts and carcinoma, pheochromocytoma, and other tumors. Different VHL kindreds display different risks of developing particular tumors, so a clinical classification system has developed. Individuals with VHL type 1 have susceptibility to characteristic VHL tumors but not pheochromocytoma, whereas individuals with VHL type 2 are at risk for developing pheochromocytoma. In subtype 2A renal cell carcinoma is rare, in subtype 2B the risk for this cancer is high, and in families with subtype 2C affected individuals manifest only pheochromocytoma. Case 1: At age 4, this now 12 year old child developed nightsweats, headache, hypertension and weightloss. A pheochromocytoma was discovered and removed. At age 6 similar symptoms developed and a contralateral pheochromocytoma was found and removed. His father and sister each had bilateral pheochromocytoma in childhood. A germline missense mutation in VHL was found in this child, a G to T base change at nucleotide 463 (V84L). This mutation has been described once before, in a smaller pedigree that manifested only pheochromocytomas (Crossey, et al. (1995). Journal of Medical Genetics. 32(11): 885-6). Case 2: A 12 year old child with headaches was found to be diaphoretic, tachycardic, and hypertensive by the school nurse. Emergent inpatient evaluation revealed bilateral pheochromocytoma. A germline missense mutation in VHL not shared by his parents was found, a C to G change at nucleotide 703 (Q164E). This mutation has not been described previously. Notably, despite the presence of a germline VHL mutation, all four of our patients have remained free of other VHL manifestations. Using genotype to predict VHL phenotype remains complex. Missense mutations are usually associated with type 2, whereas other types of mutations (truncating, null, frameshift) manifest as type 1; a few mutations correlate with specific VHL subtypes. Currently we lack the ability to use genotype to predict the restricted phenotype of VHL 2C, so standard VHL tumor surveillance must be recommended for children with pheochromocytoma due to germline VHL mutations. Further collection and clarification of genotype-phenotype correlations will ultimately lead to a better understanding of the molecular pathophysiology of this complex neoplasia syndrome, and allow for refined prognostic and screening recommendations for VHL-affected individuals.

Disclosure(s): None

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Sex reversal in a patient with partial monosomy of 9p.

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Introduction: Partial deletion of 9p was described since 1973 and more than 80 cases have been reported. It manifests with typical clinical features including trigonocephaly; upward slanting palpebral fissures; short, broad and webbed neck; flat nasal bridge;anteverted nostrils and long philtrum. Abnormal genitalia have been reported in approximately 70% of 46,XY 9p deleted patients and a number of reports describe 46,XY partial or complete gonadal dysgenesis associated with deletions of 9p. Case report:We describe a newborn, first pregnancy of a 23 year old mother with epilepsy of unknown origin since childhood treated during pregnancy with valproic acid. The father was a 24 year old, healthy man.The baby was born by a C-section with normal amniotic fluid, placenta and three vessels in the umbilical cord.At birth she had a weight of 2,570 grams and was 47.5cm long. At physical examination she was hypotrophic, normocephalic with a wide anterior fontanelle, oblique palpebral fissures, ocular hypertelorism, broad nasal bridge, bilateral cleft lip and palate and small and low set ears. Her neck was broad and short. Her genitalia were female. She had camptodactyly of both hands and right postaxial polydactyly. Both feet had anomalous position of fingers.The echocardiogram showed severe bivalvular insufficiency and the renal sonogram reported multiple renal cysts. The karyotype showed: 46,XY,der(9)t(6;9)(q15;p22) mat.FISH studies demonstrated the presence of SRY. Conclusion: Multiple congenital anomalies have been associated with unbalanced structural chromosomal anomalies. The clinical phenotype presented by this patient was a combination of the partial monosomy of 9p and partial trisomy of 6q.Sex reversal has been reported in patients with partial monosomy 9p and two candidate genes (DMTR-1 and 2) have been found in 9p24.3. This region contains at least five genes. Sex determination in humans is a complex mechanism which requires the presence and function of many autosomal and sex chromosomal genes.

Disclosure(s): None

CLINICAL GENETICS POSTERS

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A unique autosomal dominant type II distal arthrogryposis with congenital heart disease and cryptorchidism. *J Al-Aama*[*1], *S Nikkel*[1]. [1]*Department of Genetics, Children's Hospital of Eastern Ontario, Ottawa, Ontario, Canada.*

Type II distal arthrogryposis was classified into 5 types (A to E) by Hall et al. (1982) according to the associated abnormalities present. We report a family with features of type II autosomal dominant arthrogryposis present in three generations with male to male transmission that does not fit into any of these classes. The propositus is a baby boy born by spontaneous vaginal delivery at 36 weeks to a 22 year old G_7 P_1 mother with several first trimester miscarriages. On examination his birth weight was 2920 grams (3-10^{th} centile), length 49cm (3-10^{th} centile) and head circumference 34cm (10^{th} centile). He was found to have multiple contractures at birth. These included flexion contractures of both shoulders and elbows, with limitation of elbow extension to 150-160 degrees. There was camptodactly especially of the fourth and fifth fingers which was exaggerated with extension of the wrist. In the lower limbs there were moderate flexion contractures bilaterally at the hips, knees and toes, but to a lesser degree than the upper limbs. Both ankles were stiff and held in dorsiflexion. There were no overt abnormalities of the spine. The mouth was small with trismus limiting opening. There was a submucous cleft and micro-retrognathia. The nose had a prominent bridge and the ears showed deficient helix with excessive folding. The neck was short with pterygium. He had hypoplastic nipples and unilateral cryptorchidism. Echocardiogram revealed a ventricular septal defect and an ASD. Karyotype was 46,XY. The father was met later and had mild to moderate joint contractures at the shoulders, elbows, wrists and feet. In addition he had downward slanting palpebral fissures, a small mouth with trismus, micrognathia, pterygium coli and pseudocamptodactly. He gave a history of surgery for bilateral club feet and a unilateral undescended testis. He also gave a history of a murmur in childhood which was due to an aortic cusp abnormality. He reported that his father and brother have similar features, including limitation of range of movement at the major joints, unilateral cryptorchidism and congenital heart disease. On literature review, we did not find a known autosomal dominant form of arthrogryposis that can describe this family. They appear to have a type II distal arthrogryposis, but do not fit into any of the known classifications. We believe this family has a unique form of autosomal dominant type II distal arthrogryposis associated with congenital heart disease and cryptorchidism.

Disclosure(s): None

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Importance of early diagnosis in multiple endocrine neoplasia type 2B: a case report. *R Arce*[*1], *V del Castillo*[1], *L de la Torre Mondragó*[2], *L Ruíz Reyes*[3], *D Carrasco Daza*[4], *M Tusié Luna*[5], *T Guerra García*[5], *S Cabrera Vázquez*[5], *E Lieberman Hernández*[1]. [1]*Human Genetics Investigation Department,* [2]*General Surgery Department,* [3]*Endocrinology Department,* [4]*Pathology Department, Pediatrics National Institute,* [5]*Molecular Biology and Genomic Medicine Units, INCMNSZ. Biomedical National Institute, UNAM. México.*

Multiple Endocrine Neoplasia type 2B(MEN 2B) is an autosomal hamartoneoplastic syndrome dominantly inherited, is the least common variety of MEN 2. It is characterized by the occurrence of medullary thyroid carcinoma, pheocromocytoma, hyperplasia of the intestinal autonomic nerve plexuses, characteristic facies due to mucosal neuromas, and skeletal and ophthalmic abnormalities. The onset of thyroid and adrenal tumors tends to be early and with an aggressive behaviour. The MEN 2B phenotype is caused by a mutation in the RET proto-oncogene, this gene lies on chromosome 10q11.2 and encodes a transmembrane tyrosine kinase receptor for glial cell-line derived neurotrophic factor. Case Report A 3-year-old female with frequent vomit, abdominal distension, alternating episodes of constipation and diarrhea and diminished tearing. The patient has a characteristic facial phenotype, with thick blueberry lips and anteverted eyelids. The rectal biopsy demonstrates the presence of focal lymphoid hyperplasia. Considering the facial phenotype, the gut motility disorder and the decreased tearing, MEN 2B was suspected. The diagnosis was confirmed by RET mutation testing where the Met918Thr mutation in exon 16 was found. Prophylactic thyroidectomy was done, the histopatologic analysis revealed the presence of medullary thyroid carcinoma in situ. Screening of calcitonine and chatecolamines is done periodically. Abdominal tomography has been normal. The physician has to be aware of the facial phenotype this syndrome has, this enables him to establish an early diagnosis, give the patient prompt treatment and improve the prognosis of survival.

Disclosure(s): None

CLINICAL GENETICS POSTERS

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Consanguinity as a confusing factor in Charcot Marie Tooth type 1A. *M Artigas*[1], S Moreno[1], A Alonso[1], A Valiente[1], E Monròs[2], M Nadal[3], A Pérez-Juana[1], M Ramos[1]. [1]Genética. Hospital Virgen del Camino, [2]Genètica. Hospital San Joan de Déu, [3]Genètica. Institut de Recerca Oncològica.*

Charcot Marie Tooth type 1A (CMT1A) is a neuropathy inherited as an autosomal dominant trait. In some families consanguinity can be a confusing factor. PSS is a 54 year old patient with a family history of Charcot Marie Tooth disease, with some members diagnosed at the molecular level, having the common duplication of the peripheral myelin PMP22 gene located on chromosome 17p11.2-p12. Consanguinity is present, PSS parents being uncle and niece. PSS presented with unstable gait two years ago. Nerve conduction velocities are slow. Cranial MRI shows a vermian atrophy. Molecular diagnosis of inherited ataxias has not demonstrated any mutation in the autosomal dominant ataxias but has showed that he is a carrier of Friedreich's ataxia, the most common hereditary ataxia. Family history is negative for this disease. After reviewing the extended family tree, and the presenting clinical phenotypes, PSS has undergone molecular analysis of CMT1A. Surprisingly genetic analysis by microsatellites indicates that he has a 17p11.2 deletion, found in hereditary neuropathy with liability to pressure palsies (HNPP). Probably both parents share the same haplotype, thus mimicking a deletion in the 17p11.2-p12 region, and being not possible to demonstrate a duplication in the same region. FISH analysis is being performed to confirm this hypothesis.

Disclosure(s): None

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Mosaic trisomy 13, report of one case with abnormal skin pigmentation. *R Avendaño*[1], V Del Castillo[1], L Velasco[1], B Blanco[1], C Salas[1], R Ruíz-Maldonado[2], B Asch[1], A González Del Ángel[1]. [1]Departamento de Investigación en Genética Humana, Instituto Nacional de Pediatría, [2]Departamento de Dermatología, Instituto Nacional de Pediatría, México.*

INTRODUCTION: Trisomy 13 or Patau syndrome its among the most frequent chromosomal abnormalities, clinical features include microcephaly, holoprosencephaly, microphtalmia, cleft lip and palate, polydactyly, congenital heart defects, renal malformations, severe mental retardation and early demise; mosaic trisomy 13 is a rare entity, as in other cases of chromosomal mosaicism the clinical manifestations are usually milder, although this, the correlation between phenotype and percentage of trisomic cells have not been established. We report a single case of mosaic trisomy 13 with minor dismorphism, borderline mental development and abnormal skin pigmentary pattern. CASE REPORT: Female, 3 years old, second child of nonconsanguineous, parents, the mother was 21 years old, with history of first trimester spontaneous miscarriage, the father was 22 years old, the family history was unremarkable. Pregnancy was uneventful, obtained by spontaneous delivery at 36 weeks of gestation, with a birth weight of 3000g, no height documented, she remained one month at intensive care unit with episodes of hypoglycemia and neonatal hyperbilirrubinemia. She was referred to our service at the age of 1 year 6 months, with diagnosis of Ito´s hypomelanosis. Gessell test was performed reporting 80% of development in the motor area, 75% in the adaptative area, and 70% in language. At physical examination weight and height were at 25th centile, cephalic circumference on 3rd centile, bilateral epicanthic fold, strabismus, small ears, normal palate and fifth finger of hands with clinodactily; she had an abnormal pigmentary pattern consisting of hyperpigmented linear and whorly lines, disseminated at trunk and limbs, without midline involvement. Cerebral CT, renal US and echocardiography were normal. Blood cytogenetic analysis showed 46, XX/47, XX +13 mosaicism with the abnormal cell line in 36% of analyzed cells, karyotype of light and dark skin were also performed, reporting 9% and 92% of trisomic cells respectively. DISCUSSION: At least five reports of mosaic trisomy 13 and abnormal pigmentary pattern have been documented, in described patients, mental retardation its a constant feature, our patient is unique, because she have an almost normal development, and minimal clinical manifestations, despite the mosaicism degree of trisomy 13 cell line, which is higher than previously reported patients; also in the literature the mosaic trisomy 13 cases have a pigmentary cutaneous mosaicism, different than the observed in Ito´s hypomelanosis, this pattern is termed phylloid, which resembles an art nouveau picture and oblong macules, that don't follow Blaschko´s lines, our patient seems to have this dermatologic manifestations.

Disclosure(s): None

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An autosomal dominant syndrome with variable microphthalmia and other midline defects in three generations of a family. *TM Bardakjian*[*1], *AS Schneider*[1]. [1]*Albert Einstein Medical Center, Philadelphia, PA.*

An anophthalmia/microphthalmia Clinical Registry was established at Albert Einstein Medical Center in Philadelphia, PA in 1994. The purpose was to identify any clusters in time or place; catalog associated birth defects and to identify syndromes and inheritance patterns. In 1999 we began offering gene screening to interested families. The gene screening involves testing of 17 eye development genes in 8 different laboratories worldwide. We believe that the analysis of the Clinical Registry data will assist us in guiding the gene screening process by developing a genotype-phenotype correlation. Isolated congenital anophthalmia or microphthalmia is a heterogeneous malformation with autosomal dominant, autosomal recessive and X-linked modes of inheritance being reported as well as chromosomal and teratogenic etiologies. However, in non-syndromic cases, autosomal recessive inheritance is thought to be most likely. We describe a family identified by the Clinical Registry with three generations affected with isolated unilateral microphthalmia, as well as other midline defects, including cleft palate and anosmia. The family completed a Clinical Registry for a daughter with unilateral severe microphthalmia. Family history revealed that her father has unilateral microphthalmia with coloboma, her older sister has a cleft palate and a paternal uncle and paternal grandmother have anosmia. No renal anomalies have been noted. This may represent an autosomal dominant syndrome with variable expressivity. A search of the literature has not identified a similar family. The family's DNA has been collected with buccal swabs and is being screened in our program

Disclosure(s): None

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Primary amenorrhea, infantile uterus, alopecia, diabetes melitus, intracranial calcification, acromegalic facial features, mental retardation in two sisters:a new syndrome. *LA Bastaki*[*1], *SA Al Alawadi*[1], *KK Naguib*[1]. [1]*Kuwait.*

Here in we are reporting a Kuwaiti female with masked face,coarse acromegalic facial features,tall stature,primary amenorrhoea,absence of secondary sexual characters,alopecia,scanty eye lashes,absent eye brows,diabetes mellitus and pyramidal and extra pyramidal disorders.She has similar mildly affected younger sister but free from the neurological symptoms,while her father has late onset Parkinsos diseas.On investigation,she had mild miteral regurge.visualized ovaries and small infantile uterus.CT head showed calcification of the basal ganglia, small hypodense lesion in the left half of the pituitary gland.Hormonal profile showed high LH and FSH normal progesterone,normal E2 high para thyroid hormone,low deoxycorticosterone and normal adult growth hormone.Fasting blood sugar was high.both visual and hearing assessment were normal while the IQ assessementwas low in the proband and average /below average in the sister.Chromosomal study was normal 46,XX, These combination have no match.

Disclosure(s): The Kuwait government has provided honoraria and/or travel support for participation in this presentation.

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Further evidence that the T73I PTPN11 gene mutation is associated with a myeloproliferative disorder in Noonan syndrome. *DR Bertola*[*1], *AC Pereira*[2], *JD Carneiro*[3], *JE Krieger*[2], *CA Kim*[1]. [1]*Genetics Clinic Unit, Instituto da Criança do Hospital das Clínicas, University of São Paulo, São Paulo, Brazil,* [2]*Laboratório de Genética e Cardiologia Molecular, Heart Institute (InCor), University of São Paulo, São Paulo, Brazil,* [3]*Hematology Clinic Unit, Instituto da Criança do Hospital das Clínicas, University of São Paulo, São Paulo, Brazil.*

Noonan syndrome is an autosomal dominant disorder characterized by short stature, facial dysmorphisms (ocular hypertelorism, dowslanting palpebral fissures, palpebral ptosis), heart defect (mainly pulmonary stenosis), cryptorchidism in the males and bleeding diathesis. There have been few reports of a myeloproliferative disorder in Noonan syndrome and this association seems to be not fortuitous. The gene responsible for the syndrome was recently identified as the PTPN11, which encodes a non-receptor tyrosine phosphatase SHP-2. Mutation analysis of patients affected by Noonan syndrome with a myeloproliferative disorder showed a high frequency of a specific mutation (T73I) in exon 3. We analyzed the PTPN11 gene in a further patient with typical findings of Noonan syndrome, who presented in the first months of life with hepatosplenomegaly and peripheral blood count abnormalities and the same gene mutation (T73I) was found. This result provides further support that there is a strong genotype-phenotype correlation regarding this specific mutation in Noonan syndrome and myeloproliferative disorder.

Disclosure(s): None

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WITHDRAWN

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WITHDRAWN

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Partial duplication of the X chromosome in males: new case and review. *S Cheng*[1], K Rauen[1,2], D Albertson[2,3], D Pinkel[3], P Cotter[1,4,5]. [1]Division of Medical Genetics, Department of Pediatrics, University of California San Francisco, San Francisco, CA, [2]Cancer Research Institute, University of California San Francisco, San Francisco, CA, [3]Department of Laboratory Medicine, University of California San Francisco, San Francisco, CA, [4]Division of Medical Genetics and Department of Pathology, Children's Hospital and Research Center at Oakland, Oakland, CA, [5]Division of Genetics, US Labs Inc, Irvine, CA.*

Males with Xq duplications are rare with only 28 cases reported in the literature. Only three de novo male cases with Xq duplications are known. We report the fourth case of a male with a de novo proximal Xq duplication and review the literature. The proband's prenatal history was significant for an abnormal expanded AFP screen suggestive of trisomy 18 and abnormal ultrasound findings. Follow-up high-level ultrasound revealed clinodactyly, camptodactyly and hypospadias. Metaphase chromosome analysis from amniocyte culture, and subsequently, peripheral lymphocyte culture, showed a male karyotype containing additional material on the X chromosome. FISH with an X chromosome whole chromosome paint demonstrated signal over the entire X chromosome indicating that the additional material was derived from the X chromosome. Additional FISH analysis for XIST, with a digoxigenin-labeled XIST probe at Xq13.2, was present in a single copy, localizing the proximal breakpoint of the duplication distal to Xq13.2. Thus, the karyotype was interpreted as 46,Y,dup(X)(q13.3q24). At birth, the proband's length, weight and head circumference were all between 10-15 percentile. Phenotypic features of the proband included multiple craniofacial dysmorphia, musculoskeletal anomalies, bilateral cryptorchidism with scrotal hypoplasia, conductive hearing loss and profound generalized hypotonia. Re-evaluation, at 2 years of age, revealed significant global oral motor dysfunction, gastroesophageal reflux, central hypoventilation, cortical blindness with normal ophthalmologic exam, bilateral hearing loss, hypothyroidism, growth failure and severe developmental delay. In addition, we further characterized the duplication of the proband by array-based comparative genomic hybridization (array CGH). Array CGH analysis demonstrated one aberration in the genome as indicated by a duplication of the long arm of chromosome X. The size of the duplication was between 31-47 Mb as determined by the map position of the flanking clones on the array. The molecular breakpoint was further delineated by array CGH to Xq21.3, refining the duplicated region to Xq21.3-Xq24. Despite the paucity of data regarding proximal Xq duplications in males, a clear pattern of characteristic features can be discerned as illustrated in our case and confirmed in our literature review. Mental, *psychomotor and* growth retardation, as well as, craniofacial anomalies, muscle hypotonia, hypogonadism, cryptorchidism, feeding difficulties and endocrine dysfunction are all issues associated with proximal Xq duplications

Disclosure(s): None

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Deletion Xp22.1 with features of Marfan syndrome and skeletal dysplasia. *D Copenheaver*[*1],F Lacbawan[1]. [1]Division of Medical Genetics and Metabolism, Children's National Medical Center, Washington, D.C.*

A 12-year-old Hispanic female with diagnosis of Marfan Syndrome since birth was referred for genetic evaluation. She was known to have a moderately dilated aortic root, mitral valve prolapse and mitral regurgitation, and bilateral ectopia lentis. On examination, her height was 136.2 cm (<5th percentile of age, 50th percentile for a 9 1/2 year old) with an arm span to height ratio of 1.03. The upper segment to lower segment ratio was 1.0. Her face was long with malar hypoplasia, downslanting palpebral fissures deep set eyes, and prominent supraorbital ridges. Her ears were low-set and slightly dysplastic. She had pectus carinatum with broad chest, arachnodactyly and fifth finger clinodactyly, prominent wrist and knee joints, and pes planus. Her developmental milestones were on target and she is doing well in school. Chromosome study revealed 46, X, del (X)(p22.1), associated with a Turner Syndrome variant. The parental chromosomes were normal. Radiographic studies revealed abnormal modeling of the epiphyses and metaphyses of the long bones. There were fishmouth deformities of vertebral endplates. Diaphyseal changes occurred in both radii with medial bowing of the midshaft with cortical butressing. The latter may be explained by SHOX gene haploinsufficiency (Xp22.32) (Ogata et al., 2001). The epiphyseal changes have been described in alterations of the sedlin gene, located on Xp22.2, responsible for Spondyloepyphyseal dysplasia tarda. Mutation analysis of the fibrillin gene is pending. Genomic DNA microarray, and sedlin and SHOX gene analyses are being considered. The question arises on whether her features can be explained by deletion of contiguous genes on Xp22.2 to Xp22.32 or coexistence of separate genetic syndromes.

Disclosure(s): None

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The many faces of sonic hedgehog. *D Cordero*[*1,2], R Marcucio[1], D Hu[1], W Gaffield[3], J Helms[1]. [1]University of California at San Francisco, [2]Albert Einstein College of Medicine/Montefiore Medical Center, [3]Western Research Center, US Department of Agriculture.*

Patients with Holoprosencephaly can exhibit different clinical presentations despite carrying identical mutations in the Sonic Hedgehog gene. One mechanism that may explain the variable phenotypes observed in this disorder may be the exposure to environmental factors which can further decrease the activity in the Sonic Hedgehog pathway at select developmental time points. In this study, we use the chicken as a model system to investigate the hypothesis that the spectrum of HPE phenotypes observed in humans may arise secondary to temporal alterations in the Shh pathway. We show that temporal inhibition of Shh signal transduction by the steroidal alkaloid Cyclopamine produced a graded series of facial malformations reminiscent of those seen in HPE.

Disclosure(s): None

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Amniotic band-like sequence in the offspring of a woman with brachydactyly E. *CM Cremin*[*1], SM Nikke[1]l, MT Geraghty[1]. [1]Department of Genetics, CHEO, Ottawa, Ontario, Canada.*

The amniotic band sequence (ABS) affects about 1/1200 to 1/15000 individuals and accounts for a small but significant proportion of limb defects. The primary cause of ABS is a presumed mechanical rupture of the amniotic membrane with the formation of amniotic strands that cause secondary anomalies including constriction rings, syndactyly and intrauterine amputations. ABS is considered to be sporadic and the recurrence risk is stated to be low. However, rare familial cases with a broad range of limb anomalies have been reported. It has been suggested that these are due to an inherited defect of connective tissue in the fetal membranes. We present the case of an eight-month old girl with apparent ABS born to a mother with brachydactyly type E. The female patient was referred to the Genetics Clinic for absent digits. Physical exam of the child revealed, on the left hand, third and fourth digit amputation and a constriction ring on the thumb and fifth finger. The right hand had 2,3,4 syndactyly and absence of the distal phalanx of the fifth digit. A constriction ring was present on the thumb. There was a left club foot with pedal edema. The right foot had 2,3 distal syndactyly and absence of nails on all digits with distal hypoplasia. The mother was found to have shortening of 3,4, and 5 metacarpals on the left and 4 and 5 metacarpals on the right. She had shortened distal phalanges with nails on both hands. Both her feet were short with bilateral short third and fourth metatarsals. No constriction rings were noted in the mother nor did she have any other features of Albright hereditary osteodystrophy. Family history suggests that the maternal grandmother may also have brachydactyly E. While the findings of ABS in the child and brachydactyly in the mother may be coincidental, we considered the possibility of a single gene defect. Brachydactyly E has been described in patients with mutations in the GNAS1 and HOXD13 genes. Neither the child nor the mother had any features of pseudo or pseudopseudo-hypoparathyroidism. There have been no reports of limb absence defects in the offspring of individuals with mutations in the GNAS1 gene. Recently, mutations in the HOXD13 gene were reported in individuals with features overlapping brachydactyly type D and E. We hypothesize that the amniotic band-like sequence in our patient may be related to the brachydactyly in the mother and are analyzing the HOXD13 gene for mutations.

Disclosure(s): None

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Superior sternal cleft in a newborn. *K David*[*1], *C Barr*[1]. [1]*New York Methodist Hospital, Brooklyn, New York.*

Cleft sternum is a rare congenital malformation. Presentation may include complete or partial sternal clefting, which can be classified as superior or inferior. The underlying cause of sternal cleft is unknown. We wish to report a patient with a partial cleft sternum. We were called to evaluate a full term, appropriate for gestational age, male newborn initially thought by the NICU to have hypoplastic clavicles. On examination, the newborn was noted to have a deep and wide suprasternal notch and a 1.5 cm circular area of atrophic skin in the midline over the mid-sternum. The clavicles were palpable, but were acutely angled. A grade 2/6 systolic murmur was noted along the left sternal border. The remainder of the physical exam was non-contributory. No midline raphe or hemangiomas were noted. Family and pregnancy history were unremarkable. No fetal anomalies had been noted on ultrasound. A subsequent CT scan revealed a cleft sternum of the superior portion. Brain MRI and MRA were normal. Echocardiography revealed a patent ductus arteriosus and a patent foramen ovale, but no arterial anomalies. Primary repair of the sternum was performed at approximately six months of age. By 10 months of age, he was developing normally and had not developed any external hemangiomas. Cleft sternum, with or without an overlying area of atrophic skin, may be an isolated finding. However, it is associated with the PHACES phenotypic spectrum (posterior fossa brain malformations, hemangiomas, arterial anomalies, coarctation of the aorta and cardiac defects, eye abnormalities, and sternal clefting with or without abdominal raphae), which includes the sternal malformation/vascular dysplasia (SM/VD) association. We are reporting this case because of its rarity. Recognition of the phenotypic spectrum associated with cleft sternum is important to ensure testing for associated anomalies. In addition, surgical repair appears to be most effective during the neonatal period.

Disclosure(s): None

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Meir-Gorlin syndrome in a postpuberal Mexican female. *V del Castillo*[*1], *C Villarroel*[1], *A González del Angel*[1]. [1]*Departamento de Investigación en Genética Humana. Instituto Nacional de Pediatría. México, DF. México.*

In 1959, Meier et al. described a patient with microtia, micrognathia, absent patellae and arthrogryposis of the joints. In 1975, Gorlin et al. presented a patient with similar symptoms and short stature. Additional cases were reported in the literature and in 1991, Cohen proposed the term ear-patella-short stature syndrome and Bole in 1994, suggested the eponym Meir-Gorlin syndrome (MGS). MGS is a rare autosomal recessive disorder, characterized by pre and postnatal growth retardation, delayed skeletal development, bilateral microtia, and hypoplasia/aplasia of the patellae; additional clinical findings are some craniofacial features including microcephaly, prominent nose, maxillary and mandibular hypoplasia, full lips, retrognathia, highly and arched or cleft palate, as well as genitourinary tract anomalies, skeletal alterations and deafness. The most serious aspects of MGS are feeding and respiratory problems in early infancy. Here, we describe a 15 year-old female, second child of healthy, nonconsanguineous parents, her birth weight was 2800g and delayed growth development was referred during infancy, but she had medical advice until 12 years because of a deformity in her left arm due to an osteochondroma that was surgically removed. She had normal sexual development. Physical examination showed height, weight and head circumference below third percentile, sloping forehead, midface hypoplasia, beaked nose, micrognathia, height palate, full lips, scoliosis, normal nails, genu valgum and hypoplasia of the patellae that was confirmed by radiographic examination, that also showed slender bones, delayed bone maturation and flattening of the epiphyses. Although our patient did not have low birth weight, she has other features of MGS and represents, to our knowledge, the first Mexican case. This syndrome should be differentiated from several well known syndromes with proportionate short stature, small ears and skeletal anomalies, particularly with absence/hypoplasia of the patellae. Most of the cases are described in pediatric ages and only two cases reported in the literature are in postpuberal females that also have normal sexual development. Skeletal anomalies are important manifestations of the syndrome, including blount osteochondritis dissecans and bilateral aseptic necrosis of the lateral femoral condyles; however, the presence of an osteochondroma has never been described, whether it is a component feature or a coincidence, it is difficult to establish. This syndrome has been postulated similar to the short ear mouse phenotype which is a recessive murine osteochondrodysplasia due to mutation within the bone morphogenetic protein 5 gene (BMP5), that is located in chromosome 6 in humans. The analysis of this gene in one patient was normal without detecting any mutation.

Disclosure(s): None

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The Utah dystrophinopathy project: a genotype/phenotype database and patient registry. *K Dent*[1], J Mendell[2], A Pestronk[3], J Florence[3], W King[2], C Wall[1], L Kerr[1], D Dunn[1], RB Weiss[1], KM Flanigan[1]. [1]University of Utah, Salt Lake City, Utah, USA, [2]Ohio State University, Columbus, Ohio, USA, [3]Washington University, St. Louis, Missouri, USA.*

The Utah Dystrophinopathy Project consists of a large genotype/phenotype database and registry of dystrophinopathy patients including those with Duchenne and Becker muscular dystrophies, X-linked dilated cardiomyopathy, and symptomatic carriers. The primary objectives of this database are to (1) determine the influence of dystrophin sequence variation on disease phenotype, and (2) identify novel dystrophin mutations and mutation mechanisms associated with disease. **Methods**: All dystrophinopathy patients seen by three investigators at large muscular dystrophy centers are enrolled. Study subjects undergo a standardized, prospective clinical examination by a trained clinical evaluator, based on that established in natural history studies. Data on clinical history, disease course, past diagnostic testing, medications, and clinical laboratory testing are gathered on a thirty-page dataform, which is scanned into a dedicated database. All participants have blood samples drawn for direct sequence analysis of the dystrophin gene by the SCAIP method (Flanigan, et al, AJHG 2003; 72:931-939). Muscle specimens from selected patients are analyzed by dystrophin immunoblot, immunofluorescence, RT-PCR, or other studies. **Results**: Patient enrollment is ongoing, with an expected accumulation of 900 patients over the next two years. To date we have 91 individuals enrolled in the database. Dystrophin gene sequence data (including mutations and polymorphisms) will be posted on a public website (www.genome.utah.edu/dystrophysnps/). Clinical/genotype correlative data will be analyzed by a variety of statistical methods. **Discussion**: The availability of affordable direct sequence analysis of the entire dystrophin gene allows studies of the influence of dystrophin sequence on disease phenotype (including mutation context, and polymorphisms). This unique data set will also allow us to catalogue mutations, and generate testable hypothesis of gene and protein function and organization. Clinical/genotype correlation data obtained may provide families with anticipatory guidance in terms of clinical presentation and management. We intend to expand the consortium of participating centers to obtain greater geographical representation, and invite participation of a larger number of patients and families.

Disclosure(s): None

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A child with multiple congenital anomalies, dysmorphic features, and a rare skeletal dysplasia. *E Elias*[1]. [1]Children's Hospital, Univ of Colorado School of Medicine, Denver, CO.*

A 13 year old girl presented for genetic consultation because of short stature, unusual features and multiple anomalies. **History:** She was the AGA product of a 37 wk gestation to a 32 yr old healthy mother whose pregnancy was complicated by polyhydramnios. Pierre-Robin anomalad with severe airway obstruction necessitated tracheostomy in the newborn period. Congenital heart disease (PDA, ASD) also required repair during early infancy. Her subsequent medical course has been notable for chronic URI's with conductive hearing loss, strabismus, growth hormone deficiency, and development of severe thoracic scoliosis. She has moderate cognitive disabilities. The family history is unremarkable. **Physical exam:** Her weight is at the 5th%, height below the 2nd% and OFC above the 98th% for age. Her skin reveals a prominent venous pattern on her trunk. Her facies are distinctive with facial asymmetry, hypertelorism, broad nasal bridge and notched nasal tip, dysplastic pinnae with a preauricular pit, repaired cleft palate and microagnathia. Her clavicles had thickened medial ends, and her digits were dysplastic. **Laboratory findings:** Her karyotype was normal, 46,XX. Subtelomeric FISH probe studies showed no evidence of deletion. Her xrays were strikingly abnormal, with a markedly sclerotic and thickened calvarium, and linear striations of all long bones and pelvis. **Discussion:** This child's physical features and xray findings are consistent with the diagnosis of Osteopathia Striata with Cranial Sclerosis. This is a rare skeletal dysplasia felt to be inherited as either an x-linked or autosomal dominant trait. Progressive cranial sclerosis may have neurologic sequelae secondary to bony encroachment on cranial foramina causing nerve compression. Macrocephaly, hypertelorism, Pierre-Robin anomalad, congenital heart disease and abnormalities of digits have been reported as associated findings, as have hearing loss and cognitive disabilities. The distinctive and unusual xray findings were the key to a diagnosis in this patient, allowing for more accurate genetic counseling, and better medical anticipatory management.

Disclosure(s): None

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X-linked lissencephaly with absent corpus callosum and ambiguous genitalia: an Egyptian family with variable presentation. *E Elsobky*[1], S Elsayed[1], H Hasan[1], A Parsian[2]. [1]Medical Genetic Center, Cairo, Egypt, [2]Birth Defect Center, University of Louisville, USA.*

Lissencephaly is a severe brain malformation manifested by a smooth cerebral surface, abnormally thick cortex, enlarged ventricles and often agenesis or malformation of corpus callosum. X-Linked lissencephaly with absent corpus callosum (XLAG) is a newly recognized syndrome that was first described in 1994. to our knowledge, only 9 cases have been reported worldwide. in this paper we describe an Egyptian family with this syndrome including two carrier mothers, one carrier daughter, and several affected children with variable presentation including the index case with typical phenotype. A candidate gene for XLAG have been suggested as a causative factor in 11% of cases. No mutations of this gene have been found in our family.

Disclosure(s): None

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Wolf-Hirschhorn syndrome: weight gain patterns and natural history review. *L Escobar*[1], M Heiman[1]. [1]Medical Genetics - Developmental Pediatrics Center, St. Vincent Children's Hospital, Indianapolis, IN.*

Wolf-Hirschhorn syndrome, is a well recognized genetic condition caused by a deletion of the critical region, 4p16.3. Described in 1961, Wolf-Hirschhorn syndrome (WHS) has been well delineated, however the natural history of this disorder continues to present challenging questions to clinicians. Recent information has changed the way we look at individuals with WHS, previously thought to have severe to profound disabilities, typically not developing speech and having minimal communication skills. Recent studies indicate that a small number of individuals may have significantly better developmental prognosis than previously thought (Battaglia et al., 2001). As with many other genetic conditions, the increasing knowledge we have about the syndrome is reflected in the way we treat patients. New recommendations for medical management and health supervision for individuals with WHS were proposed by Battaglia and Carey in 1999. The authors proposed guidelines for treatment such as correcting congenital heart defects and aiding those with hearing loss. Battaglia and Carey note that despite adequate calorie and protein intake, all patients with WHS show slow weight gain (<2%). Several possible causes including oral facial clefts, poorly coordinated sucking, aspiration and gastroesophageal reflux are suggested. The authors also state that the consideration of gastrostomy tube placement is highly recommended for improvement in weight gain and general health status. We present here a series of twenty-five patients with WHS who despite aggressive nutritional therapy continue to present poor growth and short stature. Close to 50% of our patients received a gastrostomy tube with variable responses, that seem to be independent from collateral morbidity such as gastroesophageal reflux. Patients that did not receive G-tubes presented similar results with variable weight gain. We also present results from a survey of parents of children with WHS. Data on their feedings, weight gain and other compounding factors were collected. In our series 50% of patients reported having a congenital heart defect, 40% reported renal anomalies, and almost 75% reported seizures. Various other factors such as tracheostomy, diaphragmatic hernia, bladder exstrophy, and imperforate anus were reported by our survey population as well. Our data raise significant questions regarding the nutritional approach for the child with WHS. We discuss the risks and benefits of aggressive treatments including the use of gastrostomy tubes and high caloric intake. We also review the natural history of this disorder and current expectations. The data presented here clearly shows the need for further delineation of the natural history of WHS, the need of standardized growth curves for affected children and the need of follow up protocols based on WHS phenotypic findings and clinical course.

Disclosure(s): None

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Natural history and clinical phenotype of patients with Smith-Magenis syndrome due to RAI1 gene mutations. *BM Finucane*[1], CI Scott, Jr.[3], EH Zackai[4], SH Elsea[2]. [1]Genetic Services, Elwyn Training and Research Institute, Elwyn, PA, [2]Genetics Graduate Program, Michigan State University, East Lansing, MI, [3]Division of Medical Genetics, duPont Hospital for Children, Wilmington, DE, [4]Division of Human Genetics and Molecular Biology, Children's Hospital of Philadelphia, Philadelphia, PA.*

Smith-Magenis syndrome (SMS) is a well-described chromosomal disorder associated with characteristic facial dysmorphism, mental retardation, sleep disturbance, and behavioral abnormalities. The condition is associated in almost all cases with a cytogenetically visible deletion of band 17p11.2. The critical region in deleted patients spans nearly 1 million base pairs and includes at least 2 dozen genes. Recently, we reported frameshift mutations in a single gene (RAI1) within the SMS critical region in 3 non-deleted patients with clinical features of SMS (Slager et al., 2003. Nat Genet 33:466-8.) Detailed clinical information and photographs at different ages have allowed us to document the natural history of the syndrome in these 3 individuals, now young adults, and to make phenotypic comparisons with SMS deletion cases. All 3 patients with RAI1 mutations manifested characteristic SMS facial features, including relative prognathism and a tented upper lip, as well as brachycephaly. All had a history of disordered sleep patterns. Cognitively, the mutation patients functioned within the mild to moderate range of mental retardation, as do most people with 17p11.2 deletions. Our patients also exhibited key behavioral features of the syndrome, including onychotillomania (self-mutilation of finger- and toenails), polyembolokoilamania (insertion of foreign objects into body orifices), and self-hugging. These unusual behaviors are rarely observed in people without the syndrome and are considered behavioral hallmarks of SMS. By contrast, none of our 3 patients had structural heart, kidney, or palate abnormalities, nor did they exhibit the severe myopia which sometimes occurs in those with cytogenetic deletions. All 3 had normal stature. Although moderate hypotonia and delayed motor milestones are universal among young children with 17p11.2 deletions, 2 of the 3 mutation patients had normal motor development. In the 3rd young man, infantile hypotonia and motor delays may have been related to severe Arnold-Chiari malformation rather than a direct effect of his gene mutation. In addition, all 3 of our patients were morbidly obese, in contrast to the normal weight variance found among SMS deletion patients; it remains to be confirmed whether obesity is a related phenotypic feature in RAI1 mutation patients. Although SMS is generally considered a contiguous gene deletion syndrome, we found a striking degree of clinical overlap between the mutation patients and those with visible cytogenetic deletions. We conclude that most of the major phenotypic features of SMS, including its unique behavioral manifestations, are related to mutations, deletions, and potentially other abnormalities affecting RAI1 gene function.

Disclosure(s): None

CLINICAL GENETICS POSTERS

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Unusual phenotypes associated with microdeletion 22q11.2. *L Florez*[1], P Rojas[1], D Moscatello[1], Y Lacassie[1,2]. [1]Department of Pediatrics, Louisiana State University Health Sciences Center, New Orleans, Louisiana, [2]Children's Hospital, New Orleans, Louisiana.*

The phenotypes associated with the microdeletion 22q have been expanded from the original description of the Velocardiofacial and DiGeorge syndromes, to the Conotruncal anomaly face syndrome, the autosomal form of the Opitz GBBB syndrome, and to familial and isolated cases of congenital heart disease. All these conditions have been encompassed under the acronym CATCH 22. The overlap of manifestations among those syndromes is well recognized. Cases presenting as Robin, Potter, and holoprosencephaly sequences, CHARGE association and Oculo-auriculo-vertebral spectrum have also been described. Numerous authors have emphasized the wide variability of expression associated with these conditions, even among familial cases. Reports of very mildly and severely affected patients have broadened the phenotypic spectrum. Unusual anomalies, including bifid nasal tip, asymmetric crying facies, limb anomalies, idiopathic thrombocytopenic purpura, celiac disease, and neural tube defect have been described as rare features of the 22q deletion. We report two patients with a microdeletion 22q11.2 and unusual anomalies. The first patient, in addition to DDM/MR, feeding difficulties and mild immune deficiency in childhood, presents with severe ocular colobomas, prognathism, patulous lower lip, hypothyroidism, penoscrotal pterygium, short fingers and extensive vitiligo. The second patient, besides cleft palate, microcephaly, joint laxity, elbow subluxation and imperforated anus, presents triphalangeal right first digit, polydactylic left thumb and patches of aplasia cutis congenita on the scalp. These two cases further expand the phenotypic spectrum of anomalies associated with the 22q11.2 microdeletion, making imperative the consideration of this diagnosis even in the presence of unusual phenotypes.

Disclosure(s): None

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Narrowing the critical region and expanding the phenotype of duplication 15q syndrome in an adult with dual diagnosis. *C Forster-Gibson*[1], P Walsh-Bergin[2], C Evans[1], K Harrison[3]. [1]Department of Family Medicine, Queen's University, Kingston, Ontario, Canada, [2]Royal Ottawa Health Care Group, Brockville Psychiatric Hospital,Brockville, Ontario, Canada, [3]Department of Pathology and Molecular Medicine, Queen's University, Kingston, Ontario, Canada.*

We describe a dysmorphic 44 year old female with a de novo, partial duplication of the distal long arm of chromosome 15. Genetic assessment was requested when she was 42. Early historical records were unavailable. The propositus was dysmorphic and morbidly obese. She had a dual diagnosis of severe developmental disability and psychiatric illness. Her psychiatric diagnosis was of Organic Mood Disorder-Bipolar Type and she suffered from hyperphagia and self-abusive behaviours. She did not meet the criteria for diagnosis of panic disorder. Medical problems have included hyperuricemia, cataracts, cholecystitis, osteoarthritis of the knees and degenerative changes secondary to obesity, and anemia. A recent CT scan of the head was normal. Cytogenetic analysis and FISH studies revealed a karyotype of 46, XX, dup(15)(q24.1q25.3).ish dup(15q)(wcp15+). Review of 25 individuals with 15q duplications including the same region of 15q as our case, identified 10 features that were present more than 69% of the time. These included mental retardation; postnatal growth deficiency; micrognathia; puffy cheeks; prominent, bulbous nose; long or well defined philtrum; midline crease of lower lip; broad nasal bridge; upslanting palpebral fissures and down-turned corners of the mouth. Information was available on at least 10 individuals for each of these common features. Of these, our propositus had mental retardation, puffy cheeks, prominent bulbous nose, broad nasal bridge and down-turned corners of the mouth. She also had some physical characteristics that have not been previously described, including coarse facial features; hirsutism; thick, coarse scalp hair; synophrys; smooth lips and broad hands. The identification of common physical features and mental retardation in individuals with a common region of duplication of chromosome 15q (q24.1-q25.3) suggests that a recognizable phenotype for duplication of this critical region exists. Furthermore, the history of psychiatric illness (other than panic disorder) in our propositus suggests that psychiatric illness may be a feature of this condition. Further description of adults with this duplication is needed.

Disclosure(s): None

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Conversion analysis in genetic testing: a novel clinical platform for comprehensive mutation detection in inherited disorders.
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Identification of germline mutations in a sensitive and specific manner presents a continuing challenge. Humans are diploid; therefore, mutations in one allele are often masked by the presence of the normal sequence present on the other copy of the chromosome. In addition, the spectrum of disease-causing mutations is diverse and it is difficult to design a single test that can detect all type of mutations. Furthermore, the accurate interpretation of a mutation as pathogenic cannot always be deduced from just sequence information. Here we present Conversion Analysis, a clinical platform based on analysis of cDNA in haploid templates that can address the above issues. Mutation analysis on haploid templates, rather than on diploid templates, overcomes the diploidy problem and obscured mutations are unmasked. Conversion Technology converts a sample from diploid to haploid state by isolating individual alleles in somatic cell hybrids. From each sample a series of stable hybrids is generated that contain the human chromosome complement in the haploid state. Hybrids that contain the alleles (haploid) of interest are identified by genotyping and selected for further analysis. Mutation analysis is performed by evaluation of expression of the cDNA for each allele, followed by sequencing of cDNA, when required. Altered expression levels or molecular weight of RT-PCR products is diagnostic for single or multiple exon deletions or duplications, splice-site mutations, chromosomal rearrangements that affect the gene of interest, promoter mutations, and whole-gene deletions. Expression analysis permits unequivocal interpretation of splice-site and expression mutations. Sequence analysis of the coding region on full-length RT-PCR products can identify missense, nonsense mutations and small deletions and insertions. In a recent study (Casey et al., in press), Conversion Analysis showed a significant increase (~50%) in the sensitivity of genetic testing for HNPCC compared to genomic DNA sequence analysis from diploid samples. The increase in sensitivity was due not only to the identification of mutations masked in diploid samples, but also to the use of expression analysis to allow functional interpretation of splice-site, expression, and some missense mutations, which were of unknown significance based on sequence alone. As it is applicable to genes on all chromosomes and as many human genes are expressed in the hybrids, Conversion Analysis could be used to improve performance for genetic analysis for large number of inherited diseases. As a single platform, Conversion Analysis can facilitate the logistics, performance, and quality control for genetic reference laboratory testing, precluding the need to integrate multiple platforms performed in tandem or to proceed through reflex testing sequentially to detect different type of mutations.

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Achondroplasia-hypocondroplasia complex in a Mexican patient.
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Acondroplasia (ACH) is the most common short-limb skeletal dysplasia with an autosomal dominant inheritance. Most of the cases result from a de novo mutation in the fibroblast growth factor receptor 3 gene (FGFR3) that causes a substitution of an arginine for a glycine at position 380 (G380R/G1138A). The most frequent clinical manifestations are rhizomelic limb shortness, enlarged head with frontal bossing, midface hypoplasia, and short, broad hands with a trident configuration. Hypocondroplasia (HCH) is considered an allelic disease of ACH with a less severe phenotype. The mutation observed in about 70% of HCH patients is a transversion at nucleotide 1620 (C1620A or C1620G). The phenotype of patients with the acondroplasia-hypocondroplasia complex (ACH-HCH complex) is not well defined, because there are only three patients reported in the literature with a confirmed diagnosis by molecular studies. We report a Mexican patient whose mother has ACH and the father HCH, neither had received genetic counseling or had been submitted to molecular testing before the patient's birth. The index case was studied at the National Institute of Pediatrics when he was 5 months of age, on physical examination he had an enlarged head with a very large anterior fontanel, frontal bossing, midface hypoplasia, small chest, protuberant abdomen, rhizomelic limb shortness, short broad hands with a trident configuration and a significant respiratory distress. X rays showed shortness of the long bones, wide metaphyses and reduced interpedicular distance of lumbar vertebrae. At 8 months of age he required foramen magnum decompression. He has been hospitalized eleven times due to bronchopneumonias and in one occasion he had a cardiac arrest. He is now four years old and has a severe developmental psycomotor delayed. The clinical manifestations and evolution of the patient suggested that he could had ACH-HCH complex. Molecular studies confirmed that the patient inherited the ACH and HCH mutations from his parents, being a compound heterozygote (G1138A/C1620G). The clinical evolution of the patient indicates that the phenotype of the ACH-HCH complex is more severe with important neurological and respiratory complications than observed in ACH or HCH patients. As far as we know, only three ACH/HCH patients with confirmed molecular diagnosis have been reported in the medical literature, one of them had mental retardation but as in our patient, he suffered cardiorespiratory complications and in other patient the brain CT scan suggested cerebral dysplasia. It is not possible to affirm that mental retardation is a consistent finding in the ACH/HCH complex and follow up studies are needed to further delineate natural history of this entity.

Disclosure(s): None

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Delayed diagnosis in two symptomatic MPS I patients.

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MPS I, an autosomal recessive disorder that is caused by a deficiency of the enzyme, α-L-iduronidase, constitutes a wide clinical spectrum that includes severe and attenuated forms. Patients with the severe phenotype (Hurler) are usually diagnosed within the first 2 years of life, while patients presenting with the attenuated forms (Hurler-Scheie and Scheie) can easily be misdiagnosed. This abstract presents two illustrative cases. Patient A is a 23-year old female with an attenuated form of MPS I. Her symptoms began at 7 days of life with hip dislocation. During adolescence, she experienced frequent bouts of diarrhea and skeletal complications, including kyphosis, hip dysplasia, and persistent joint stiffness requiring orthopedic intervention. Because she lacked the classic symptoms associated with the severe form of MPS I, e.g. coarse facial features and upper airway obstruction, her condition remained undiagnosed for over 20 years. At age 21, she was referred to a pediatric cardiologist for evaluation of a murmur. Because of this pediatric cardiologist's experience managing patients with MPS I, the disorder was suspected based on the presence of valvular dysplasia. The diagnosis of MPS I was confirmed by the lack of α-L-iduronidase activity. Patient B was 40 years old when she was diagnosed with MPS I. Her symptoms began in primary school when she experienced difficulties in coping with sporting activities. During childhood and adolescence, she developed kyphoscoliosis, hip dysplasia, and a moderate claw-hand deformity with bilateral carpal tunnel syndrome. She also had a moderate degree of valvular insufficiency secondary to mitral and aortic valve dysplasia, was amenorrheic, had hepatomegaly, and uncontrollable diarrhea. The correct diagnosis was made at the time this patient underwent a laminectomy for spinal cord compression. Discussion: Both cases share a long history of unrecognized disease related symptoms. Each patient was followed by multiple specialists (pediatrician, internist, orthopedic surgeon, and rheumatologist) prior to diagnosis. With the passage of time, their symptoms progressed to the point of severe disability. In November 2002, both patients began enzyme replacement therapy with Aldurazyme® (laronidase). Since the initiation of treatment, both patients have reported improvements in quality of life as well as multiple disease-related symptoms, including increased mobility and reduced frequency of diarrhea. With the introduction of enzyme replacement therapy, early diagnosis and treatment of MPS I may help prevent irreversible damage. Increased awareness of the pattern of symptoms associated with MPS I will be required to facilitate timely diagnosis.

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Ectrodactyly, patent ductus arteriosus, and bicuspid aortic valve: is it part of the spectrum of reported "patent ductus arteriosus, bicuspid aortic valve with hand anomalies?"

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A combination of patent ductus arteriosus (PDA), bicuspid aortic valve (BCAV), and hand anomalies (MIM 607411) was reported by Gelb et al. (1999) in seven members of a family in three generations. The Char syndrome (MIM 169100) was excluded by molecular studies. Here we report a newborn baby boy with the same combination of congenital anomalies, except that the unilateral hand anomaly was more severe in our patient, as ectrodactyly. The baby was born at 29 weeks' gestation by C-section delivery due to placenta previa. Mother is 25 years old, G3, P1, SAb1. Gestational history is significant for vaginal bleedings between the third and 16 weeks of pregnancy. The reason for the bleeding was thought to be due to demise of a twin, aborted at fourth months of pregnancy. The family history is non-contributory. The clinical manifestations at three months of age include ectrodactyly of the right hand with missing 2nd and 3rd fingers with digitalization of the thumb and slightly shortened right forearm, PDA, and BCAV. The rest of the skeletal survey was normal. In addition he has a right auricular pit (the mother also has a unilateral auricular pit), mild retrognathia, mild hypospadias and bilateral direct inguinal hernia. No apparent amniotic band marking are identified. The patient does not seem to have characteristic features of Char syndrome. We agree with Gelb et al. that the "PDA, BCAV, and hand anomalies" present a unique heart-hand syndrome, with our case expanding the spectrum of finding to include more severe hand anomalies (ectrodactyly), hypospadias, and inguinal hernia.

Disclosure(s): None

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Development of a single-tube multiplex assay for the detection of exonic deletions and duplications in the MSH2 gene of hereditary non-polyposis colorectal cancer. *FM Hantash*[1], W Sun[1], R Bender[2], CM Strom[1]. [1]Molecular Genetics Department, [2]Hematology/Oncology Department, [1,2] Nichols Institute, Quest Diagnostics Inc. San Juan Capistrano, CA.*

HNPCC is a dominant disease that affects approximately 30,000 Americans. Mutations in the MSH2, MLH1, and MSH6 genes among others can cause the disease. Various classes of mutations have been described to cause HNPCC including point mutations, small deletions/insertions and exon deletions/duplications. Our lab has designed a sequencing assay to detect mutations in MSH2, MLH1 and MSH6 genes. However, up to 30% of HNPCC mutations can be due to DNA rearrangments including single or multiple exon deletions or duplications. We designed a single tube, multiplex semi-quantitative fluorescent PCR assay for the detection of exon deletions/duplication in the MSH2 gene. Fluorescently-labeled primers amplify 16 fragments representing all MSH2 exons in a single reaction as well as an internal control. Amplified fragments are analyzed on an ABI 3100 Genetic Analyzer followed by data analysis using Genemapper software (ABI). By comparing the profile of amplified fragments in the linear PCR range to the reference internal control amplicon, we can accurately detect the presence of deletions/duplications. We tested known homozygous and heterozygous deletion samples in MSH2 and demonstrated the ability of the assay to detect the various DNA deletions. A similar assay is being developed for detection of deletions/duplications in MLH1 and initial testing of the MLH1 single tube multiplex assay shows the ability to detect a heterozygous deletion in one sample and a heterozygous duplication in another. Our assays are an improvement on similarly described assays in that all exons are analyzed in a single reaction per gene allowing ease of use, the automation ability of the assay to handle larger sample volumes, and the potential ability of the assays for automated results interpretation. The described single tube assays are economical and simple and will complement existing sequencing assays for the characterization of mutations in HNPCC.

Disclosure(s): Authors are employees of and have stock options with Quest Diagnostics.

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Variable early onset familial cardiomyopathy: mitochondrial disease or Alstrom syndrome?

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Dilated cardiomyopathy in the newborn period has a large differential diagnosis including myocarditis, muscular dystrophies, and inborn errors of metabolism. Alstrom syndrome is an autosomal recessive disorder that manifests as early-onset dilated cardiomyopathy, progressive vision and hearing loss, morbid obesity, hypogonadism, insulin resistant diabetes, and renal failure. We report 4 siblings with Alstrom syndrome with intra-familial variable onset, severity, and spectrum of manifestations. Sibling 1 had significant dilated cardiomyopathy (less than 12% shortening fraction) in the first week of life. She was non-dysmorphic, and developed nystagmus by 3 months of age. Initial metabolic tests showed increased levels of urinary α keto-glutarate and serum pyruvate, low total and free plasma carnitine, normal acylcarnitine levels, and elevations of several plasma amino acids. At 13 months of age she developed cardiac failure, required a heart transplant, and died two weeks later. Endomyocardial biopsy showed non-specific myocardial cell hypertrophy. Sibling 2 (male), now 11 years-old, had significant dilated cardiomyopathy requiring treatment at 5 months of age with partial resolution at 18 months of age. A recent recurrence of his cardiomyopathy has required re-institution of medication. Sibling 3 (female), now 8 years-old, has had stable, mild cardiomyopathy since birth, and patient 4, (male), has normal cardiac function at 7 years of age. Each of the 3 surviving siblings has, over time, developed nystagmus, photophobia, achromatopsia, morbid obesity, hypogonadism (in males), acanthosis nigricans, and sensorineural hearing loss. None has yet developed diabetes or renal dysfunction. Their cognitive function is normal. Alstrom syndrome is caused by mutations in the ALMS1 gene. The gene has unknown function, but has wide-ranging effects and may be involved in mitochondrial pathways. An endomyocardial biopsy performed in sibling 3 showed combined deficiency of all complexes of the electron transport chain. Analysis of the ALMS1 gene in our patients is in progress. It is important to consider Alstrom syndrome in the differential of neonatal and infant-onset dilated cardiomyopathy to provide early diagnosis and intervention. This may prevent or retard syndrome-associated disease complications, and allow for timely and appropriate genetic counseling and prenatal diagnosis.

Disclosure(s): None

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Antenatal natural history and perinatal autopsy correlates of unusual case of cloacal extrophy: anhydramnios, COEM, and urinary ascites.

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CASE REPORT: Maternal history of medication exposure and abnormal maternal serum analyte screen were the indications for an ultrasound at 19 weeks EGA. Multiple fetal anomalies were noted. There was no evidence of spontaneous rupture of membranes. The family did not wish to terminate the pregnancy and declined invasive fetal testing. **FETAL IMAGING:**Fetal findings included intrauterine growth retardation, severe oligohydramnios, meningocylocele, hydrocephaly, ascites/omphalocele, no visualization of the bladder, nor gender suggested COEM. The progressive nature of the fetal ascites and anhydramnios were associated with findings predicitvie of pulmonary hypoplasia. Realtime video of fetal sonography facilitated the complex medical ethical decision process. **PERINATAL AUTOPSY:** She delivered at 28 weeks, livebirth with immediate neonatal death due to respiratory failure. An autopsy performed by the clinical geneticists and pathologist confirmed the prenatal suspicion: cloacal extrophy and urinary ascites due to apparency renal-pelvic rupture, omphalocele, imperforate anus, extrophy of bladder, bidfid genitalia, split symphysis, diaphragmatic hernia, adn severe pulmonary hypoplasia. **NATURAL HISTORY:** Most reported fetal cases of Cloacal Extrophy have been associated with polyhydramnios. Massive urinary ascites resulting from disruption of the pyelectasis secondary to distal obstruction is the most likely explanation for the anhydramnios and fetal ascites as well as pulmonary hypoplasia. The perinatal hospice approach was based upon family valvues in full consideration of the dismal prognosis.

Disclosure(s): None

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Monozygotic twins discordant for cystic microphthalmia, abnormalities of first branchial arch and midline structures: a variant of oculocerebrocutaneous syndrome? *CA Kim*[1], LM Albano[1], LR Sadeck[2], CR Leone[2], GF Gattas[3], DR Bertola[1]. [1]Genetics Clinic Unit, Instituto da Criança do Hospital das Clínicas, University of São Paulo, São Paulo, Brazil, [2]Department of Pediatrics, Instituto da Criança do Hospital das Clínicas, University of São Paulo, São Paulo, Brazil, [3]Insitututo Oscar Freire, University of São Paulo, São Paulo, Brazil.*

Oculocerebrocutaneous syndrome (OCC), described in 1981 by Delleman and Oorthuys, is characterized by cystic microphthalmia, complex brain malformations and typical skin lesions (appendages, focal hypoplastic skin defects). We report on a newborn baby, product of a twinning gestation (separate amnions, chorions and placentae) from healthy and non-consanguineous parents. The proband was born at 32 weeks of gestation, with a birth weight of 1730g, length of 42cm, OFC of 27cm and Apgar score of 8/9/9. The physical exam showed: asymmetric cranial vault, narrow palpebral fissures and apparently anophthalmia, hypoplastic nose, central cleft lip and palate, left microtia and preauricular tags bilaterally; cardiac murmur; cryptorchidism and mild clinodactyly of the 5th fingers. Complementary exams disclosed: bilateral microphthalmia, with a cystic mass on the left; sphenoidal encephalocele, large cyst in the parieto-temporal region, third and forth ventricles not identified and absence of the septum pelucidum; ASD of 8mm. Abdominal ultrasound, spine X-ray and G-banded karyotype were normal. The patient died at 6 months of age due to respiratory distress. His monozygotic twin brother (determined by DNA markers) was clinically normal. The patient reported here shows some of the features of OCC syndrome, but lack the typical skin findings. The only skin abnormality was the presence of preauricular tags. The etiology of OCC syndrome is still unclear, but several authors suggested that a disruption of the anterior neuroectodermal plate is the pathogenic mechanism. All reported cases have been sporadic and consanguinity has been described in a few patients. The phenotypic discordance in our monozygotic twins shows strong evidence that nongenetic factors play a role in this condition.

Disclosure(s): None

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The copper plate system: effective use of DHPLC for clinical genetics and pharmacogenetics. *K Kosaki*[1], T Utaka[1], H Samejima[1], H Fujita[1], N Yahagi[1], T Takahashi[1]. [1]Dept of Pediatrics, Keio University School of Medicine.*

The more the molecular geneticists unravel the genetic basis of inherited diseases or susceptibility to adverse drug reactions, the more the practicing clinical geneticist get frustrated because they are confronted with a situation in which molecular diagnostic tests cannot be offered to the patients simply because of the cost and labor associated with such testing. A cost-effective and automated strategy needs to be implemented. Denaturing high-performance liquid chromatography (DHPLC), the new technology, based on temperature-modulated liquid chromatography and a high-resolution matrix was implemented in a way suitable for clinical application. Primers were designed so that all of the primers would have the same annealing temperature. In this way, all the exons can be amplified simultaneously in a single PCR machine. Primer pairs were aliquoted on a 96-well format PCR plate referred to as COPPER plate (Condition-Oriented-PCR primer-Embedded-Reactor). The COPPER plate was accompanied with a corresponding computer programs optimized for DHPLC analysis of the particular PCR product. COPPER plates and accompanying computer programs were developed for analysis of the PTPN11 gene (Noonan syndrome), the JAGGED1 gene (Alagille syndrome), and the FGFR2, and the TWIST genes (craniosynostosis syndromes). All the exons could be analyzed sequentially by the optimized method and all the operation were completely automatic once the operator sets the plate and starts the "gene-specific" analysis program. The COPPER plate system was further developed for genotyping of major polymorphisms of drug metabolizing enzymes including CYP2C9, CYP2C19, and thiopurine methyl transferase. COPPER plates for The DHPLC technology, when complemented with the COPPER plate system, offers an ideal tool for mutation analysis in clinical applications. The key aspects to setting the operating parameters will be discussed.

Disclosure(s): Transgenomic, Inc. has sponsored research activities relevant to this presentation.

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Ocular findings in Canavan's disease. *KL Bergwerk*[2,3], K Koslowe[1], U Yinon[1], J Merrick[3]. [1]Goldschleger Eye Research Institute, Sackler Faculty of Medicine, Chaim Sheba Merdical Center/Tel Hashomer, Tel Aviv University, [2]Cedars-Sinai Medical Center, Los Angeles, CA, [3]National Institute of Child Health and Human Development, Israel.*

Introduction: Canavan's disease (spongiform leukodystrophy) is a rare neuro-degenerative disease with childhood onset, commonly found in Ashkenazi Jews, for which there is no current therapy. Previously there has been no extensive previous discussion in the medical literature of the ocular abnormalities encountered in this population. The purpose of this paper is to document the ocular findings in this group. Methods: This is a retrospective observational case series report of 7 Canavan's disease patients examined with portable equipment in residential facilities. The examination included: transiluminator-assisted examination of the external structures, pupillary response, cycloplegic retinoscopy, direct ophthalmoscopy, cover tests and visual tracking. Results: Of the 7 patients, 4 were male and 3 were female, with an age range of 6-13 years (mean = 9.5). The refractive status of all the Canavan's patients was within normal range. Cover testing revealed that 3/7 had significant exotropia. Pupillary examination with the swinging flashlight test demonstrated that 5/7 had abnormal pupillary responses. All 7 had optic nerve pallor with 3 cases of optic nerve hypoplasia. Tracking was subnormal in 3 and virtually absent in 4. Conclusion: Significant ocular abnormalities are encountered in Canavan's disese. Although this disease has devastating neurologic involvement, maximal efforts should be made to provide appropriate intervention to enrich quality of life.

Disclosure(s): None

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Ocular findings in Rett's syndrome. *K Bergwerk*[2,3], K Koslowe[1], U Yinon[1], J Merrick[3]. [1]Goldschleger Eye Research Institute, Sackler Faculty of Medicine, Tel Aviv University, Chaim Sheba Medical Center, Tel Aviv University, [2]Cedars-Sinai Medical Center, Los Angeles, CA, [3]National Institute of Child Health and Human Development, Israel.*

Introduction: Rett's syndrome (Cerebroatrophic hyperammonemia) is a rare sex-linked neuro-degenerative disease, characterized by initial normal development, followed by rapid mental and physical deterioration. Previously there is only limited discussion in the medical literature of the ocular abnormalities encountered in this population. The purpose of this paper is to document the findings in a cohort of Rett's syndrome patients. Methods: This is a retrospective observational case series report of 7 Rett's syndrome patients examined with portable equipment in residential facilities. The examination included: trans-illuminator-assisted examination of the external structures, papillary response, cycloplegic retinoscopy, direct ophthalmoscopy, cover tests and visual tracking. Results: All patients were female, with an age range of 9-21 years (mean = 14.7 years). Refraction revealed 3/7 had astigmatism in the amblyogenic range, and 2/7 had significant spherical refractive errors. Two patients had strabismus, with one each of esotropia and exotropia. While the optic nerves appeared normal in all 7, only 2 had minimal tracking ability. Conclusion: Significant ocular abnormalities are encountered in Rett's syndrome. Although this disease has devastating neurologic involvement, maximal efforts should nevertheless be made to provide appropriate therapy in this condition.

Disclosure(s): None

CLINICAL GENETICS POSTERS

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Chromosome 2 in the genetics clinic. *BG Kousseff*[1]. [1]University of South Florida Regional Genetics Program, Tampa FL.*

While there are numerous reports of anomalous chromosome 2, karyotype/phenotype correlations are lacking. Between 1/2/81 and 12/31/02 through busy (2002 patient volume 2797) prenatal and pediatric genetics clinics at the University of South Florida 42, 795 probands/families were evaluated. Forty-three had anomalous chromosome 2. Seventeen had translocations; 3 of them translocation 2;12 with different breakpoints. Two had translocation 2;5 and one of them was de novo with trisomy 21. The remaining 12 shared only chromosome 2. Six had complex chromosome rearrangements (CCR) with 3 or more breaks. There were 10 inversions, 8 pericentric and 2 paracentric. Six were maternal and 1 paternal. One of the de novo pericentric inversions was with trisomy 21. A maternal pericentric inversion was found in an individual with Neurofibromatosis type I (NF-1). Two had partial deletion 2q and 4 had partial duplication 2q, all with different break points. One each had deletion 2p, duplication 2 p and mosaic 46,XY/47,XY,+mar (2 cen ->2q12) respectively. A pregnant woman had a history of miscarried fetuses with 45,X and 93,XXXX, +2, respectively. The probands were 31 Caucasians, 7 African-Americans, 4 Hispanic and 1 East Indian. Twenty-five were seen in the pediatric clinic and 17 through the prenatal clinic. The six CCR probands showed 3 breakpoints in 4 and 1 each had 5 (Clin Genet 42: 135-42, 1992.) and 8 breaks (Amer J Med Genet 26: 771-782, 1987) respectively. The study illustrated the wealth of cytogenetic, clinical and outcome findings of patients with anomaluous chromosome 2. It also emphasized the need for similar studies attempting to define karyotype/phenotype correlations.

Disclosure(s): None

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Domino liver transplantation as both temporary resolution and definitive treatment for a neonate with factor V Leiden hepatic veno-occlusive disease. *L Krueger*[*1,7], A Tsai[2], G Griffin[3,7], S Toth[4], A Casas-Melley[5,6,7], K Falkenstein[5,7], C Marando[1,7], S Dunn[5,6,7]. [1]Molecular Genetics, Cellular & Tissue Transplantation, Nemours Biomedical Research, [2]Department of Surgery, York Hospital, York, PA , [3]Division of Hematology and Oncology, [4]Technology Applications, Pyrosequencing, Incorporated, Westborough, MA , [5]Division of Transplantation, [6]Division of General Surgery, [7]Alfred I. duPont Hospital for Children, Wilmington, DE .*

Liver transplantation as a curative measure for factor V Leiden (fVL) induced thrombophilia is rare in adults. Indications for hepatic replacement for children with thrombophilia caused by fVL are undetermined. We report a neonatal case with a prenatal diagnosis of hepatic vein occlusion secondary to fVL-induced thrombosis. Transplantation was indicated because of acute hepatic decompensation secondary to veno-occlusive disease. Although live-donor (LD) liver transplantation was considered, the size constraints of the neonate precluded the use of an adult segmental allograft. The patient received an interim transplant with a segment of a liver from a child with primary oxalosis that was genotypically wild type for factor V. The allograft functioned well, and no new thrombotic events occurred despite the discontinuation of anticoagulation during the post-operative period. Subsequently, a definitive maternal LD liver transplant (genotypically normal factor V) was performed at four months. The patient continues to do well and has survived for 20 months without recurrent thrombosis. Although the child remains heterozygous for the fVL mutation, coagulation studies confirm that he is phenotypically normal. Liver transplantation with a wild type donor corrects fVL thrombophilia. As more coagulopathies and metabolic diseases become amenable to LD transplants, genetic diagnosis and precise molecular monitoring will be actively required. Given that it was the availability of the domino transplant that allowed our patient to proceed to a living donor transplant, it may be beneficial to develop standards for determining when the outcome of transplantation is preferable to alternative management. Consensus among practitioners and implementation of this strategy await agreement on appropriate clinical justifications, risk-to-benefit analyses, documentation of favorable outcomes and other significant factors. This case underscores the risks associated with the fVL allele for thrombophilia within the liver transplant population. The presence of this allele is approximately 7% in Caucasians and variable in other populations. Although thrombotic events are clearly multi-factorial, fVL is the most common inherited form of thrombophilia. Clearly the role of liver transplantation for thrombophilia in the absence of intrinsic liver disease is controversial. In fVL patients in whom the risk of thrombosis or actual thrombosis and its sequlae are significant, hepatic transplantation must be carefully considered. Additionally, recent anecdotal data suggests that in individuals requiring hepatic transplantation for other reasons, the effect of a donor liver with an abnormal fVL allele may lead to frank disease. This calls attention to the possibility that recipients of hepatic allografts from donors with fvL may not be without consequence. These patients sustain a new “transplantable” life-long risk for suffering the complications of fVL.

Disclosure(s): None

CLINICAL GENETICS POSTERS

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Clinical diagnosis of Down syndrome using dermatoglyphics: a wonderful but underused method. *Y Lacassie*[*1,2]. [1]*Department of Pediatrics, Louisiana State University Health Sciences Center, New Orleans, Louisiana,* [2]*Children's Hospital, New Orleans, Louisiana.*

In 1926, Dr. Harold Cummins coined the name “dermatoglyphics”. He was the first to recognize, in 1936, that patients with Down syndrome have distinct finger prints and palmar configurations. Since then his findings have been corroborated and expanded, even among patients of different racial groups, by many other researchers from different countries. Different scores have been delineated. Between 1973 and 1975, while working at Johns Hopkins, I had the opportunity to improve the Hopkins Composite Score created by Digamber S. Borgaonkar et al. The original Hopkins score had been devised as a quick and easy method by which the clinical diagnosis of Down syndrome patients could be confirmed by a predictive discrimination analysis of the dermal patterns. The Hopkins Composite Score was the best dermatoglyphic test at that time. Although we introduced some new traits, the number of patterned areas was substantially reduced and the codes simplified. The size of the sample was increased and dependence between different related areas was considered. This Hopkins Revised Score (1976) allowed us to correctly diagnose 99 percent of a new population of patients with full trisomy 21 from Louisiana and Santiago, Chile. This score performed much better than the other tests with which it was compared, showing it to be an excellent diagnostic aid. This score was named the Revised Hopkins LS-Score. In the last 25 years, the use of this dermatoglyphic score has allowed the correct diagnosis in patients with full trisomy and mosaicism, even in very premature babies. In this presentation, I will explain the use of the Hopkins LS Score, present some clinical cases stressing the importance of the evaluation of dermatoglyphics as part of the physical examination, and show its efficacy not only in Down syndrome but in several other genetic syndromes and malformations.

Disclosure(s): None

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Macrocephaly–cutis marmorata telangectasia congenita: how far to expand the clinical phenotype? *JL Lauzon*[*1], *D McLeod*[1]. [1]*Department of Medical Genetics, University of Calgary, Calgary AB, Canada.*

Macrocephaly-Cutis Marmorata Telangectasia Congenita (M-CMTC) is becoming a well recognized overgrowth syndrome since it first descriptions by Moore and Clayton-Smith. As the syndrome's name suggests the main features are macrocephaly and cutis marmorata. Other important features include CNS malformations (hemimegalencephaly, ventricular asymmetry and/or hydrocephalus), hypotonia, generalized and segmental overgrowth, mid face capillary malformation, and syndactyly/polydactyly. We present a case of a 10 month old boy with many features of M-CMTC without cutis marmorata. Our patient was born to non-consanguineous patients of Bosnian descent. There were no pregnancy complications. He was delivered at 37 weeks gestation by C-section. His birth weight was at the 90th percentile and his head circumference of 40 cm was well above the 97th percentile. He was noted to be hypotonic at birth and continues to be so. He is currently unable to sit independently. On physical exam he had significant macrocephaly (head circumference > +6 SD) and generalized macrosomia. His facial features include frontal bossing, a large anterior fontanelle, down-slanting palpebral fissures, infraorbital creases, prominent ears, and a philtral nevus flammeus. He is also noted to have inverted nipples and 2-3 toe syndactyly bilaterally. He has had excision of a right post auricular hemangioma and an admission to hospital for cyanotic episodes of unknown cause for which he continues to require oxygen. Cutis marmorata has never been noted. Investigations have included a normal karyotype (600-650 band-resolution), normal bone age and baseline metabolic investigations. An initial CT scan of his head was normal at 2 months of age but he has since developed significant hydrocephalus requiring surgery. We are aware of three other patients in the literature that have been reported as having M-CMTC without cutis marmorata. All of these patients share many of the other features seen in patients with M-CMTC. Features shared by these patients include macrocephaly, prominent forehead, facial nevus flammeus and CNS abnormalities including hydrocephalus. Syndactyly is found in three of these patients (including our patient). This suggests that cutis marmorata is but one feature that may be seen in this syndrome that shows variable phenotypic expression. Alternatively, this and the three other patients described in the literature may have an undescribed syndrome.

Disclosure(s): None

CLINICAL GENETICS POSTERS

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Clinical features of two individuals with monosomy 1p36 and Angelman syndrome.

P Lenglet[1], A Rope[1], D Viskochil[1], H Torisu[2], T Yamamoto[3]. [1]Department of Pediatrics, Division of Medical Genetics, University of Utah, Salt Lake City, Utah, [2]Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan, [3]Division of Medical Genetics, Kanagawa Children's Medical Center (KCMC), Yokohama, Japan.*

We report on 2 unrelated girls with monosomy 1p36 and Angelman syndrome due to an unbalanced transmission of a maternal balanced chromosomal translocation. To our knowledge the combination of Angelman syndrome in an individual with deletion 1p36 has not been previously described. This presents a rare opportunity to compare and contrast manifestations of the well-described entities on a divergent genetic background. Case 1 presented with generalized hypopigmentation, ataxic movements, intractable seizures with characteristic electroencephalographic abnormality compatible with Angelman syndrome, large anterior fontanelle, severe psychomotor retardation and seizures. Her karyotype was 45, XX, der (1) t(1;15)(p36.31;q13.1),-15 derived from maternal translocation. Case 2 was ascertained in the newborn period with the presentation of respiratory distress, apnea, feeding difficulties, hypotonia, and multiple minor anomalies. Additional evaluation revealed cardiomyopthay, ventricular septal defect, bilateral optic nerve hypoplasia, gastroesophageal reflux, and seizures. The patient's karyotype was 45,XX, der(1) t(1;15)(p36.1;q11.2),-15.ish der(1)t(1;15)(CEB108/T7-,SNRPN-,D15S10-). Methylation analysis revealed a paternal-specific DNA pattern consistent with Angelman syndrome. In both cases, the unbalanced chromosome rearrangement was inherited from a maternal balanced translocation. Both are noted to have seizures, psychomotor retardation, and multiple minor anomalies with overlap of dysmorphic features. An overlap of clinical features in Angelman and deletion 1p36 syndromes has been noted. In this early stage of analysis we are unable to determine if these will have an additive effect. Anticipatory guidance for individuals with Angelman and monsomy 1p36 syndrome should include cardiac, ophthalmologic, audiologic and neurologic evaluation.

Disclosure(s): None

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Ataxia and oculomotor apraxia (AOA): belated diagnosis in two sibs.

JG Leroy[1,2], F Roelens[2], J De Meester[2], M De Rammelaere[2], E Devos[2], P De Bleecker[3]. [1]Baylor College of Medicine, Department of Molecular & Human Genetics, [2]MPI "Dom. Savio", Gits, Belgium, [3]Ghent University Hospital, Ghent, Belgium.*

Over twenty-five years ago cerebellar ataxia was diagnosed in a dozen children in a special school for the physically handicapped in Western Belgium. In about half of them physical and neurological examination, recurrent pulmonary infections, analysis of serum markers and a steadily progressive course with fatal outcome before or in adolescence, supported the diagnosis of ataxia telangiectasia, which could not yet be confirmed by demonstrating mutations in the ATM gene. In two of the longer surviving unrelated patients, preadolescent onset Friedreich ataxia was recognized on clinical grounds and confirmed by showing homozygosity for the GAA trinucleotide expansion in intron 1 of the FRDA gene encoding frataxin. Two male siblings, members of the original patient group, were children of consanguineous parents. From admission they had significant oculomotor apraxia associated with ataxia, severe scanning of speech and nearly absent DTRs. They have not suffered from recurrent grave infections and maintained aided ambulation until the 30th birthday. They became wheelchair-bound due in part to peripheral neuropathy. MRI showed cerebellar atrophy, but normal supratentorial anatomy. Normal intelligence was unaltered. Albuminaemia was low but within the control range. Also the cholesterolaemia was within normal limits. In both patients the homozygous transition 837G>A was found in exon 6 of the aprataxin gene, located on chromosome 9p13 resulting in the non-sense mutation W279X and the premature translation stop of the HIT/Zn-finger protein, aprataxin. This result was obtained in the laboratory which characterised the AOA1 gene (Moreira et al,2001, Nat Genet 29: 189) and has since reported on the AOA2 gene located on 9q34 (Moreira et al, 2003, AJHG, 73, S174) associated with a type of ataxia-oculomotor apraxia with a later clinical onset. Much earlier molecular sorting of childhood ataxia, now available, will be challenged mainly by its genetic heterogeneity.

Disclosure(s): None

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Folate anatagonist (methotrexate) embryopathy syndrome in a 14 years patient. *J Llerena, Jr*[*1,2,3], C Barbosa[1], F Vargas[4], J Cabral de Almeida[1,2]. [1]Departamento de Genética Médica, Instituto Fernandes Figueira, FIOCRUZ - Rio de Janeiro, Brazil, [2]Unidade de Citogenética Humana/IBCCF[a]/UFRJ – Rio de Janeiro, Brazil, [3]Serviço de Genética Médica, Faculdade de Medicina, UNIGRANRIO - Rio de Janeiro, Brazil, [4]Divisão de Genética/CPQ, INCA - Rio de Janeiro, Brazil.*

An obstetric ultrasound performed at 6th month gestation revealed hydrocephalus. A 46,XY karyotype was detected in amniocytes. At birth, a male patient was born at 8 months gestation weighting 1.800g and measuring 40 cm and subsequently submitted to a peritoneal ventricular derivation. At 4 months of age a severe craniosynostosis was surgically corrected. He walked at 5 years old and febrile convulsions until 7 years of age were frequent. At 14 years old he was referred to our unit for clinical evaluation. The patient was found to be mentally retarded with severe speech handicap. At physical examination he was very short and microcephalic (both below the -2SD). A conspicuous phenotype was observed comprehending cranial, facial, hands and feet malformations. Craniofacial abnormalities included abnormal calvaria with abnormal skull shape. Striking dysmorphic features with hypertelorism, small palpebral fissures, abnormal shape of the eyebrows, hypoplastic midface, and abnormally shaped and placed ears. Both hands had been surgically corrected for partial syndactylies; both thumbs were long, specially the right one. Bilateral oligodactyly of feet with only three rudimentary toes present. His external genitalia was normal for his age. A 3D CT scans detected several craniolacunae. His mother's gestation was unplanned; she had a left breast cancer surgically removed and followed a three chemotherapy cycle with methotrexate protocol unaware of her 16 weeks pregnancy. Methotrexate is a methylated derivative from aminopterin that acts reducing DNA synthesis through competitive inhibition of folic acid reductase. The critical susceptible teratogenic period in pregnancy is considered to be about the eighth to ninth week of gestation. At least 24 well documented cases are known in the literature and hydrocephalus, abnormal calvaria, craniolacunae, abnormal skull shape, and craniosynostosis are considered as cardinal clinical features. Hand and feet abnormalities and variable degree of mental retardation have been reported. Increased use of this drug to treat different conditions such as cancers, psoriasis and rheumatoid arthritis are current. For this reason and regarding that unplanned pregnancies are considered to be more than half of all recognized pregnancies (Wise, 2001 – BMJ 322, 1510) the necessity of the clinical awareness of these observations seems imperative.

Disclosure(s): None

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Complex psychiatric phenotype associated with duplication 8q22q24. *J Macayran*[*1], M O'Conner[2], J Gray[2], B Ciarimboli[2], P Rao[3,1], K Dipple[4,1]. [1]Department of Pediatrics, University of California, Los Angeles., [2]Department of Psychiatry and Biobehavioral Medicine, University of California, Los Angeles., [3]Department of Pathology and Laboratory Medicine, University of California, Los Angeles., [4]Department of Human Genetics, University of California, Los Angeles.*

Family history is one of the strongest risk factors for the development of child psychiatric disorders. Much work has been done to find the genetic basis of these complex disorders including cytogenetics, linkage, and whole-genome scans. Chromosomal rearrangements are often used to help find possible positions of candidate genes that predispose to illness. We report a patient with a complex psychiatric symptom presentation including attention deficits, hypomania, behavior disturbances, developmental delay, and mild dysmorphisms with duplication of 8q, that arose as an unbalanced translocation due to a maternal 15p8q translocation. This 7-year-old Caucasian male was born at 42 weeks to a 19-year-old gravida 3, para 0, spontaneous abortion 2 mother. Breech presentation required external version. Delivery was normal. Birth weight was 4.8kg (>95th centile). Motor milestones were met on time, but receptive and expressive language delays were present. A hearing deficit was discovered and corrected, although he continues to require speech therapy. He demonstrated significant developmental deficits in adaptive and cognitive functioning, with a low-average IQ. Behaviorally, he presents with severe attention deficits, hypomania, impulsivity, aggressive behavior, and hypersexuality. Due to his developmental delays and behavioral disturbances, he is enrolled in special education classes. Family psychiatric history is significant for ADHD, dyslexia and potential mood disturbance with mania. A younger maternally-related half-sister is normal. Maternal family medical history is significant for spontaneous abortions of male offspring. Paternal psychiatric and medical history is unknown. Hospitalization occurred at seven years for aggressive and hypersexual behaviors. Head circumference 52cm (50th centile), with bitemporal narrowing. Height 135.8cm (>95th centile) and weight 30.4kg (90-95th centile). Eyes were hypoteloric. There was a thin vermillion border. Fifth digits and toes had bilateral clinodactyly. Nails were hypoplastic and there were broad first toes bilaterally. Neurologic examination was normal except severe distractibility, hyperactivity, and poor speech. Cytogenetic analysis revealed 46,XY,add(15)(p11.2). Mother's karyotype revealed a balanced translocation 46,XX,ins(15;8)(p11.2;q22.3q24) demonstrating an insertion of an interstitial segment of 8q material on 15p. When passed onto the propositus, along with two normal chromosome 8s, the result is partial trisomy for 8q. This rearrangement was confirmed by whole chromosome 8 painting and FISH analyses with the c-myc oncogene (8q24) and ETO (8q22). Futher fine mapping of the breakpoints with analysis of other family members will help to determine the role of 8q duplication in psychiatric disorders.

Disclosure(s): None

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A cryptic full mutation in a male with a classical fragile X phenotype. *JJ MacKenzie*[1,2], *IV Sumargo*[1,2], *SA Taylor*[1,2]. [1]*Department of Pediatrics, Queen's University, Kingston, Ontario, Canada,* [2]*Department of Pathology, Queen's University, Kingston, Ontario, Canada.*

Fragile X syndrome (FRX) is the most common inherited cause of mental retardation. The incidence is approximately 1/4000 males and half as many females. Mosaicism has been reported in 12-14% of male cases. Indications for molecular genetic testing include specific physical features, developmental delay, autism and a positive family history. Classically, cases of FRX can be detected by analysis of the FMR-1 gene using DNA extracted from peripheral blood. We present a 47 year old male with the typical FRX phenotype referred for an evaluation of developmental delay and psychiatric disease. FMR-1 analysis had previously been carried out on DNA extracted from peripheral blood using PCR and Southern blot analysis. The results revealed that the proband carried a premutation sized allele of 58 CGG repeats. Due to the compelling clinical phenotype, testing was performed on DNA extracted from skin fibroblasts, which yielded a repeat size of 500 CGG repeats, a full mutation. Mosaic cases of FRX have been reported but rarely without detectable mosaicism in peripheral blood. The symptoms in the reported individual were severe such that the mutation had a significant phenotypic effect even though the premutation allele was unmethylated and presumably functional. We concur with others that testing of ectodermally derived tissues may provide improved diagnosis and perhaps better insight into the overall prognosis of the affected individual. This case demonstrated the need to consider further study on other tissues when there is a strong clinical suspicion of fragile X syndrome.

Disclosure(s): None

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Bilateral microtia with absence of external auditory meati, Mondini type malformation, duodenal atresia, thyroid hemiplasia and biliary atresia: a new syndrome? *GH Maegawa*[1], *D Chitayat*[1,4], *H Whyte*[2], *M Thomas*[4], *P Kim*[3], *J Kim*[2], *PJ McNamara*[2]. [1]*Division of Clinical and Metabolic Genetics,* [2]*Neonatal Intensive Care Unit,* [3]*Paediatric Surgery Department of Hospital for Sick Children, Toronto, Canada,* [4]*Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Toronto, Canada.*

We report a female born at 36 weeks gestation with following multiple abnormalities coarse facial features, bilateral microtia with absence external auditory meati, duodenal atresia (DA), intestinal malrotation, anterior displacement of the anus, left hemiplasia of the thyroid and biliary atresia. The patient was born to East Indian healthy and non-consanguineous parents. The couple's first pregnancy was terminated for sirenomelia while the second pregnancy resulted in a healthy son. The patient was the product of the couple's third pregnancy. The mother is a 32-year-old whose pregnancy was initially uncomplicated. MSS was done at 20.3 weeks and showed an increased risk for Down syndrome (1:356). Amniocentesis showed a normal female karyotype (46, XX). A third trimester ultrasound showed IUGR and double bubble indicative of DA. Her birth weight was 1550 g (<3rd centile), length was 45 cm (10th centile) and OFC was 30.5 cm (< 2nd centile). At birth, microcephaly and bilateral microtia with absence of the external auditory meati were noted. A left eye esotropia was evident. There was an asymmetric cry with deviation of the mouth to the right side, micrognatia and anterior displacement of the anus. Surgery was performed on day 3 and showed complete DA with a blind-ended jejunum (long pedicle) in the left lower quadrant and intestinal malrotation with Ladd's bands. Cholescintigraphy (HIDA SCAN) revealed no evidence of biliary drainage suggesting biliary atresia. The TSH was elevated at 32.5 (0.5-5.00 mU/L) and thyroid scintigraphy revealed poor left lobe uptake. The neck ultrasound confirmed hemiplasia of the left thyroid lobe. The brain MRI/MRS was normal. The CT-scan for petrous bone showed bilateral "Mondini" type deformity, bilateral absence of external auditory canals and internal fused ossicles. The associations of DA with intra and extra-hepatic biliary atresia have been reported (Martinez-Friaz et al 1992; Anneren et al 1998; Danesino et al 1999). However, the association with ear anomalies and the thyroid malformation has not been reported. We thus suggest that this constellation is a hitherto new syndrome.

Disclosure(s): None

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Autism and novel duplication in 15q11-13 GABRB3 region. *L Martin*[*1], J Johnson[2], W Wells III[2], L Meisner[2]. [1]Northwest Illinois Regional Perinatal Center-Rockford Health System, Rockford IL, [2]Wisconsin State Laboratory of Hygiene, University of Wisconsin, Madison, WI.*

Autism is classically described as a type of pervasive developmental disorder (PDD)characterized by repetitive and stereotypic behaviors, impairment in interpersonal and social relationships and communicative skills. We report here a 7 year old boy who presented with developmental delay (DQ 50 at 5 years),aggressive behavior with anger control issues, and a history of autistic like behaviors: not cuddly as a baby, speech delay, no friend, sleep disturbance with a lack of sufficient REM sleep. Family history is remarkable for maternal uncle and maternal great uncle with speech delay, odd behaviors,and "slow intellect". Physical examination was noteworthy for short stature (50%tile for 4 year old at 6 10/12 years) and deep set eyes. **CYTOGENETICS:** High resolution karyotype: 46,XY. VysisToTelVysion telomere panel did not detect any deletions or rearrangements affecting the telomeres. Analysis of the Prader-Willi/Angelman locus utilizing the FISH assay with SNRPN and D15S10 probes did not detect any duplication or deletion. Similarly, the UBE3A/D15S10 probe used together with a 15q telomere specific control, demonstrated two signals in all metaphase and interphase cells examined. However, with the probe for GABRB3, which is within 15q11-13 but distal to UBE3A, there were three signals in all 30 interphase cells examined, although the duplication was not visible on metaphase cells due to spatial compaction. **DISCUSSION:** Autism is an apparently complex genetic disorder where only 5-10% of cases are felt to be "secondary" autism. 1-3% of children have been found to have duplications in the 15q11-13 region, most often seen with isodicentric 15q or, more rarely, a duplication detected with the SNRPN gene, implicating a more proximal region than was observed here. Using linkage analysis, Buxbaum and others have demonstrated an association between autistic disorder and the GABRB3 gene, though others have not. This is the first known report of a patient with a duplication of this locus on chromosome 15.

Disclosure(s): None

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Cystic hygroma due to duplicated region on chromosome 9 derived from a maternal pericentric inversion. *L Martin*[*1,2], T Lynn[1], C Vitali[2], I Haroun[1], G Headley[1,2], M Tyrkus[2], R Hume[1,2]. [1]Northwest Illinois Regional Perinatal Center-Rockford Health System, [2]Rockford Memorial Hospital, Rockford, IL.*

CASE REPORT: We present a patient who was initially ascertained via an abnormal maternal serum analyte screen. A comprehensive ultrasound at 18 weeks EGA revealed an isolated, unilateral, multiseptated cystic hygroma. Following genetic counselling and amniocentesis (46,XX), the family elected to conservatively manage the pregnancy. At 32 5/7 weeks, the patient presented with pre-term labor, massive polyhydramnios and cystic hygroma (14 cm X 18 cm) requiring EXIT maneuver at c-section to allow endotracheal intubation prior to cord ligation. Infant at birth had the cystic hygroma, peripheral edema and normal nails. Tracheal obstruction due to left nuchal/mediastinal mass necessitated subsequent surgical resection. Aspiration of the cystic hygroma fluid was performed.**FETAL IMAGING:** Fetal echocardiography revealed no cardiac anomalies except hyper echoic focus in the left ventricle. Color-flow mapping confirmed unilateral multiseptated (cystic hygroma) mass from the midline of the mandible to the posterior neck, down the axillary lymphatic chain. **CYTOGENETICS:** Cystic hygroma fluid was submitted for high resolution karyotype. In all cells, a duplication of chromosome 9 was seen: 46,XX,dup(9)(q21.1q22.2).Examination of maternal chromosomes clearly revealed 46,XX,inv(9)(p11q13) in all but 2 of 20 cells. **DISCUSSION:** This is the first reported case involving this particular interstitial duplication, though, translocations and deletions have been seen distal to this region. Fetal mosaicism, maternal contamination, or spatial compaction may explain the amniocentesis karyotype: 46,XX. A cross over within the inverted segment seen in the maternal complement is likely to explain her offspring's chromosome 9 duplication.

Disclosure(s): None

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WITHDRAWN

CLINICAL GENETICS POSTERS

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Autosomal dominant Robinow syndrome with variable neurologic features. *E McPherson*[1], C Zaleski[1], P Giampietro[1]. [1]Marshfield Clinic, Marshfield WI.*

We report a father and daughter with Robinow syndrome, characterized by macrocephaly, hypertelorism, brachydactyly, short arms and mild short stature. The proposita presented to genetics because dysmorphic features were noted during a hospitalization for complications of status epilepticus. She had a history of macrocephaly with white matter atrophy documented at age 7 months, a seizure with residual hemiparesis at 1 year, and mild developmental delay and continued epilepsy with hypoxic encephalopathy due to complications of seizures at 2 1/2 years. Karyotype including subtelomere FISH and metabolic evaluation including VLCFA were normal. The father had first presented in childhood for evaluation of possible fetal dilantin syndrome. His mother had taken Phenobarbital and Dilantin for a seizure disorder throughout her pregnancies, but since both she and her son had hypertelorism, "fetal face" and mild short stature, dilantin embryopathy was considered unlikely and no specific diagnosis was made at the time. Another child in the family had died with hypoplastic left heart At re-evaluation the diagnosis of Robinow syndrome was made in the proposita and her father. This was based on macrocephaly, facial appearance and short limbs with supporting evidence of mild madelung deformity on X-rays of proposita and a minor conotruncal anomaly on ECHO in the father. Family photos from the original clinic visit of the father suggested the same diagnosis in the paternal grandmother. Of particular interest in this family are the neurologic complications, which are not a usual part of Robinow syndrome. Both dominant and recessive forms of Robinow syndrome exist and are similar clinically except that more severe skeletal anomalies are found in the recessive type. Recently the recessive form was found to be due to mutations in the ROR2 gene at 9q22, but the dominant form has not been mapped. Up to 20% of Robinow patients may have mental retardation, and no distinction has been made between dominant and recessive cases with respect to developmental delays. Seizures have not been previously reported, although developmental brain dysplasia was reported in one Robinow patient and external hydrocephalus in another. The proposita had mild developmental delay and "white matter atrophy" on MRI prior to her seizure onset and hypoxic episode. The father has no history of seizures but had learning disabilities in childhood, now complicated by sequelae of a head injury, and the maternal grandmother had "idiopathic" epilepsy with onset in early childhood. Since there is no other cause evident for the epilepsy or learning disabilities in affected family members, it seems likely that these neurologic problems may be part of the variable phenotype of autosomal dominant Robinow syndrome.

Disclosure(s): None

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3M syndrome: case report. *P Medina*[1], C Esmer[1], C Villarroel[1], V del Castillo[1], A González-del Angel[1]. [1]Departamento de Investigación en Genética Humana. Instituto Nacional de Pediatría, México.*

The 3M syndrome has an autosomal recessive inheritance, is characterized by prenatal and postnatal growth retardation, triangular facies, prominent forehead, bulbous and anteverted nose, thick lips, abundant eyelashes, mid-facial hypoplasia, characteristic radiologic alterations and normal intelligence. Only forty patients have been described since 1975. We present the case of twelve-year-old female twin sisters products of the second gestation from non-consanguineous young parents. The girls both weighted 1600 g and measured 40 cm at birth. They were referred at 10 years old due to short stature and dysmorphies. The clinical findings included short stature, wide forehead, triangular facies, bilateral ptosis, mid-facial hypoplasia, bulbous nose, anteverted nostrils, long filtrum, thick lips, prognathism, normal ears, short neck, low hair implantation, wide chest wall, mammary Tanner IV, normal female genitals Tanner III. Thoracic limbs with brachydactyly and bilateral clinodactyly of the fifth fingers. Karyotyping in peripheral blood lymphocytes was performed by GTG banding and the chromosomes turned out to be normal in 100 metaphases. Their bone age agreed with their chronologic age. Their pelvic and abdominal US were normal as well as thyroid function, estrogenic hormone profile, parathyroid hormone, calcium, and phosphorus levels. Blood type markers and the HLA study suggest monozygocity on the patients. The radiologic findings in both girls showed high vertebral bodies with reduced anteroposterior diameter, thin metacarpal and long bones. The forementioned findings confirms the diagnosis of 3M syndrome. These cases corroborate that the radiologic findings can define the diagnosis of the syndrome, which is important since there are not any laboratory data or molecular studies which can explain neither the etyology nor the pathophysiology of this syndrome. Diferential diagnosis must be performed with other syndromes such as Bloom, Russell-Silver, Dubowitz, Mulebrey and Fetal alcohol syndrome.

Disclosure(s): None

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Shades of gray: Roberts phocomelia versus TAR syndrome. *DT Miller*[1], D Marsden[1], V Kimonis[1], DJ Harris[1]. [1]Division of Genetics, Department of Medicine, Children's Hospital, Boston, MA .*

A male infant, with reportedly five normal prenatal ultrasounds, was delivered at term to healthy non-consanguineous parents. There were no prental exposures. Birth weight was 8 pounds 6 ounces. Absence of both upper extremities and flexion contracture and deformity of both lower extremities were noted at birth. Leukocytosis (WBC 64,500/mm3) and thrombocytopenia (15,000/mm3) were noted on the first postnatal day. Physical exam findings included normal OFC (38cm; 95th percentile), downslanting palpebral fissures, high palate, poorly fromed externa; ear, and sacral dimple Radiographs confirmed the presence of hands with short first metacarpals and fused fourth and fifth metacarpals, clinodactyly of the index finger, bilateral absence of humerus/radius/ulna, and normal vertebrae. Lower extremity radiographs demonstrated congenital dislocation of the knee with tibial with tibial hypoplasia. Ultrasound of the spine was negative for a tethered cors. A horseshoe kidney was seen by ultrasound and confirmed on MRI. Echocardiogram demonstrated a small atrial septal defect. Head ultrasound and brain MRI showed no anatomical abnormalities. Peripheral blood and bone marrow karyotypes were 46,XY with no evidence of premature separation of the heterochromatin. Diepoxybutane chromosome breakage studies were normal. In summary, upper limb phocomelia and milder lower limb defects are seen in both Roberts SC phocomelia syndrome and thrombocytopenia-absent radius (TAR) syndrome. A discussion of salient features of both syndromes and implications for future development is presented.

Disclosure(s): None

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Causes and characteristics of patients with abnormal sex differentiation and development.

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Background : Abnormal sex differentiation and development may present ambiguous genitalia in newborn or lack of secondary sexual maturations in puberty. A prompt and accurate diagnosis should be established to minimize medical, psychological and social complications. The purpose of this study is to evaluate the etiology and clinical characteristics of patients with abnormal sex differentiation and development. Method : We analysed 34 patients with complaint such as ambiguous genitalia or lack of pubertal development. Twenty-nine patients were less than 3 years old. Seventeen were considered or reared as females and 17 as males. The diagnostic evaluation consisted of physical examination, hormonal analysis, cytogenetics, sonogram, genitogram and gonadal biopsy. Result : Among the 34 patients, 11 were hypogonadism, 9 male pseudohermaphroditism (6 hypospadia, 2 androgen insensitivity syndrome), 6 female pseudohermaphroditism(4 congenital adrenal hyperplasia), 1 mixed gonadal dysgenesis, 4 micropenis and 3 congenital anomaly. Sex of rearing and gender assignment were all concordant. The chief complaints of all 3 congenital anomaly were amenorrhea and mental retardation. Of 3 patients, 1 had ring of chromosome 15, 1 had 46,XX/46,XY and 1 normal female karyotype. Conclusion : A newborn with ambiguous genitalia needs to rule out congenital adrenal hyperplasia. And careful clinical inspection and chromosome study are important to establish whether the sexual defect is a manifestation within the contest of a congenital anomaly. The causes and characteristics were variable, so patients with abnormal sex differentiation and development should be managed with an entire multidisciplinary approach.

Disclosure(s): None

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Langer mesomelic dysplasia in a patient with 45, X/46, X, idic(X). *E Moran*[1], K Hovanes[2], M Perle[3], S Kaffe[4], J Pappas[1,3]. [1]NYU-Hospital for Joint Diseases, New York, NY, [2]Esoterix Endocrinology, Calabasas Hills, CA, [3]New York University, School of Medicine, New York, NY, [4]Mount Sinai Services at Elmhurst Hospital Center, Queens, NY.*

Langer mesomelic dysplasia (LMD), a distinctive form of dwarfism, is characterized by marked mesomelic and rhizomelic limb shortening and micrognathia. We present a ten year old female with phenotypic and radiographic features of LMD and 45, X[17]/46, X, idic(X)(pll.23)[3].ish idic(X)(p11.23) (wcp X+, DXZ1x2); a karyotype associated with Turner syndrome. Our patient is a product of a full term gestation, para 2, gravida 2, delivered by repeat C-section. Short stature was evident at birth. Parents were from Bangladesh and non-consanguineous. Clinical examination was remarkable for short stature, height less than third percentile, mesomelic shortening of all limbs, micrognathia and cardiac murmur. Radiographs demonstrated marked shortening/thickening with metaphyseal flaring of femur, tibia, fibula, humerus, ulna and radius bilaterally. The tibia/fibula were more significantly involved than femurs with attenuation of proximal fibula. The ulna/radius were more significantly involved than humerus with marked attenuation of distal ulna. X-rays of skull, spine, pelvis, hands were normal. These skeletal features are not consistent with Turner syndrome. Cardiac evaluation revealed mild aortic dilatation with minor aortic valve anomaly and residual perimembranous VSD. Renal ultrasound was normal. DNA analysis for SHOX deficiency demonstrated lack of heterozygosity, indicative of whole SHOX gene deletion. Sequencing of exons failed to detect a point mutation in remaining allele. Maternal height is less than third percentile with clinically apparent mesomelia. Paternal height was normal. Chromosome analysis demonstrated 46, XX and 46, XY karyotypes. Mutation analysis on both parents failed to detect any SHOX abnormalities. Zinn (2002) postulated that LMD is caused by homozygous SHOX defects. Haploinsufficiency of one copy of short-stature homeobox gene (SHOX) located in the pseudoautosomal region of Xp22 and Yp11.3 has been demonstrated to cause Leri Weil Dyschondrosteosis (LWD) (Belin, 1998). LWD is a dominant disorder characterized by mild to moderate short stature, mesomelia and Madelung deformity. Our patient represents a severe skeletal phenotype consistent with LMD and haploinsufficiency of the SHOX gene.

Disclosure(s): None

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Novel neurometabolic condition responsive to folinic acid supplementation. *F Scaglia*[1], P Moretti[1], T Sahoo[1], K Hyland[2], T Bottiglieri[2], G Miller[3], V Ramaekers[4], N Blau[5]. [1]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, [2]Institute of Metabolic Disease, Dallas, Texas, [3]Department of Pediatrics, Baylor College of Medicine, Houston, Texas, [4]Department of Pediatrics, University Hospital, Aachen, Germany, [5]Division of Clinical Chemistry and Biochemistry, University Children's Hospital, Zurich, Switzerland.*

Two distinct transport system of folates across the mammalian cell membranes have been described. The first system is represented by the reduced folate carrier (RFC), which mediates a low affinity, high-capacity system for the uptake of reduced folates at high (μM) concentrations. A defect in this transporter may lead to hereditary folate malabsorption (MIM 229050). The second system, a family of membrane-associated folate binding proteins (FBPs), mediates a high-affinity, low capacity system and operates at low (nM) concentrations. The folate binding protein-1 (FBP-1) is localized at the basolateral surface of the choroid plexus, and has a high binding affinity for 5-methyltetrahydrofolate (5-MTHF), the biologically active form of folates in cerebrospinal fluid (CSF) and blood. A disorder of folate transport affecting this system was recently described in five children with a slowly progressive neurological disorder manifesting with psychomotor retardation, cerebellar ataxia, dyskinesia, pyramidal signs, and seizures. Treatment with folinic acid resulted in substantial clinical recovery and normalization of CSF 5-MTHF. The molecular etiology of this defective transfer of folate across the blood-brain barrier (BBB) is not known. We evaluated a five-year-old girl who presented at age 6 months with a progressive neurological condition that included developmental delay, regression of motor skills and language, loss of purposeful hand movement, cerebellar ataxia, dyskinesia, pyramidal signs, and occasional seizures. The child had a chronic progressive clinical course with fluctuations in severity. By age 5 years she was wheelchair bound, unable to use her arms, and on tube feedings. CSF analysis revealed low values for 5-MTHF. Consistent with this abnormality, we also found significant elevations of CSF homocysteine and S-adenosylhomocysteine, and decrease of S-adenosylmethionine. CSF neurotransmitters and pterin levels were normal. Folate and B12 levels and metabolism were normal in blood and peripheral tissues. Oral treatment with folinic acid resulted in normalization of the CSF abnormalities and remarkable clinical improvement with restoration of gait, hand use, and oral feeding. The biochemical abnormalities observed in this patient suggest a defective transfer of folate across the BBB. To further characterize this condition, we are currently analyzing the RFC and FBP genes, testing the binding of folate to FBPs, and measuring the intracellular flux of folates.

Disclosure(s): None

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Delineation of the phenotype of trisomy 6p/ monosomy 12p (unbalanced translocation) including novel nasal and ophthalmologic features. *M Morrisey*[*1,4,5]*, J Bateman*[1,3,4,5]*, V Durairaj*[1,4,5]*, P Kelley*[2,5]*, E Elias*[3]*. [1]Department of Ophthalmology, University of Colorado, Denver, CO, [2]Department of Otolaryngology, University of Colorado, Denver, CO, [3]Departments of Pediatrics and Genetics, The Children's Hospital, Denver, CO, [4]Rocky Mountain Lions Eye Institute, Aurora, CO, [5]The Children's Hospital, Denver, CO.*

An infant is described with dysmorphic features, poor growth, multiple congenital anomalies, and novel nasal and ophthalmologic findings to better define the phenotype associated with her unique chromosomal abnormality. **History:** KS was the 1.85 kg product of a full-term gestation to a 37 yr old G4, P1, Ab2, healthy mother. An unbalanced translocation was identified on amniocentesis, with the karyotype 46 XX, der(12)t(6:12)(p21.1;p13), and confirmed on postnatal analysis. Maternal karyotype revealed a balanced 6/12 translocation. **Physical Exam:** She was a tiny, dysmorphic infant. The examination revealed plagiocephaly, microcephaly, left coronal suture synostosis, flattening of the left temple, and narrowing of the nasal root. Her palate was highly arched, lips thin and chin microagnathic. She had right unilateral atypical choanal atresia and moderate sensorineural hearing loss with normal pinnae. She had central hypothyroidism, an atrial septal defect, 2/6 heart murmur, respiratory problems, gastroesophageal reflux disease and vesicoureteral reflux. Exam of extremities revealed unusual digits with spatulate distal phalanges and dysplastic nails; she was hypotonic in her trunk and mildly hyperreflexic in her lower extremities. Ophthalmologic abnormalities included nystagmus, severe bilateral ptosis, high myopia, optic nerve hypoplasia, and a decreased foveal reflex with macular granularity. On ocular ultrasonography, the globes had normal axial length; a vitreous strand extended to the right optic nerve head and a moderate amount of fluid surrounded each optic nerve. The electroretinogram was abnormal. Her feeding problems necessitated fundoplication and gastrostomy tube placement. Continued feeding problems and increased respiratory work led to the repair of her unilateral choanal atresia. Computerized tomography revealed not a persistent buccal membrane as is usual in choanal atresia, but a duplicated nasal septum as the cause of her nasal obstruction. **Discussion:** Partial duplication of 6p has been described in the literature and has a recognizable phenotype including intrauterine growth retardation, mental retardation, microcephaly, ptosis, and dysmorphic features. We report a patient with partial duplication 6p and similar features, but further expand the phenotype to include a detailed description of unusual ophthalmologic and choanal abnormalities. Microphthalmia, reported in other dup 6p cases, was not present in this patient. Partial monosomy 12p does not have a defined phenotype and is generally a lethal abnormality. Detailed description of phenotype and medical course in patients with rare chromosomal abnormalities is important as it can lead to improved medical management and prognostic accuracy.

Disclosure(s): None

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Triallelic inheritance of BBS genes was not evident in an Amish family with McKusick-Kaufman syndrome. *T Nakane*[*1]*, LG Biesecker*[1]*. [1]NHGRI/ NIH.*

Recently, it has been hypothesized that, for full penetrance of Bardet-Biedl syndrome (BBS), two mutations on one BBS locus are not enough, three mutations on two BBS loci are required (a triallelic inheritance model). We found an Amish family with McKusick-Kaufman syndrome (MKS), where three children were affected and had homozygous MKKS (BBS6) mutations (H84Y and A242S on both alleles) and their father was a carrier. Interestingly, their mother was a homozygote for the same MKKS mutations, but not affected, that is, she was apparently non-penetrant. This explains why this family had four of five children affected (one affected sibling died at a young age and an unaffected sibling did not participate in the study). Genotyping and sequencing of BBS1, BBS2, BBS4 and BBS7 of the family revealed no mutation in the coding region and the intronic splice junctions of these genes in the parents. Unaffected individuals having two mutations on one BBS locus (apparently non-penetrant homozygotes) are important sources for further studies to explore the inheritance of BBS/ MKS and to identify the modifier genes of BBS/ MKS. These data have interesting implications for our currently held concepts of modifiers, penetrance, and modes of inheritance.

Disclosure(s): None

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Incidence of Smith-Lemli-Opitz syndrome in Canada: results of a 36 month surveillance program. *MJ Nowaczyk*[*1,2], JS Waye[1,2], L Hunnisett[2], JD Douketis[3,4]. [1]Dept. of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada, [2]Hamilton Regional Laboratory Medicine Program, Hamilton, Ontario, Canada, [3]Dept. of Medicine, McMaster University, Hamilton, Ontario, Canada, [4]St. Joseph's Hospital, Hamilton, Ontario, Canada.*

Background. Smith-Lemli-Opitz syndrome (SLOS) is a potentially treatable inborn error of metabolism consisting of multiple malformations, mental retardation, and behavioral abnormalities. It is caused by the deficiency of 7-dehydrocholesterol reductase coded by DHCR7 gene and results in a cholesterol-deficient state. Objectives. To determine the incidence and prevalence of SLOS in Canada; to determine the percentage of mild cases of SLOS; and to determine the age of diagnosis of atypical and mildly affected patients. Methods. All physician members of the Canadian Pediatric Society were surveyed monthly for 36 months by a means of a national surveillance study. A clinical identification tool was designed to capture a broad spectrum of patients with SLOS and its phenocopies. Clinical information was obtained on all reported cases; unconfirmed cases were investigated by the measurement of plasma 7-dehydrocholesterol (7DHC) or DHCR7 mutation analysis. Results. Eighty five cases of possible SLOS were reported. Of these 35 were shown biochemically to have SLOS. Twenty two were diagnosed during the surveillance period: 14 newborns with SLOS and four older patients. All newborn patients were reported by more that one physician participating in the surveillance. All cases of SLOS were born to Canadians of Caucasian-European ancestry. Conclusion. The incidence of severe SLOS in Canada was 1:70,358. The point prevalence of SLOS (mild and severe) was 1 in 849,027 on July 1, 2001. The severity of 18% of patients was classified as mild; the mean age of diagnosis of mildly affected patients was 5.28 years.

Disclosure(s): None

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Autosomal recessive ischiospinal dysostosis with rib gaps and nephroblastomatosis. *R Olson*[*1], G Pai[1]. [1]Children's Hospital, Medical University of South Carolina, Division of Genetics and Developmental Pediatrics, Charleston, SC.*

Spranger et. al. reported in 2001 (Clinical Dysmorphology 10: 19-23, 2001) a sporadic case of Ischiospinal Dysostosis with Rib Gaps and Nephroblastomatosis in a single Caucasian male infant. The proband was born to a healthy 23-year-old couple after term gestation during which polyhydramnios and cystic enlarged kidneys were identified. The proband was noted to have mild dysmorphic feature including, high forehead, macrocephaly, hypertelorism, depressed nasal bridge, short neck and trunk, mild pectus excavatum, protuberant abdomen, lordotic gait, and palpably enlarged kidneys. His psychomotor development has been normal after an initial period of gross motor delay. A skeletal survey showed rib gaps, ischial hypoplasia, and vertebral clefts. An autosomal recessive mode of inheritance was suspected. Chromosomal analysis showed a normal male karyotype. Both kidenys were biopsied at age 8 months and showed nephrogenic rests consistent with bilateral nephroblastomatosis. Serial ultrasound examination has shown no evidence of Wilms tumor to date. During the second pregnancy of the couple prenatal studies were initiated in the second trimester. Sonography showed fetal renal enlargement and cysts similar to those seen in the proband. At birth the female infant showed remarkably similar facial features to those observed in her brother without macrocephaly and prominence of the forehead. A skeletal survey showed rib gaps, ischial hypoplasia and vertebral clefts identical to those found in her brother. Abdominal ultrasound confirmed fetal sonographic findings and subsequently renal biopsy was also found to be similar to that of her brother. Her growth and development are normal to date at age 7 months. The family history is negative for parental consanguinity. Neither parent or any close relatives are known to have skeletal or renal anomalies. These findings are consistent with autosomal recessive inheritance with lack of expression in the heterozygote.

Disclosure(s): None

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Normal intelligence and gender identity issues in a child with diploid-triploid mixoploidy.

JC Palumbos[1], JC Carey[1], CO Leonard[1], S Varga[1], E Drozdborysiuk[1], P Bayrak-Toydemir[1], J Spiegel[1]. [1]University of Utah, Salt Lake City, Utah.*

Background: Diploid-triploid mixoploidy is associated with a variable phenotype including streaky hyperpigmentation, significant asymmetry of limb length, dysmorphic facial features, and syndactyly. Most cases in the literature are reported to have developmental delay, although at least one child has had low average developmental skills. Objective: We describe the clinical, neuropsychological and chromosomal findings in a now 4 ½ year old girl with diploid-triploid mixoploidy who has normal intelligence, behavioral problems and gender identity issues. We will review the published cases that address developmental and behavioral outcome in diploid triploid mixoploidy. Results: A.P. is a normally grown infant born vaginally at term after a pregnancy complicated by ultrasound identification of an abnormality of the feet. On physical examination, there was a coarse face, prominent occiput, and macroglossia. The genitalia were ambiguous with clitoromegaly; ventral groove and introitus on the perineum. There was macrodactyly of the right foot with wide spacing of both great toes and enlargement of the right second toe. An atrial septal defect was present. Blood karyotype revealed 5/21 cells with 69,XXY (triploidy) and 16/21 cells with 46,XX. Fibroblast karytotype later showed a similar degree of mosaicism. The patient underwent clitoral resection, vaginoplasty and gonadectomy at 7 months. Pathology was consistent with a true hermaphrodism, with a right ovary and a left ovotestis. Histology showed early gonadoblastoma with dysgerminoma prompting close monitoring by oncology. Streaky pigmentation and significant asymmetry developed after infancy. Early developmental milestones were normal, but language was delayed. Behavioral problems began at 3 ½ years with increasing separation difficulties, sleep difficulties, emotional and behavioral outbursts, inability to learn from consequences and poor attention span. Neuropsychological testing showed a non-linear neurocognitive profile. On the Wechsler Preshool and Primary Scale of Intelligence (WPPSI-III), the Verbal IQ was 98, Performance IQ 127 and Full Scale IQ 111. However, there were weaknesses in the areas of attention, motor functioning, and executive functioning. The patient expressed significant gender identity issues during the evaluation and at home. She prefers male play partners, takes on male characters in imaginative play, and perseveres in stating her desire to be male and have male genitalia. Conclusions: The documentation of this patient is important for the observation of normal intelligence and gonadablastoma at an early age in a child with diploid-triploid mixoploidy. Developmental functioning is likely related to the level of mosaicism in the brain. The significance of the patient's gender identity issues at this young age is not yet clear, but could be a result of truly ambiguous genetic identity.

Disclosure(s): None

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Significant aneuploidy in two tissue types and mild phenotype in a child with tetrasomy 9p.

HE Roberts[1], H Creswick[1], P Papenhausen[2]. [1]Children's Hospital of The King's Daughters, Division of Medical Genetics, Norfolk, VA, [2]LabCorp of America, Department of Cytogenetics, Research Triangle Park, NC.*

Tetrasomy 9p is a rare chromosomal aberration reported in approximately 33 cases. The severity of the phenotype varies significantly. Most authors agree that the degree of mosaicism affects survival rates. However, it is unclear if the extent of mosaicism impacts the clinical phenotype. Case Report: The patient was born to a 31-year-old G2P1 Caucasian mother and a 38-year-old father. Prenatal care, starting at approximately 8 weeks gestation, included two normal ultrasounds. Delivery of a female infant at 41 weeks was by cesarean section secondary to failure to progress. Birth weight was 7 lbs 4 oz, length was 19.5 inches and OFC was unknown. She was discharged in 3 days without any complications. Mild axial hypotonia/developmental delay occurred during infancy. Chromosome analysis from peripheral blood lymphocytes done at 1 year showed mosaic tetrasomy 9p in 70% of the cells: 47,XX,+idic(9)(q21.1)[14]/46,XX[6]. Parental chromosomes were normal. Genetics evaluation done at 14 months at another academic center showed growth parameters to be at the 5-10th percentile. Mild facial asymmetry, enlarged midline upper frenulum, developmental delay and axial hypotonia were noted. Brain MRI, abdominal ultrasound, skeletal survey, echocardiogram and BAER were normal. The patient started physical and occupational therapies at 15 months of age. Speech therapy began at 21 months when the family moved to Virginia. The child's developmental progress improved significantly, and all therapies were discontinued at approximately 3 years of age. She was referred to our academic center for a follow-up genetics evaluation. At 3 1/2 years of age, our patient was at the 10-25th percentile for height, 25-50th percentile for weight, and 5th percentile for OFC. Cognition was normal. Physical features included mild facial asymmetry, deep set eyes, small ears, flat nasal bridge, bulbous nose and enlarged midline upper frenulum. Bilateral fourth toe clinodactyly, overlapping left second and third toes and plantar furrows with an increased gap between the first and second toes were also noted. Ophthalmologic evaluation revealed mild astigmatism. FISH studies from a buccal smear confirmed the origin of the supernumerary chromosome in 64 of 149 interphase cells analyzed (43%). Additional site-specific FISH studies are currently ongoing. The clinical phenotype among published tetrasomy 9p cases is quite variable. This is the first report of a child with significant aneuploidy in two tissues with mild clinical manifestations. This information should be useful for prenatal/postnatal counseling. However, all reported cases should be reviewed to highlight the broad clinical spectrum that exists. This report also underscores the importance of obtaining two tissue types for chromosome analysis in patients with tetrasomy 9p.

Disclosure(s): None

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Persistent Mullerian structures in a male with multiple anomalies: a new association with Robinow syndrome or undescribed condition? *E Peach*[*1], R Hopkin[1]. [1] Division of Human Genetics, Cincinnati Children's Hospital Medical Center Cincinnati,Ohio.*

An 11month male infant was evaluated for dysmorphic features including short stature, hypertelorism, down-slanting palpebral fissures, a broad forehead, mid-face hypoplasia, and a short nose with anteverted nares. Further work up revealed a patent urachus, cryptorchidism, malrotation of the colon, and bilateral partial fallopian tubes. He had a normal karyotype of 46,XY. Testicular biopsy, testosterone levels, and antimullerian hormone levels were also normal. The most unusual finding in this patient is the persistence of Mullerian structures in a male. Several syndromes have been associated with this finding including: persistent Mullerian duct syndromes type I and II, leutenizing hormone or chorionic gondotropin receptor syndrome, absence of SRY, Bardet-Biedel syndrome, camptomelic dysplasia, androgen insensitivity syndrome, and Frazier syndrome. Our patient's findings are inconsistent with these diagnoses. The facial features of our patient are suggestive of either Robinow or Aarskog syndromes. Neither of these has persistence of Mullerian structures as a common feature. However, we were able to find one previous report of this finding in a patient with Robinow syndrome. Based on this report and our patient we suggest persistence of Mullerian structures is a rare complication of Robinow syndrome. Molecular research studies for mutations in the ROR2 gene associated with the autosomal recessive form of Robinow syndrome are currently pending.

Disclosure(s): None

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Confined placental mosaicism for trisomy 2.

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Background: Mosaicism, the existence of two or more cells lines in a single conceptus, is detected in 1-2% of pregnancies karyotyped by chorionic villus sampling (CVS) at 10-12 weeks gestation. Most cases of mosaicism detected at CVS are confined to the placenta. Confined placental mosaicism (CPM) is described as an aneuploid cell line, observed in extra-embryonic tissue, but apparently not present in the fetus. Complications of CPM reported in the literature include perinatal loss, intrauterine growth restriction (IUGR), oligohydramnios and may include heart defects, renal failure and hypothyroidism if uniparental disomy has occurred. The literature has several reports of fetal IUGR and CPM for trisomy 2. We report a case of confined placental mosaicism for trisomy 2 affecting 100% of placental cells. **Case Report:** Our patient, a 43-year-old G2P0 African-American woman, was seen for genetic counseling because of a previous trisomy 18 pregnancy. She elected to undergo CVS at 11+6 weeks gestation. Following long term culture, the karyotype was determined to be 47,XY+2 in 100% of cells. Amniocentesis was performed at 15+0 weeks gestation. Chromosome analysis by the in situ method revealed a 46,XY complement in all 24 colonies. Molecular analysis of polymorphic DNA markers for chromosome 2 revealed biparental inheritance. The findings of a fetal anatomy scan and echocardiogram at 20 weeks gestation were normal. The pregnancy was complicated by oligohydramnios and progressive IUGR. Labor was induced and a non-dysmorphic male weighing 1279g (<3%tile) was delivered at 35 weeks. Standard chromosome analysis of cord blood revealed an apparently normal male chromosome complement in all cells analyzed. **Conclusion:** A fetus with a completely trisomic placenta, normal karyotype on amniocentesis and biparental inheritance of chromosome 2 may be at increased risk for oligohydramnios and IUGR, but otherwise have a normal outcome at birth. This case supports the belief that CPM for trisomy 2 can be a cause of IUGR. The infant will be followed to assess growth and development.

Disclosure(s): None

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Methotrexate teratogenicity masquerading as TAR syndrome.

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Folic acid antagonists are known causes of malformations in humans. Methotrexate and its precursor Aminopterin are folic acid antagonists which inhibit dihydrofolate reductase, resulting in cell death during the synthetic phase (S-phase) of the cell cycle. Methotrexate is now used as a prescribed abortifacient during the 6th to the 8th week of gestation. A black female newborn was noted to have bilateral aplasia of radius and fibula and prenatal-onset growth deficiency. She had shortened, bowed ulnas. The humeri and shoulder girdle were normal. Each hand had a thumb and three digits, with complete syndactyly of two of the three digits. The tibias were shortened, and there was bilateral talipes equinovarus. She had borderline low platelet count (>100,000 plt/m2) during the first few days of life, which normalized at one week. She was diagnosed with Thrombocytopenia-Absent-Radius (TAR) syndrome. No prenatal history of methotrexate exposure was given. During a hospitalization for septic arthritis at age three, history was obtained by the pediatric team of attempted abortion by injection. She was also noted at this time to have dysmorphic facial features, including long, narrow facies with prominent forehead, sparse eyebrows, malar hypoplasia, small mandible, and unilateral ptosis. The obstetrical medical records revealed that the mother received a single 100mg injection of methotrexate at seven weeks of gestation. The threshold dose for teratogenicity is 10 mg. The mother did not follow-up and did not seek prenatal care until six months later. In light of this exposure history and her characteristic facial features her diagnosis of TAR syndrome was revisited. The TAR syndrome shares with this patient the following features: bilateral aplastic radii, ulnar abnormalities, and presence of thumbs. The patient did not have the following features of TAR: abnormal humeri or the hematologic abnormalities of TAR syndrome. We conclude that this patient's phenotype and history of methrotexate exposure make a diagnosis of methotrexate teratogenicity. We recommend thorough review of obstetrical records for fetal exposure to methotrexate when faced with a small for gestational age newborn with facial and limb anomalies.

Disclosure(s): None

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Management issues in Bardet-Biedl syndrome.

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Bardet-Biedl syndrome (BBS) is an autosomal recessive disorder with complex inheritance, whose features include obesity, polydactyly, retinal dystrophy, and genitourinary anomalies. Although a well-described syndrome, there is little information available about medical management of patients with this disorder. We describe 4 patients with BBS with significant medical and behavioral issues, and discuss controversies regarding management. Our 4 cases ranged in age from 6 years to 25 years; they were 3 female, 1 male. All cases met diagnostic criteria for BBS; 3/4 had polydactyly, 4/4 had retinal dystrophy, 2/4 had major genitourinary anomalies, and 4/4 had severe obesity. Most patients reported onset of obesity in infancy, with only 1/4 reporting a slim build until 5 years of age. Body Mass Index (BMI) was extremely elevated for age, ranging from 30.4 in a 6 y.o. female to 46.9 in a 13 y.o. male. 2/4 displayed severe behavior problems regarding food, including temper tantrums over food, stealing food, and eating raw food. All 4 patients had difficulty losing weight on an outpatient basis, although 2 had been able to lose weight during inpatient hospitalizations with strict control of diet. 2/4 had never vomited, similar to patients with Prader-Willi syndrome (PWS). Three patients had significant snoring during sleep, and 1 patient had severe obstructive apnea and hypoventilation requiring use of BiPAP. All 4 patients had mild cognitive handicaps. The most severely involved individual, a 13 y.o. male, had life-threatening complications of obesity including pseudotumor cerebri, severe hypoventilation, cardiomegaly, and chronic somnolence. He was being considered for gastric bypass surgery. Exercise options were limited in teen-aged and older patients due to the combination of obesity and visual impairment. Few guidelines exist in the medical literature regarding dietary or medical management of patients with BBS. The metabolic rate and caloric need of these individuals has not been determined. We note multiple similarities to PWS, including food-seeking behaviors and lack of vomiting, and speculate that management strategies useful for patients with PWS may be helpful in selected patients with BBS. We suggest strict external controls on food intake, including locks on cabinets and refrigerators, as well as a regular exercise program which provides accommodations for visual impairment. Patients should be screened with sleep studies for obstructive apnea/hypoventilation. There is no information on whether patients with BBS can successfully undergo gastric bypass surgery. Additional study is needed to determine effective management for patients with this complex disorder.

Disclosure(s): None

CLINICAL GENETICS POSTERS

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Analysis of the GABA-A receptor alpha 1 subunit gene in Japanese patients with acute encephalitis or encephalopathy with refractory, repetitive partial seizures. *N Shiroma*[*1], S Fukuyama[1], K Katsuren[1], T Ota[1]. [1]Department of Pediatrics, Faculty of Medicine, University of the Ryukyus, Okinawa, Japan.*

Acute encephalitis or encephalopathy with refractory, repetitive partial seizures (AERRPS) is a rare disease caused by unknown etiology. In some case, overdose benzodiazepine which acts on GABA-A receptor, is effective to control of seizure. Recent studies conformed that some mutations in GABA-A receptor alpha1 subunit (GABRA1) gene alter the functional properties of GABAA receptors. We speculated GABA-A receptor was implicated in the pathogenesis of AERRPS.

Disclosure(s): None

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Visceroskeletal malformation sequence masquerading as mesomelic chondrodysplasia.
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A 14 year old male with normal psychomotor developmental history presented for genetic assessment for possible skeletal dysplasia; he had very short lower extremities. Obstetric history had been normal, parents non-consanguineous, and there was no potentially teratogenic exposure. He was considered normal as an infant, until hospitalization with overwhelming meningococcal sepsis. Situs inversus and asplenia were detected during that protracted critical illness, which involved necrosis of some fingertips and toes, requiring amputations. After recovery, he continued to grow normally except that he had growth arrest in long bones of the legs. Fibulae continued growth beyond tibiae; therefore epiphysiodeses were performed. At the time of genetics consultation, radiographs revealed flared metaphyses, bowed fibulae, very short tibiae; spine and tubular bones of the upper extremities were entirely normal. Various skeletal dysplasias result in widening of diaphyses and flaring of the metaphyses; relatively long fibulae produce bowing of the legs, but in mesomelic dysplasias there is also anomalous growth of radius and ulna. In the present case, the radiologic findings are strongly suggestive of a skeletal dysplasia, but do not allow for a syndromic diagnosis because the other bones are spared. Here, the pathophysiology involves disruption of the central epiphyses as part of the meningococcal sepsis episode. The symmetry of the defect had suggested skeletal dysplasia, but served rather as a distraction. In fact, the syndromic problem for this patient is limited to the visceral anomalies, not including skeletal anomalies. The latter are secondary to the sepsis episode, by an organism for which he was at previously unrecognized risk as a consequence of asplenia. Thus, the superficial appearance of a skeletal dysplasia results from atypical presentation of an infant with asplenia.

Disclosure(s): None

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Detection of Smith-Magenis in infancy. *SE Smith*[*1], D Marsden[2], JM Stoler[3]. [1]Brigham and Women's Hospital, Harvard Medical School, Boston, MA, [2]Boston Children's Hospital, Harvard Medical School, Boston MA, [3]Massachusetts General Hospital, Harvard Medical School, Boston MA.*

The incidence of Smith-Magenis syndrome, a microdeletion syndrome involving 17p11[1], is reported to be ~1 in 25,000, although the true incidence may be higher due to under diagnosis[2]. Older children present with a well described phenotype including a broad square shaped face, brachycephaly, prominent forehead, synophrys, up-slanting palpebral fissures, deep set eyes, broad nasal bridge, marked mid-facial hypoplasia, short, full tipped nose with reduced nasal height, prognathia, fleshy everted upper lip with a "tented" appearance and down turned corners[3]. Classic behaviors, sleep disturbances, and moderate to severe mental retardation tend to present later in childhood[4]. In infancy, the index of suspicion lies mainly in congenital abnormalities, dysmorphic features, and a hypotonic/complacent affect. Two infants of similar age presented with the diagnosis of Smith-Magenis syndrome based on cytogenetic studies. The first infant was evaluated at 39 days of life and had severe tracheomalacia, large VSD with pulmonary hypertension, severe gastroesophageal reflux, and hypersomnolence. Dysmorphic features included bitemporal hollowing, a beaked nose with a short columella, long philtrum, and a thin upper lip with no tenting, shaped in a cupid's bow. He had a depressed mid-face and significant joint contractures. Our second patient was evaluated at 26 days of life and also had severe tracheomalacia and hypersomnolence. She had Pierre Robin sequence with a cleft of the hard and soft palate, hypotonia, and strabismus. There was no cardiac disease. She had a round face, up slanted palpebral fissures, long philtrum, tented thin upper lip shaped like a cupid's bow and a full tipped nose. Both babies had posteriorly rotated, low set ears with marked thickening of the ear lobes, elongated but flattened foreheads, and epicanthal folds. After comparing their clinical features to the described phenotype in the literature, it is not clear whether these infants would have been detected at such a young age based on physical exam alone. From this study we conclude that infants with Smith-Magenis infants are mildly dysmorphic but the phenotype may vary slightly from the classic description in the literature. Since early detection leads to early intervention and awareness that presumably would benefit these children as they mature, practitioners should, therefore, consider FISH for 17p11 after a normal karyotype in dysmorphic infants with hypotonia and complacency.

Disclosure(s): None

CLINICAL GENETICS POSTERS

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Mowat-Wilson disease with unusual clinical manifestations.

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Mowat-Wilson disease is a form of syndromic Hirschsprung disease with associated microcephaly, mental retardation, and distinctive facial features. Mutations or deletions involving ZFHX1B (SIP1) are causative. Other associated congenital anomalies include congenital heart disease, hypospadius, renal tract anomalies, and agenesis of the corpus callosum. We report a case of a 13 year-old Caucasian female with typical features of Mowat-Wilson disease (Hirschsprung disease, severe mental retardation, microcephaly, VSD, and distinctive facial features) who was identified during evaluation for cystic fibrosis testing due to positive sweat chloride tests after recurrent episodes of pseudomonal pneumonia. DNA sequencing of the ZFHX1B (SIP1) gene revealed a novel nonsense mutation predicted to be pathologic for Mowat-Wilson disease. Cystic fibrosis (CFTR) DNA analysis was negative for the eighty-seven mutations analyzed. This may represent a unique manifestation of the spectrum of ZFHX1B mutations. As features of cystic fibrosis have not previously been reported in association with Mowat-Wilson disease, it may also be due to as yet unidentified CFTR mutations in this patient which await further delineation on complete sequencing.

Disclosure(s): None

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Selection avoidance may underlie common diseases.

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Various studies have suggested involvement of methylenetetrahydrofolate reductase (MTHFR) C677T homozygosity in the etiologies of orofacial clefts, cardiovascular defects, neural tube defects and chromosomal defects. We genotyped 82 orofacial cleft patients and 107 controls from West Africa for MTHFR C677T. The frequency of 677TT homozygosity was 0 in patients and 0.026 in controls. Frequencies of the C677T allele were 0.03 in patients and 0.065 in controls. In northwest Louisiana, we studied the polymorphism in 2000 newborn infants and 1000 pregnant women. There was 677TT homozygosity in 2% of African-Americans, 13% of Caucasians, and 23% of Native Americans. Frequencies of the C677T allele were 0.104 in African-Americans, 0.344 in Caucasians, and 0.591 in Native Americans. Adding our data to the known population frequencies of the C677T allele and of orofacial clefts, forms a smooth cline from Africa (low levels) across Asia (medium levels) to the Americas (high levels). Similar data are being found for alleles of other genes associated with common diseases (IGHG2, IL8, MBL, etc). We re-analyzed data of Rosenberg et al on frequencies of 9 alleles of neutral marker D13S1493, using a diversity score (0.1(1/Maximum-Minimum)). In Africa (Bantu), the score was 0.5. As the frequencies were followed across the populations, some dropped out while others became prominent, yielding a diversity score in America (Karitiana) of 0.2. Considered together, these data suggest that selection optimized allele frequencies in the sessile population of primieval Africa. As humans emerged from Africa, they were able to avoid selection in some instances by moving away from selective influences. The final populations have less genetic diversity, as well as accumulations of alleles that predispose to common diseases.

Disclosure(s): None

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Partial monosomy 11q with partial trisomy 12q: rare or missed cases? *M Ueda*[1], S Yang[2], J Karkera[1], F Lacbawan[1,2]. [1]Medical Genetics Branch, NHGRI, NIH, Bethesda, Maryland, [2]Children's National Medical Center, Washington D.C.*

We report a 10-month-old male with microcephaly, frontal bossing, left microphthalmos, posteriorly rotated small ears with irregularly helices and high arched palate, VSD, left cryptorchidism, and imperforate anus. He also had short sternum, vertebral anomaly, single palmar creases, radial anomaly with proximally placed thumbs, flat and clubfeet, sandal gap and hypotonia. VATER association was the initial working diagnosis at birth, and original karyotype was reported as normal. A karyotype with DEB was done in a different laboratory and revealed 46, XY, add(11) (q23). Parental karyotype demonstrated a paternal balanced translocation t(11;12) (q23;q24.1). Thus, the patient was determined to have a partial monosomy of 11q and a partial trisomy of 12q. The same chromosomal abnormality was present in the deceased sibling and in two paternal cousins on retrospective review of their chromosome studies. Several of the clinical features in our case are shared with Jacobsen syndrome (11q deletion) and partial trisomy 12q syndrome (Tengstrom, C., 1985). A single report of two siblings with comparable translocation (Lukusa, T., 2003) showed discordant phenotype; however, growth and developmental retardation, hypotonia, and abnormal behavior/autism were common. In contrast, seizures, which occurred in our patient, were not a problem in the reported siblings. A detailed elucidation of the breakpoints performed with a genomic DNA microarray (Spectral Genomics) revealed the breakpoints to be very close to11q23.3 and 12q24.1. Notable genes in the deleted region of 11q are PKNOX2, BARX2 and FEZ1. PKNOX2 and BARX2 are both homeobox genes which play important roles in the regulation of transcription, especially during the embryonic development. FEZ1 is required for normal axonal bundling and elongation within axon bundles. The trisomic region on chromosome 12 includes genes such as CMKLR1, DTX1 and LHX5. CMKLR1 plays a role in bone metabolism. DTX1 is a regulator of Notch signaling pathway involved in neurogenesis, lymphogenesis and myogenesis. LHX5, another homeobox gene, participates in the regulation of neuronal differentiation and migration during nervous system development. It is clear that these genes involved in this unbalanced translocation are critical for embryonic development and may explain the phenotype. Furthermore, we wonder if this chromosome abnormality is truly rare or are cases missed because of the subtle chromosome changes on routine karyotype. We also recommend that for patients diagnosed with VATER association, it would be prudent to closely examine chromosomes 11 and 12.

Disclosure(s): None

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Myhre syndrome in a female with previously undescribed symptoms. *M van Steensel*[1], C de Die-Smulders[2], P Steijlen[1]. [1]Dermatology, University Hospital Maastricht, [2]Clinical Genetics, University Hospital Maastricht.*

Myhre syndrome (OMIM 139210) is a characteristic phenotype consisting of mental retardation, pre- and postnatal growth deficiency, peculiar face with prominent mandibular prognathism, seeming muscle hypertrophy, decreased joint mobility, cryptorchidism, early-onset mixed deafness of mixed conductive and sensory type, and unspecific skeletal abnormalities. Cardiac anomalies have also been described. The syndrome has so far been described in one female and six males. Some overlap with Moore-Federman syndrome (OMIM 127200) has been discussed and it has been suggested that GOMBO syndrome (OMIM 233270) is actually Myhre syndrome. However, mental retardation in GOMBO is severe. Also, ocular abnormalities are found in both Moore-Federman and GOMBO syndromes, differentiating them from Myhre syndrome. Here, we report on a female suffering from what appears to be Myhre syndrome with some additional symptoms. The patient, a 16-year old female, was born to non-consanguineous Dutch parents. A younger brother is healthy. She presented to our department because of peculiar stiff skin. Since birth, she suffered from increasing limitation of motion of the large joints. Her psychomotor development was mildly delayed. At age four years, she underwent a pharyngoplasty for nasal speech and at age 6 she suffered from an episode of constrictive pericarditis. Bilateral deafness was noted around the same age. Her general health was good. Upon examination, we noticed decreased elasticity of the skin with hypertrophic scar formation, generalized limitation of joint mobility and short stature with a seemingly muscular build. She had a peculiar face with mandibular prognathism, maxillary hypoplasia, small mouth and deformed external ears. Skeletal X-rays demonstrated thickened calvaria and other previously described abnormalities. However, we also found several fusion vertebrae. A spinal MRI demonstrated narrowing of the caudal spinal canal. Echocardiography and an abdominal ultrasound showed no abnormalities. A skin biopsy showed no abnormalities. The karyotype was 46, XX. We diagnosed the patient with Myhre syndrome based on the prognathism, retardation and muscular build with limitation of joint motion. Stiffness of the skin and fusion vertebrae have not been previously described in Myhre syndrome. There is one report of abnormally thick skin caused by accumulation of collagen in dermal tissue but stiffness is not mentioned there. The stiffness of the skin suggests that Myhre syndrome may be a metabolic disorder as similar skin abnormalities are found in I-cell disease. Our findings expand the phenotype of Myhre syndrome and further confirm its existence as a separate entity. The occurrence in females and males supports an autosomal dominant inheritance although recessive inheritance cannot be ruled out presently.

Disclosure(s): None

CLINICAL GENETICS POSTERS

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1p36 deletion syndrome and neuroblastoma: another example. *F Vargas*[1,2], S Ferman[3], J Llerena, Jr[4], H Ramos[4], J Cabral de Almeida[4]. [1]Divisão de Genética / CPQ, Instituto Nacional do Câncer (INCA), Rio de Janeiro, Brazil, [2]Unidade de Genética, Universidade do Rio de Janeiro (UNIRIO), Rio de Janeiro, Brazil., [3]Serviço de Oncologia Pediátrica, Instituto Nacional do Câncer (INCA), Rio de Janeiro, Brazil., [4]Departamento de Genética Médica, Instituto Fernandes Figueira (FIOCRUZ), Rio de Janeiro, Brazil.*

More than 50 patients with 1p36 deletion syndrome have been described to date. Hypotonia, moderate-severe developmental retardation, speech delay and common dysmorphic features allow the clinical diagnosis. Genotype–phenotype correlation is difficult. It is known that approximately one third of neuroblastomas tumours show deletion of 1p36, usually associated with poor clinical outcome. This loss of heterozygosity suggests the presence of at least one tumor suppressor gene in 1p36 in the development of neuroblastoma. We studied an 18 year-old patient with the clinical features of 1p36 deletion syndrome who also developed neuroblastoma. Family history was unremarkable. Neuroblastoma IV-S with an abdominal mass and metastases in liver and bone marrow was diagnosed at the age of three months and treated with neo-adjuvant chemotherapy, radiotherapy, followed by surgery at age 9 months. She was mildly hypotonic, with retarded motor development and significant speech delay (first words at age four). Clinical evaluation showed horizontal eyebrows, dysplastic pinnae, synophrys, high arched palate, and small teeth. She is mildly retarded, has a pleasant personality, and is able to read and write, however, with difficulties. She has never had seizures. Prometaphase chromosomal preparation from peripheral blood at one year old raised the suspicion of partial monosomy of 1p36 but, was eventually considered normal. Recent cytogenetic re-evaluation at 18 years old searching 1p36 monosomy was negative for this deletion. We have then performed FISH studies using a set of subtelomeric probes for 1pter (14-e10) and 1qter (160-n23) that were kindly provided by Dr. J. Flint (Oxford, United Kingdom). Monosomy of 1p36 was unequivocally identified and the karyotype defined as de novo 46,XX.ish del(1)(p36)(x1 14-e10-). Microsatellite studies in the patient and her parents revealed no deletion for markers D1S214 and D1S2663. As for marker D1S2660 was not informative. Our case and a previously reported one by Biegel et al. (1993) of a constitutional 1p36 deletion in a child with neuroblastoma support the localization of a neuroblastoma tumour suppressor locus to this region. The mild degree of developmental delay in this patient suggests that the spectrum of cognitive delay in this syndrome may be greater than initially thought. Further molecular study of this patient is important, and might be helpful in identifying the putative tumour suppressor gene (or genes) associated with neuroblastoma, thus shedding some light on the etiology of this childhood malignancy.

Disclosure(s): None

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Genome abnormalities in malignant peripheral nerve sheath tumors in neurofibromatosis 1 are consistent between allelic imbalance analysis and comparative genomic hybridization (CGH) microarray. *D Viskochil*[1], M Hang[1], M Liew[1], B Cowley[1], H Zhou[2], C Coffin[2], A Brothman[1]. [1]Department of Pediatrics, University of Utah, Salt Lake City, UT, [2]Department of Pathology, University of Utah, Salt Lake City, UT.*

In neurofibromatosis type 1 (NF1), a significant number of plexiform neurofibromas have double inactivation of the NF1 gene. Malignant transformation is associated with a wide variety of genetic changes affecting non-NF1loci. We applied two methods to assess genetic imbalances in peripheral nerve sheath tumors; allelic imbalance analysis of a subset of genetic markers and comparative genomic hybridization (CGH) microarray analysis. We evaluated 2 peripheral nerve sheath tumors in one individual to establish a genetic signature of clonal expansion. A low-grade MPNST (T2000) was removed from the left thigh and two years later a high-grade MPNST (T2002) was removed from the pelvis of a female with NF1. Each tumor was randomly dissected into 4 and 5 areas for immunohistochemical analysis and DNA extraction. Quantitative genotyping at 31 selected informative markers spanning the genome was performed. DNA derived from two areas from each tumor were combined respectively and subjected to CGH microarray. Quantitative genotyping analysis revealed allelic imbalance at 20 of 41 loci in T2000 and 25 of 41 in T2002. Loss of heterozygosity was detected in 4 loci of T2000 and 7 loci of T2002. Data from the CGH microarray confirmed the genetic imbalances and deletions were detected at the sites in which loss of heterozygosity was detected by quantitative genotype analysis. There were consistent genetic imbalances for both tumors using either allelic imbalance analysis or CGH microarray, with the higher grade MPNST showing increased allelic imbalance. These shared genetic signatures indicate that CGH microarray is a valid approach to detecting genomic imbalances and further demonstrates that both tumors may have arisen from common precursor cells, either as an extension or metastasis.

Disclosure(s): None

CLINICAL GENETICS POSTERS

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Evidence supporting the multifactorial threshold model (MTM) in autism. *C Wolpert*[*1], *J Grubber, S Donnelly*[1], *H Cope*[1], *R Abramson*[2], *H Wright*[2], *J Gilbert*[1], *M Cuccaro*[1], *MA Pericak-Vance*[1]. [1]*Duke Center for Human Genetics, DUMC Durham, NC,* [2]*WS Hall Psychiatric Center Columbia, South Carolina.*

The genetic basis of autism is complex with autism occurring more frequently in males (male: female ratio of 4:1). The multifactorial threshold model predicts that a higher genetic load is necessary to cause the disorder in the less frequently affected sex. Thus, it was hypothesized that females with autism, when compared with autistic males, index a higher genetic load, resulting in increased clinical severity as measured by an earlier age of onset (AOO) and greater developmental impairment. The data set consisted of 234 families ascertained for genetic studies, including 139 families with one affected individual with autism, but no family history of autism (Fhx-)) and 95 families with a positive family history of autism (Fhx+). All individuals met clinical research diagnostic criteria for autism. The Vineland Adaptive Behavior Scales (VABS) was used to assess adaptive functioning. Lower VABS scores reflect greater severity. In the overall data (Fhx- subjects plus 1st diagnosed affected from Fhx+ families) females (N=56) showed lower VABS communication (p=0.03) and adaptive behavior composite scores (ABC; p=0.03) than males (N=178). Fhx- females (N=28) had significantly lower VABS scores than Fhx- males (N=111) on communication (p=0.002), daily living skills (p=0.013) and ABC (p= 0.009) scores while no gender differences were noted in Fhx+ families. No differences were noted between Fhx- and Fhx+ groups when collapsed across gender. Earlier AOO is also an indicator of greater severity. ADI-R items 5 and 94 measure AOO based on parent report and interviewer judgment, respectively. No significant differences between male and female AOO were noted in the overall data set for items 5 (p=0.11) and 94 (p=0.11). However, when stratified by family history status, Fhx- females showed a significantly earlier AOO than Fhx- males (item 5, p =0.03; item 94, p=0.05). No differences were noted in Fhx+ families. Finally, no AOO difference was noted between Fhx- and Fhx+ groups when collapsed across gender. In summary, females from Fhx- families show an earlier AOO and more impaired adaptive behaviors than Fhx- males. We believe these indices may have etiologic relevance in isolated or sporadic cases of autism. Furthermore, AOO, sex, and family history data have potential for use in statistical modeling to further refine the search for genetic factors associated with autism. The current findings suggest a complex relationship between severity, gender, and family history status in individuals with autism.

Disclosure(s): None

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Hereditary spherocytosis and renal tubular acidosis: a case report. *E Yokoyama*[*1], *V del Castillo*[1], *S Nieto Martínez*[2], *J Carrillo-Farga*[3], *Y Sánchez-López*[4], *F Perea*[4], *B Ibarra*[4], *A González-del Angel.*[1]. [1]*Departamento de Investigación en Genética Humana. Instituto Nacional de Pediatría,* [2]*Departamento de Hematología. Instituto Nacional de Pediatría,* [3]*Instituto de Hematopatología, México, DF,* [4]*División de Genética, CIBO, CMNO, IMSS, Guadalajara, Jal., México.*

Hereditary spherocytosis (HS) is usually an autosomal dominant hemolytic anemia, in which defects in spectrin or the proteins that attach spectrin to the membrane (ankyrin, band 3, protein 4.2) lead to spheroid, osmotically fragile cells that selectively trapped in the spleen. Some forms of HS are associated with mutations in EPB3 gene that encode band 3 or also known as red cell anion exchanger, that cause in heterozygous state typical HS associated with partial deficiency of band 3 and protein 4.2. In addition, certain mutations in EPB3 gene are responsible of dominant distal renal tubular acidosis (dRTA) without HS. We present a male patient, 2 years old, with HS and RTA, who is the third child of healthy parents who denied consanguinity, he also has two older healthy brothers. Obtained after normal pregnancy by vaginal delivery, weight 2,700gr, height 42cm, Apgar score is unknown. He was attended at our Institute due to the diagnosis of HS. During physical examination we observed dolicephaly, wide forehead, downslanted palpebral fissures, broad and depressed nasal bridge, bulbous nose, large filtrum, downturned mouth, cup ears with posterior angulation, short neck, normal thorax wall with a cardiac murmur of mitral predominance, hands with transversal equivalent and convex nails. He had breath-holding spells since 4 days old with 2 events of tonic-clonic seizures. He also has an auricular septum defect of 3mm, osteum secundum type. Since 8 moths old RTA was diagnosed, treated with bicarbonate PO with a good response. The diagnosis of HS was done by peripheral blood smear that showed spherocytes, reticulocytes of stress, echinocytes and the osmotic fragility test had an increased response. Because of associated clinical manifestations we performed karyotype from leukocytes that was normal. Both parents had a hyperchromic population of red blood cells without spherocytes and osmotic fragility test with an increased response. The HS has genetic heterogeneity and the majority of the mutations reported have been in heterozygous state. It was called to our attention that our patient has HS and RTA and only one other case has been reported by Ribeiro et. al. with both manifestations and a band 3 Coimbra mutation (GTG→ATG; V488M) that was in homozygous state. Because of the similarities between our patient and the reported case by Ribeiro et. al. we consider necessary to realize the molecular analysis of EPB3 gene.

Disclosure(s): None

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Mutation analysis of TBX5 in families with Holt-Oram syndrome: novel mutations, polymorphism and the absence of TBX5 mutations. *M Zaragoza*[*1], *G Sun*[1], *C Price*[1], *J Seidman*[3], *C Seidman*[3], *T Huang*[1,2]. [1]*Dept. of Pediatrics, Division of Human Genetics, University of California, Irvine, CA,* [2]*Dept. of Developmental and Cell Biology, University of California, Irvine, CA,* [3]*Dept. of Genetics, Harvard Medical School and Howard Hughes Medical Institute, Boston, Massachusetts.*

Holt-Oram syndrome (HOS; OMIM#142900) is an autosomal-dominant condition with congenital cardiovascular malformations and radial ray skeletal abnormalities. Cardiac defects range from atrial or ventricular septal defects to complex CHD; skeletal anomalies include thumb abnormalities such as triphalangeal thumbs to severe radial or ulnar defects. Mutations in TBX5 at 12q24.1 cause HOS. TBX5 is a member of the T-box gene family that encodes a transcription factor involved in both cardiogenesis and upper limb development. To understand the role of TBX5 in HOS, we sequenced the coding regions and the intron/exon boundaries in families referred with the diagnosis of HOS. We identified three novel splice-site mutations (Int2AS(–3)C→A, Int3AS(-1)A→T and Int7DS(-3)G→A) in four different families and a polymorphism (A to G) in the non-translating region in a Chinese family. Furthermore, we found no mutations in TBX5 in two families with evidence for linkage to 12q. Including the three new mutations, 41 different mutations have been described to date with six (15%) different splice-site mutations. These studies demonstrate that splice-site mutations significantly contribute to HOS. Further analysis is needed to determine if TBX5 polymorphism has a role in the phenotype of HOS and to search for other factors in those families without a mutation in TBX5.

Disclosure(s): None

CYTOGENETICS POSTERS

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Deletions involving 1p36: incidence and categories among 8723 patients analyzed by G-banding and 2257 patients analyzed by subtelomeric FISH probe. *A Adeyinka*[*1], E Thorland[1], S Adams[1], B Dawson[2], S Jalal[1]. [1]Cytogenetics Laboratory, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester MN, [2]Molecular Genetics Laboratory, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester MN.*

Deletion of 1p36 is a recently delineated contiguous gene deletion syndrome characterized by dysmorphic features, developmental delay and psychomotor retardation. This terminal deletion is often difficult to detect cytogenetically. Thus, the first few reports of 1p36 deletions were ascertained in patients with unbalanced translocations. Over a ten-year period (1993-2003), 8723 patients with unexplained mental retardation, developmental delays and/or mild to moderate dysmorphism had chromosome analysis by G-banding in our laboratory. Of these, four individuals were identified with rearrangements of 1p36- three had inversions of chromosome 1 and one had a der(1)t(1;13) product of an unbalanced translocation. FISH analysis using the P73 or P58 locus specific probe confirmed a change in the location of 1p36 in all three cases with inversion and loss of 1p36 in the unbalanced translocation. No cases of pure terminal deletion were identified. On the other hand, among 2257 consecutive patients with idiopathic mental retardation, developmental delay and/or mild to moderate dysmorphism that were studied from 2001 to 2003 using subtelomeric FISH probes, eight patients with anomalies of 1p36 were identified. Six had terminal deletions of 1pter and two had unbalanced translocations resulting in a derivative chromosome 1 with del(1)(pter). Three of the six patients with del(1)(pter) and one of the two with unbalanced translocations had loss of the p73 probe signal, whereas the other four retained the P73 locus, implying a breakpoint proximal to 1p36.3 in some of the cases. Molecular studies are underway to better delineate the extent of the deletions with possible genotype-phenotype correlations. The present findings confirm the difficulty of ascertaining 1p36 deletions by routine G-banded chromosomes, as this method will likely pick up only cases with structural rearrangements other than terminal deletions. Based on the more sensitive FISH subtelomeric probes and our large series of 2257 patients, del(1)(p36) appears to be responsible for 0.35% (95% confidence interval 0.2-0.7%) of idiopathic mental retardation, developmental delays and/or dysmorphism. Assuming that 2-3% of the population is affected by mental retardation of which 40% cannot be explained, the expected frequency of 1p36 haploinsufficiency would be between 1 in 20,000 and 1 in 65,000- a frequency far fewer than 1 in 5,000 reported in 2003.

Disclosure(s): None

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Xp duplication with STS gene amplification in a mildly retarded patient with minor dysmorphies. *G Arteaga*[*1], V del Castillo[1], L Gómez[1], K Nieto[1], L González[2], S Cuevas[2], C Esmer[1]. [1]Departamento de Investigación en Genética Humana, Instituto Nacional de Pediatría, [2]Servicio de Genética, Hospital General de México, México D.F.*

The present study describes a female infant with segmental aneusomy resulting from partial duplication of the short arm of the X-chromosome. She has mild dysmorphic features, congenital heart anomalies, muscular hypotonia, and is mentally retarded. Molecular cytogenetic analysis using FISH was performed employing a probe for the steroid sulfatase (STS) gene. The presence of several copies of STS was evident. The quantitative PCR analysis of the STS and KAL genes showed higher doses for the STS gene, and normal doses for the KAL gene. The X-chromosome inactivation pattern showed selective inactivation of the abnormal chromosome in 98% of the analyzed cells. The STS assay in leukocytes was higher in our patient than in the normal control (>30%). The mechanism by which clinical anomalies arose in this child with dup(Xp) is not well understood, although it could be due to the presence of several copies of the STS gene and possibly to other close genes involvement. Additional theories are the selective X-inactivation of dup(Xp) and the existence of a tissue-dependent mosaic inactivation pattern. The participation of environmental or epigenetic factors in the pathogenesis of the abnormal phenotype should be considered. This amplification is possibly the result of an aberrant recombination mediated by segmentary duplications and reciprocal to deletions that result in X-linked icthyosis.

Disclosure(s): None

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Isochromosome Yp identified in three males with developmental delay. *SM Chiu*[1], R Fetni[2], AM Duncan[2], F Halal[1], J Ortenberg[1], LJ Russell[1]. [1]Department of Medical Genetics, [2]Department of Cytogenetics, Montreal Children's Hospital, Montreal, Canada.*

Isochromosome Yp is one of the most common structural anomalies of the Y chromosome. It contains two short arms and absence of most or all of the long arm of the Y chromosome. Most are dicentric and found in mosaic form with a 45,X cell line. The phenotype of idic (Yp) ranges from phenotypic males with azoospermia or genital hypoplasia, to individuals with ambiguous genitalia, or females with some features of Turner syndrome. Developmental delay is not usually associated with rearrangements of the Y chromosome. However, one review of 46 cases from 1995 reported mental retardation in 3/20 patients (15%). The exact incidence of developmental delay in idic (Yp) is not known since many of the published cases do not report this finding. We present three additional males with non-mosaic idic (Yp) each also having developmental delay. Case 1 was seen at 20 months for evaluation of significant gross motor delay, moderate language delay and mild hypotonia. His genitalia were normal aside from right-sided cryptorchidism. The karyotype was 46,X,i(Y)(p10). Paternal chromosomes and Fragile X studies were normal. Case 2 was referred at 15 months of age because of severe language delay and gross and fine motor delays. There was a history of intrauterine growth retardation and congenital microcephaly; the father was also microcephalic. On exam, his genitalia were normal. Head CT and Fragile X studies were normal. Karyotype showed 46,X,idic(Y)(q11.2); paternal chromosomes were unavailable. Case 3 was initially seen 7 months of age due to hemihypertrophy of the right arm. On exam, genitalia were normal. At 3 years of age, he had mild speech and motor delays as well as aggressivity and oppositional behaviors. MRI of the brain was normal. Karyotype showed 46,X,idic(Y)(q11.23); paternal chromosomes were normal. The developmental delay in our three cases and those in the literature could represent ascertainment bias, since each child was referred because of a problem in development or congenital anomaly. However, they may also reflect a true association with idic (Yp). Recent sequencing of the Y chromosome has allowed the identification of more than 70 genes, many whose functions are still unknown. We raise the possibility that duplication or deletion of specific genes on the Y chromosome, such as seen in idic (Yp), could adversely affect brain development. In order to address this issue, we recommend 1) larger scale prospective studies on patients diagnosed prenatally 2) molecular studies to analyze breakpoints and to identify gene deletions or duplications, and 3) careful characterization of phenotype, including developmental problems.

Disclosure(s): None

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Cytogenetically unrelated clones in hematologic malignancies. *S Cho*[1], J Huh[1], C Seong[2], W Chung[1]. [1]Dept. of Laboratory Medicine, Mokdong Hospital, Ewha Womans University, Seoul, South Korea, [2]Dept.of HematoOncology, Mokdong Hospital, Ewha Womans University, Seoul, South Korea.*

Background : Cytogenetically unrelated clones are uncommon in hematologic malignancies because their origins are monoclonal. We evaluated the incidence and characteristics of patients with cytogenetically unrelated clones in the hematologic malignancies. Methods : Four hundred and three patients diagnosed with hematologic malignancies were included in this study ; 100 cases of AML, 44 ALL, 8 mixed lineage leukemia, 98 chronic myeloproliferative disorder (CMPD), 28 MDS, 7 CLL, 74 Non-Hodgkin's lymphoma (NHL), and 44 multiple myeloma (MM). Results : The overall incidence of cases with cytogenetically unrelated clones was 1.2%. Of AML patients, 1% was unrelated clones, MM 2.3%, NHL 1.4%, CLL 14.3%, and CMPD 1%. A 66-years-old female with AML had 46,XX,add(11)(q23) / 46,XX,add(1)(p36.3); a 69-years-old female with MM, 46,XX,+der(1)t(1;13)(p12;q12),-13 / 61-65,X,-X,+5,+7,-8,-12,-13,add(14)(q32),+15,-16,-20,+19,-22,-22; a 56-years-old male with NHL, 47,XY,der(1)t(1;11)(p36.1;q13),t(1;11)(p36.1;q13),+5 / 45,XY,t(3;12)(q21;q24.3),-21; a 72 years-old female with CLL, 46,XX,del(13)(q22) / 47,XX,+12; a 55-years-old female who treated with imatinib mesylate (Gleevec) for CML, 46,XX,t(9;22)(q34;q11.2) / 47,XX,+8. Discussion : The clinical significance of cytogenetically unrelated clones in the hematologic malignancies has not been cleared and further evaluation would be necessary. Our CML case which showed unrelated clone in philadelphia chromosome negative cells after imatinib mesylate treatment, highlights the importance of the conventional chromosomal analysis during follow up.

Disclosure(s): None

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Pseudoautosomal trisomy Xp/Yp: second case report and proposed mechanisms to explain the phenotypic variability. *H Creswick*[*1], *J Tepperberg*[2], *S Schwartz*[3], *V Proud*[1]. [1]*Div. Medical Genetics, Children's Hospital of The King's Daughters, Norfolk, VA,* [2]*Dept. Cytogenetics, Laboratory Corp of America, Research Triangle Park, NC,* [3]*Center for Human Genetics, Case Western Reserve University, Cleveland, OH.*

Trisomy for the Xp/Yp pseudoautosomal region has been reported only at ASHG in 2002 by Mak-Tam E, et al. at North York General Hospital in Toronto who described a mother and daughter with dysmorphic features and developmental delay. FISH studies showed an additional Xpter hybridization signal at the Xq terminus in addition to the normal Xq subtelomere signal. The significance was unclear since several family members were unaffected. The authors suggested skewed X-inactivation could explain the clinical variation. We report a 16 year old white male with hypertelorism, maxillary hypoplasia, hearing loss, lacrimal duct stenosis, syndactyly, short stature and significant learning and behavioral problems including ADD, OCD, and speech delay. Pregnancy in his 34 year-old G2P1 mother was complicated by decreased AFP, but the amnio karyotype was normal, 46, XY. His maternal uncle had dyslexia. Developmental milestones included sitting at 7 months, walking at 14 months and speaking at 3 years. On physical exam at 16 years, in addition to features noted above, he was at the 10th percentile for height, 40th for weight and 90th for head circumference. He had an upswept anterior hairline, high arched palate and tapered hands with short fingers. There were asymmetric deep tendon reflexes and an MRI of the brain and cervical spine showed hypertelorism, a narrow foramen magnum and cervical spinal stenosis. Bone age was delayed. High resolution banded chromosomes, Fragile X and metabolic studies were normal, however, subtelomere FISH using an Xp/Yp VYSIS probe identified an additional Xp/Yp subtelomere signal on the q terminus of the X chromosome. FISH for the subtelomere region for the Xqter and Yqter showed normal hybridization signals for both the X and Y chromosomes. Therefore, our patient had three copies of the Xp/Yp pseudoautosomal region with no apparent loss of Xqter chromosome material. Subtelomere FISH confirmed that the abnormal X chromosome was inherited from the phenotypically normal mother. Since X-inactivation is positional and not sequence-based, skewed X inactivation could explain this clinical variation and studies are underway. Other mechanisms to explain the clinical variation include duplication and/or deletion of DNA material not identified by standard cytogenetics or FISH as well as the inheritance of maternally imprinted genes near the X chromosome subtelomere. We submit that this additional X chromosome subtelomeric material is the explanation for our patient's clinical phenotype. Additional cases are necessary to clarify the implications of pseudoautosomal trisomy Xp/Yp.

Disclosure(s): None

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DNA microarrays: is it ready for clinical use?
I Teshima[*2,4], *V Dounaevskaia*[1], *G Nie*[2], *J Ren*[2], *D Chitayat*[3,4], *R Weksberg*[3,4], *S Meyn*[3,5], *L Steele*[2], *P Ray*[2,5], *D Stephens*[6]. [1]*Faculty of Medicine, University of Toronto, Toronto, Canada,* [2]*Dept of Pediatric Laboratory Medicine, Hospital for Sick Children, Toronto, Canada,* [3]*Dept of Pediatrics, Hospital for Sick Children, Toronto, Canada,* [4]*Dept of Pediatrics, University of Toronto, Toronto, Canada,* [5]*Dept of Medical Genetics & Microbiology, University of Toronto, Toronto, Canada,* [6]*Dept of Population Health Sciences, Hospital for Sick Children, Toronto, Canada.*

BACKGROUND Microarray comparative genomic hybridization (CGH) has potential to become a powerful diagnostic tool for the future Cytogenetics Laboratory. Analysis can be genome-wide. Resolution will be greatly improved. Detection of all microdeletions and currently undetectable microduplications will become possible. Microarray CGH is also rapid and can replace labor-intensive tests such as subtelomeric FISH. This new technology is a natural extension of techniques used in the Cytogenetics laboratory and will add a new dimension to cytogenetic analysis. **OBJECTIVE** To evaluate a genomic microarray with 287 targets (GenoSensor Array 300) for genome copy number in specimens from children with known chromosome abnormalities. **METHODS** Institutional Research Ethics Board approval was obtained. Specimens were as follows: trisomies (n=5); microdeletions (n=12); unbalanced structurals (n=7); and one normal male (purchased). Test and reference DNAs of opposite sex were labeled with Cy3-CTP and Cy5-CTP respectively by random priming, and co-hybridized to the microarray. Hybridized microarrays were scanned by laser excitation and emission peaks analyzed using the GenoSensor system. Mean test/reference ratios for each target were calculated and then divided by the median test/reference ratio for normalization. Thresholds for the normal range had been determined as 0.8-1.2 for these microarrays. Non modal values with p values <0.01 were considered to be positive. Number of targets showing non modal gain (>1.2) or loss (<0.8) were determined for targets within the abnormality region. Number of targets with non modal gain or loss outside the relevant area (targets with p<0.01 and outside the normal range of 0.8-1.2) were noted as false positives. **RESULTS** This DNA microarray successfully determined genome copy number in 24/25 specimens from children with known chromosome abnormalities and one normal male. Detection failed for a subtelomeric abnormality. Sensitivity was 96.0% (95%CI of 79.6%-99.9%). Detection of all possible targets within investigated regions was not always obtained. As well, false positives were found: 57 for 25 chips (41 for 23 chips and 16 for 2 chips) for an average of 2.3 per chip. Possible explanations were polymorphisms, hybridization problems, or random error (p=0.01). **CONCLUSION and COMMENT** Evaluation of this microarray using known chromosome abnormalities indicated that while genomic copy number detection was possible, some targets used for this microarray may require reassessment and targets that resulted in false positive results need to be compiled for future review. This evaluation is only the first step for our laboratory. Evaluation of another genomic microarray using known chromosome abnormalities is currently underway. Use of genomic microarrays to assess unknown genomic imbalances in children with disabilities, with an established test as confirmation, is being planned. OAML grant # SGP02-002.

Disclosure(s): Intermedico loaned the Gensosenor to read microarray work for this presentation.

CYTOGENETICS POSTERS

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Down syndrome in Egypt: back to school.

E Elsobky[1], S Elsayed[1]. [1]Cairo, Egypt.*

Down syndrome (DS) is the most common and best-known chromosomal disorder and is the single most common genetic cause of mental retardation. Governmental care of this syndrome and other handicapping conditions has increased tremendously in the past few years to the extent that DS phenotype has became a phobia and many parents and/or physicians referred normal babies for karyotype for suspicion of chromosomal anomalies or for reassurance of their parents. On the other hand, prenatal screening is still inaccessible to most families and most cases of Down syndrome are diagnosed postnatally. In this paper we present the first and the largest study on DS patients referred from different regions and discrete all over Egypt aiming to look for possible causal factors for this high birth rate, and to study the response of parents and clinicians to the new screening programs and prenatal diagnosis. The study included 1100 patients referred as DS, 1030 cases were confirmed by cytogenetic analysis to be DS. Most of these cases (98.43 %) were diagnosed postnatally and only 1.56 % were detected prenatally while 0.01 % were products of conception. Their ages ranged from one hour to 30 years with mean of 351 days. Males represented 54.13 % while females represented 45.87 % of the studied group. Mean maternal age at conception was 31.8 years for cases with non- disjunction and 24.5 years for cases with translocation. All mothers of cases of translocation DS were under 35 years, in contrast to mothers of non- disjunction cases in which 41.48 % were above 35. Paternal age ranged from 19 to 62 years with mean of 36.5 years in non-disjunction cases and from 24-35 years in translocation cases with mean of 30.6 years. Consanguineous marriage was present in 12 % of cases. Positive family history was present in 6 % of cases. Most of cases were the first or the second in order of birth, and the most common cause of referral was dysmorphic features in live births and advanced maternal age in prenatally referred cases. Karyotype revealed that 93.98 % of cases had trisomy 21, 3.5 % of cases had translocation and 1.84 % had mosaicism. Non- classical karyotype was present in 7 cases (0.68 %). Most of the cases of translocation were t (21; 21), which was present in 51.35 % of cases, followed by t (14; 21), which was present in 40.5 % of cases, t (13; 21) in 5.4 %, and t (15; 21) in 2.7 % of cases of translocation. In conclusion, in Egypt with 1.6 million births / year and estimated risk of 2285 DS births annually, the concept pf preventive genetics should be reinforced with a national policy targeting both health professionals and general publics to offer prenatal genetic screening for all pregnant ladies and prenatal diagnosis for screen positive cases. This needs an integrated system including proper facilities, trained personnel and professional staff.

Disclosure(s): None

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Prevalence, age of onset, and characterization of seizures in 90 children and adults with isodicentric 15 syndrome.

BM Finucane[1], EW Simon[1], LW Brown[2]. [1]Genetic Services, Elwyn Training And Research Institute, Elwyn, PA, [2]Pediatric Neuropsychiatry Program, Children's Hospital Of Philadelphia, Philadelphia, PA.*

Over the past decade, a recognizable clinical syndrome has emerged in people with duplications of the Prader-Willi/Angelman Critical Region (PWACR) on chromosome 15. The most common karyotype involves a supernumerary maternally-derived inverted duplication (isodicentric 15) which results in partial tetrasomy of 15q11-13. The clinical phenotype includes a subtle but characteristic facial appearance, hypotonia, intellectual disability, and a high incidence of autism spectrum disorders. The PWACR is known to encompass a cluster of gamma-aminobutyric acid receptor subunit genes (GABRB3). The GABAergic system has been implicated in epilepsy, most recently in people with Angelman syndrome. A handful of case reports have described seizures in children with isodicentric 15 syndrome, but a systematic study of seizure prevalence and characteristics in this population has not been published. We used a questionnaire to survey parents of children and adults with isodicentric 15 involved in the U.S.-based support group IDEAS*. The questionnaire was distributed by mail to the IDEAS membership and also posted to the support group's online website. We received 90 responses from parents of individuals with a cytogenetic diagnosis of isodicentric 15. The cohort included 52 males and 38 females, ranging in age from 9 months to 24 years, with a mean age of 7.2 years. Fifty-four percent of the cohort had experienced at least one seizure. Age of onset of the first seizure ranged from birth through 18 years with a mean of 2.8 years. Fifty-five percent of those with seizures had onset by 1 year of age, with 82% having onset prior to age 5. Parents reported multiple seizure types in their children, including generalized tonic clonic (18%), absence (12%), and myclonic seizures (11%). Sixteen percent of the cohort, accounting for 29% of those with seizures, had a history of infantile spasms. Many children experienced more than one seizure type, and several had severe, intractable epilepsy. When surveyed about the impact of the child's seizures on his / her quality of life and functioning, 51% of parents reported a minor impact, 16% a moderate impact, and 33% a major impact. We conclude that seizures represent an important medical feature of isodicentric 15 syndrome. The prevalence of infantile spasms among our cohort was unusually high and suggests that isodicentric 15 syndrome could account for a significant percentage of infants experiencing these episodes. As in Angelman syndrome, abnormal GABRB3 gene expression is a likely contributor to the seizure phenotype in individuals with isodicentric 15. Additional studies are needed to further characterize seizures in this population and to study the efficacy of medications which influence GABAergic neurotransmission. * IsoDicentric 15 Exchange, Advocacy, and Support: www.idic15.org

Disclosure(s): None

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Monocentric, inverted duplication of 8p due to a maternal paracentric inversion: phenotype and proposal of a mechanism. *SR Forrester*[1], JB Ravnan[2], MC Schneider[1]. [1]Southern Illinois University School of Medicine, Springfield IL, [2]Genzyme Genetics, Santa Fe NM.*

Classically, individuals heterozygous for paracentric inversions have little risk of producing chromosomally unbalanced offspring since meiotic recombination usually produces acentric and dicentric chromosomes. We report a 17 month old boy with a monocentric chromosome 8 with an inverted, duplication in the short arm [46,XY,inv dup(8)(p23.1p12)ish dup(8)(wcp+, D8S504+, D8S574+)] whose mother is heterozygous for a paracentric inversion [46,XX,inv(8)(p12p23)]. Features of the child concordant with other reports of duplication of 8p include normal birth parameters, normal visceral anatomy, and severe encephalopathy characterized by developmental delay, brain malformations, hypotonia, and strabismus. He has similar dysmorphic features which include frontal bossing, hypertelorism, a thin upper lip with prominent lower lip, high arched palate, widely spaced teeth, and low set ears. This case is unique since it is only the third reported of an inverted, duplicated 8p arising from a paracentric inversion. Additionally, it lacks the concomitant terminal deletion that is typical of inverted, duplicated 8p. We propose a molecular mechanism of anomalous recombination in maternal meiosis that is preceded by misalignment of inverted repeat segments resulting in this pure trisomy 8p.

Disclosure(s): None

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Partial trisomy of 15pter-q15 and 10q24-qter segments in a newborn compared to the duplication 10q syndrome. *G Gowans*[1], T Berry[1], K Christensen[1], J Hersh[1], F Yen[1]. [1]Weisskopf Child Evaluation Center, University of Louisville, Louisville, KY.*

We describe a Caucasian female infant with a 47,XX,+der(15)t(10;15)(q24;q15) karyotype, resulting in trisomy of the 15pter-q15 and 10q24-qter segments. Patient was born at 36 weeks gestational age to a 30 y/o G4P2 with the pregnancy complicated by gestational diabetes. The infant has dysmorphic facial features, redundant skin to the posterior neck, wide spaced nipples, a pectus excavatum, a short sternum, external rotation of the lower left leg, and dysplastic toes of the left foot. The infant is compared to previously reported patients with duplication 10q syndrome that have the same partial trisomy of 10q24-qter. The more commonly described physical features of duplication 10q syndrome were identified in our patient including ptosis, short palpebral fissures, and abnormal foot position. A duplication of the distal segment of the long arm of chromosome 10 has been described in combination with other chromosomal duplications or deletions, but not in combination with chromosome 15pter-q15. Our patient is also compared to reported cases of partial trisomy of chromosome 15pter-q15.

Disclosure(s): None

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Frequency of the inversion encompassing the Williams-Beuren syndrome region in parents and in the population. *HH Hobart*[1], RG Gregg[2], CB Mervis[3], BF Robinson[5], KW Kimberley[1], CM Rios[1], CA Morris[4]. [1]Molecular Cytogenetics Laboratory, Dept Pediatrics, Div Genetics, U Nevada School of Medicine, Las Vegas, NV, [2]Dept. Biochemistry and Molecular Biology and Center for Genetics and Molecular Medicine, U Louisville, Louisville, KY, [3]Dept Psychological and Brain Sciences, U Louisville, Louisville, KY, [4]Dept Pediatrics, Div Genetics, U Nevada School of Medicine, Las Vegas, NV, [5]Georgia State U, Atlanta, GA.*

Williams-Beuren syndrome (WBS) is a contiguous gene microdeletion syndrome, occurring at a frequency of 1/10,000 births. By molecular cytogenetic analysis using research probes for genes within the deletion region, the overwhelming majority of cases demonstrate identical deletions, the 'classical' deletion. The classical deletion encompasses approximately 1.5Mb of DNA and 20-25 genes. Two recent studies found inversions of the classical deletion region in a percentage of a small sample of parents transmitting the deleted chromosome, but no general population data for the inversion were reported. The inversions are mediated by two sets of three differentiated blocks of repetitive sequence at both ends of the classical region. **METHODS:** A dysmorphologist examined 129 individuals with WBS and/or reviewed their medical records. When possible, lymphoblastoid cell lines were established on proband and parents. The extent of the deletion in probands was evaluated using a battery of 14 fluorescent in situ hybridization (FISH) probes. Molecular studies using polymorphic markers were performed to determine which parent transmitted the deleted chromosome. Interphase FISH analysis using cosmid and BAC probes determined inversion status of 198 unaffected individuals including 129 transmitting parents. Inversion studies used combinations of three FISH probes in three colors: one probe (yellow) hybridized to a locus outside and adjacent to the deletion region and the other two probes (red, green) hybridized to loci within the deletion region. Studies of individuals with initial results suggesting inversion were repeated using a probe outside the other end of the deletion region for confirmation. **RESULTS:** This study includes a larger sample of WBS cases and parents than previous studies, thus providing better estimates or confirmation of the earlier results. We found a paternal bias (55%) in the parent of origin of the deleted chromosome. Our frequency of inversion heterozygosity in transmitting parents, 25%, is in general agreement with previously reported studies, but a sex bias has not been reported. In our sample, 35% of the transmitting fathers were inversion heterozygotes vs. 14% of the transmitting mothers, a ratio of 2.5:1. To date from our sample of 68 non-transmitting parents, the general population frequency of inversion heterozygotes is 7.4%, with no apparent sex bias.

Disclosure(s): None

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Involvement of chromosome 6 in cases with bad obstetric history, primary and secondary infertility. *S Komandur*[*1], S Movva[2], R Varma[1], V Mohan[1], V Kodati[3], Q Hasan[1,2]. [1]Kamineni Hospitals, L B Nagar, Hyderabad, India, [2]Bhagawan Mahavir Medical Research Centre, AC Guards, Hyderabad, India, [3]Vasavi Medical And Research Centre, Lakdi-ka-pul, Hyderabad, India.*

Infertility is a common problem affecting about 13-18% of couples worldwide. Primary infertility can be defined as total amenorrhea or failure to conceive, while Secondary infertility/Bad obstetric history is reduction in actual number of offspring produced. Chromosomal anomalies have been observed in individuals belonging to all the three categories.Individuals with numerical sex chromosomal abnormalities usually exhibit primary infertility, while those with structural chromosomal aberrations are expected to produce conceptuses with a greater number of chromosomal anomalies than the general population resulting in abortion(s), stillbirth(s) and neonatal deaths, as well as children with congenital malformations. In the present study 8 individuals and 34 couples (n=68) referred for cytogenetic analysis since January 2003 to our Genetics Units were included. A detailed case history was taken for all the individuals along with a three-generation pedigree. Peripheral blood was cultured according to the modified method of Moorhead et al. Karyotyping was done after G-banding for each case and the following results were obtained. We observed a higher percent of chromosomal anomalies earlier reports, this maybe due to our stringent patient selection criteria. The study included cases after ruling out anatomical abnormalities, endocrine dysfunction, infection and other obvious reasons, which could be responsible for infertility. Hence, we have focused our efforts towards identifying only those individuals having solely cytogenetic abnormalities as the reason for infertility. To our surprise in retrospective analysis we found that 4/8 (50%) cases with primary amenorrhea and 9/68 (13.2%) individuals with fertility problems showed a chromosomal abnormality involving the 6th chromosome, however, the chromosomal breakpoint was not identical in all cases. This over-representation of chromosome 6 anomalies (ie18.4%) in our sample showing fertility impairment suggests that this chromosome maybe carrying either specific genes or important domains essential for normal fertility.

Disclosure(s): None

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Karyotyping and KaryTtutor©: the effectiveness of novel e-learning tools in teaching and training students and staff to karyotype. *A Freyer*[*2], A Kotwaliwale[3], G Lowther[1], V Dagar[3]. [1]RHSC Yorkhill Hospital, NHS Trust -- United Kingdom, [2]Humon Corporation -- California, [3]Humon Corporation -- United Kingdom.*

The healthcare industry strives to provide early detection and prevention of clinical disease. Chromosomal analysis is an effective system from which to identify a myriad of diseases such as trisomes (i.e., Down's Syndrome), unbalanced constitutional karyotypes, and chromosome rearrangements associated with malignant disease. The first level of genetic screening is often acquired through cytogenetic analysis. Early detection, the diagnosis of diseases, and the subsequent counseling of patients remain the mainstay of genetic clinics. The quality of care offered is dependent on the quality of training. Teaching diagnostic skills requires a combination of theoretical knowledge and practical experience. The variation in samples and preparation necessary requires a system that can provide unlimited practice examples. With a training system such as KaryoTutor© one can provide standardized training, thereby providing the practice and skill-set necessary for maximum efficiency. Cytogeneticists analyze chromosomes by preparing a karyotype, a size-ordered alignment of isolated chromosome pairs. The laboratory procedure for isolation of chromosomes remains protocol driven, but the process of chromosome identification requires a high degree of training. As a visually demanding skill, trainees are usually taught on both image analysis systems and directly through microscopes. Automated image analysis systems, whilst very sophisticated, cannot recognize many of the chromosomal rearrangements encountered without considerable interaction by a trained cytogeneticist. Furthermore, automated systems are ineffective as training tools, as the chromosomal identification is completed by a system rather than a trainee. Adversely, teaching students via a microscope ensures student interaction, but often requires senior laboratory staff to participate as well. The process of training new scientists to accurately perform chromosome analysis remains difficult, tedious, and expensive. A system built specifically for karyotyping can ensure higher training standards. KaryoTutor is one such system built specifically to teach karyotyping, and is not limited to “pre-coded” examples. The tutor can load any images of chromosomes in metaphase and mark the necessary chromosomes. The student can then create a karyotype on the same cell under test conditions. The tutor can also add custom ideograms. At the end of this process KaryoTutor will qualify the responses. This tool works in a standard web-browser making it possible to access metaphases and perform karyotypes remotely. A system such as KaryoTutor ensures consistent standards and adds a new level of training to the cytogenetic arena. Clinical information can be easily amended to the karyotype, thereby allowing students and staff to explore the linkages between clinical characteristics and genetic data. The skills taught become more than just learning protocol; the trainee can practice the analytical and deductive skills necessary for accurate clinical diagnosis.

Disclosure(s): The research for this presentation investigates the effects of a commercial tool from Humon Corporation on current teaching standards. Presenting author is employed as the Director of Marketing for Humon Corporation, the company which has developed the software that the research topic is based on.

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Constitutional rearrangement of the architectural factor HMGA2: a novel human phenotype including overgrowth and lipomas. *AH Ligon*[1,4], SD Moore[2,4], M Mealiffe[6,7], H Ferguson[5], M Parisi[6,7], DJ Harris[3,4], BJ Quade[1,4], CC Morton[1,2,4,5]. [1]Department of Pathology, Brigham and Women's Hospital, Boston, MA, [2]Department of OB/GYN and Reproductive Biology, Brigham and Women's Hospital, Boston, MA, [3]Department of Medicine, Division of Genetics, Children's Hospital, Boston, MA, [4]Harvard Medical School, Boston, MA, [5]Partners Center for Genetics and Genomics, Boston, MA, [6]Division of Genetics and Development, Children's Hospital and Regional Medical Center, Seattle, WA, [7]University of Washington, Seattle, WA.*

The goal of the Developmental Genome Anatomy Project (DGAP) is to identify developmentally critical genes by studying individuals with chromosomal rearrangements and at least one major congenital abnormality. The three major components of DGAP include fluorescence in situ hybridization (FISH) mapping of chromosomal breakpoints, molecular analysis of candidate genes in the vicinity of breakpoints, and creation of animal models to study novel genes identified to validate the respective phenotypes. Progress in DGAP includes 135 cases ascertained, 69 breakpoints FISH-mapped, with 33 of these positioned on the human genome map within a single BAC clone, and 10 candidate genes identified. One case for which a candidate gene has been identified is that of a 9 year-old male with a de novo pericentric inversion of chromosome 12, with breakpoints in p11.2 and q14.3. His phenotype includes facial dysmorphism, extreme overgrowth of postnatal onset, arthritis, multiple cutaneous lipomas, a stable cerebellar tumor, advanced endochondral bone and dental ages, and markedly enlarged epiphyses with platyspondyly and bilateral bowing of the lower limbs. Somatic rearrangements of HMGA2 (formerly HMGIC), a gene encoding a DNA-binding, non-histone architectural factor, play an important role in the pathobiology of benign mesenchymal tumors. Breakpoint mapping analysis showed HMGA2 to be disrupted by the q arm inversion breakpoint in this child. This rearrangement results in physical separation of the AT-hook DNA-binding domains from the acidic carboxy terminus of the protein. Similar truncations of HMGA2 have been modeled previously in mice. These transgenic animals develop somatic overgrowth and, in particular, increased abundance of both histologically normal adipose tissue and adipocytic tumors. The physical features observed in the child mirror many of those reported in transgenic mice expressing a truncated HMGA2 consisting of only the DNA-binding domains. Analysis of the p11.2 breakpoint has failed to identify any candidate genes in the immediate vicinity, with the nearest gene mapping ~82 kb away. The possibility of a position effect accounting for some of the phenotype cannot be excluded. This report represents the first example of a constitutional rearrangement affecting HMGA2.

Disclosure(s): None

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Mosaic trisomy/ring 13 causing unusual polydactyly, oligodactyly and cutaneous pigmentary abnormalities. *S Lillis*[1], L Cooley[1], M Dasouki[1]. [1]Section of Medical Genetics and Molecular Medicine, The Children's Mercy Hospitals and Clinics and the University of Missouri-Kansas City School of Medicine, Kansas City, MO.*

An African American male infant presented at birth with multiple congenital anomalies including mild dysmorphic facial features, hypo & hyper-pigmented circular skin macules, limb anomalies (single forearms bones bilaterally, resembling radii, a decreased number of osseous structures in the hands with two metacarpals, and left hand and foot post-axial polydactyly), a tethered spinal cord and small ASD. Blood chromosome analysis revealed two abnormal cell lines involving chromosome 13: mos 46,XY,der(13)t(13;13)(p11.2;q12.3)[14]. ish der(13) t(13;13) (WCP13+),13q14(RB1x3)/ 46,XY,r(13)(p11.2q12.3)[7].ish r(13)(WCP13+), 13q14(RB1x1). The trisomic cell line was found in 67% of cells examined. Cytogenetic studies done on skin fibroblasts obtained from both affected arms and a hypo-pigmented macule showed the same mosaic chromosomal abnormality with 80-90% of the cells carrying trisomy 13. No cells with normal chromosomal complement were found. This is an extremely rare post-zygotic complex multi-step cytogenetic error which had been reported in only five cases in the literature. Unlike the few other patients reported in the literature, and despite the predominance of the trisomic cell line detected in blood and skin fibroblasts, our patient's anomalies are relatively mild compared to what is expected in patients with complete trisomy 13 syndrome. Also, while both arms appear to be affected differently, the cytogenetic abnormalities were similar. The combination of the limb anomalies and the unusual cutaneous findings appear unique to this patient and add to the clinical phenotype associated with this rare syndrome.

Disclosure(s): None

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A case of a de novo balanced reciprocal translocation (7;8) with a congenital strawberry hemangioma detected by prenatal ultrasound.

I Llano[1], D Mayen[1], G Razo[1], A Berron[1]. [1]Mexico city Mexico.*

A reciprocal translocation is an interchange of genetic material between two non homologous chromosomes. They are one of the most common chromosome structural rearrengement detectable by cytogenetics, with a prevalence of 1 in 500-625 individual in the general population. They may arise de novo during gametogenesis or as a result of a balanced carrier parent. Most of the people with a balanced reciprocal translocation are phenotypically normal since there is no loss of genetic material. However, some observations suggest that we cannot rule out some submicroscopic rearrangements with deletion or duplication of material in the breaking points. We report a case of a newborn male, born of consanguineous 44 year-old father and 40 years-old mother, G5C2A2, who underwent amniocentesis for karyotype because of advanced maternal age. This result was 46,XY t(7;8)(q31.2;q24.1) in 15 cells. Parents' karyotype is reported normal in both. During gestation, the mother suffered from gestational diabetes type A1 controlled with a 1600 kcal diet starting at week 33 by LMP. Prenatal US at week 30 reports: Tumor over the internal region of the left fetal knee which in subsecuent US grew in size and wasreported on week 37 as a solid and vascularized mass without other fetal abnmormalities dtectable by US. The pregnancy was interrupted by cesarean section at week 38 with out complications. The noenatal care unit received a healthy newborn male with a strawberry hemangioma on the inner face of left knee which limited the movements, soft, vascularized and with necrosis areas. This type of lessions are benign neoformations found most frequently in infants. It appears durong the first weeks or months of life, grows quickly and after a rest phase regresses. It does not have a genetic inheritance. Newborn patients with a known balanced reciprocal translocation and with a congenital strawberry hemangioma are very infrequent in the neonatal period. The breakage points in the translocation are presumed to involve a tumor supressor gene. Could they be associated or is it only a coincidence?

Disclosure(s): None

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Clinical indications for subtelomeric studies.

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Subtelomeric studies with Vysis ToTelVysion probe panel were performed in 329 cases referred for cytogenetic analyses due to mental retardation, dysmorphic features or multiple congenital anomalies. Some patients also had a family history of mental retardation and/or multiple miscarriages. Eleven cryptic aberrations were found in cases with normal karyotypes and six rearrangements were suspected by cytogenetic analyses and the subtelomeric studies either confirmed the deletion or identified the origin of the half cryptic rearrangement. The following cryptic aberrations were detected by this technique: a deletion 4p including the telomere and the Wolf-Hirshhorn critical region in a 2 years old female with developmental delay, failure to thrive, dysmorphic features and microcephaly; another deletion 4p including the telomere only in a 5 years old male with developmental delay and hyperactivity and in his mother who has been diagnosed with bipolar disorder; a deletion 4q in a 4 years old female with speech delay and dysmorphic features; a deletion 9q in a 9 years old female with mental retardation, autistic behavior and dysmorphic features; a deletion 12q in a newborn male with cleft lip/cleft palate and congenital heart defect; a deletion 13q in a 7 years old female with borderline intelligence, speech disorder, poor motor coordination and dysmorphic features; a deletion 22q in a 4 years old female with ataxic gait, developmental delay, obesity and dysmorphic features; a deletion Yq and trisomy Yp in a 4 years old with developmental delay and radioulnar synostosis; a der(1)t(1;8) causing monosomy 1p and trisomy 8q in a 4 years old male with microcephaly, dysmorphic features, developmental delay and ataxic gait; a der(13)t(5;13) with monosomy 13q and trisomy 5p in a 4 years old female with developmental delay, microcephaly and Factor VII deficiency; a variant der(15)t(15p;Yq) in an 11 years old male with mental retardation and severe behavior problems. The other rearrangements suspected by cytogenetic analyses were: a deletion 1p in a 6 months old female with growth retardation, congenital heart defect and craniofacial asymmetry; a deletion 2q in a 2 years old female with developmental delay, dysmorphic features and brachycephaly; a deletion 17p in a newborn female with Miller-Dieker syndrome; a der(8)t(8;13)(p23;q22) in a 14 months old male with craniofacial asymmetry, developmental delay and hypotonia; a t(7;8)(q36.3;q22.3) in a child with cleft lip and palate, hypodontia, microcephaly and seizures; an inherited translocation t(4;11)(q23;p15.3) in the normal mother and her child with Beckwith Wiedemann syndrome. Phenotype/genotype correlations are the most important aspects of this report.

Disclosure(s): None

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"Unstable" mosaic chromosome 15q11-13 duplication in an adolescent female with seizures, mental retardation and hyperpigmented skin lesions. *M Lund*[1], L Cooley[1], M Dasouki[1]. [1]Sect Medical Genet & Molec Med, Children's Mercy Hosp, Kansas City, MO.*

An inverted duplication of chromosome 15 is the most common supernumerary marker chromosome in humans. They are exclusively of maternal origin. The phenotype associated with inv dup (15) generally includes seizures, mental retardation or learning disabilities, autism spectrum disorders, and minor facial dysmorphia, such as epicanthal folds and hypertelorism. A 15-year-old female presented with severe mental retardation, attention deficit hyperactivity disorder, and a history of seizures. Prior evaluations included a normal head MRI, normal female peripheral blood chromosomes and an abnormal EEG. Physical exam noted mild bilateral epicanthal folds and a slightly narrow nose. A large, somewhat discontinuous, hyperpigmented area with irregular margins was noted to extend from the left inguinal area and cover the left thigh while a smaller area was noted on the right knee with some central clearing. A skin biopsy was obtained from the hyperpigmented area and fibroblast cytogenetic analysis revealed: mos 47,XX,+inv dup (15)(q15) [11].ish inv dup (15) (D15Z1x2, SNRPNx3, GABRB3x4) / 46,XX [9].ish 15q11.2q13 (SNRPNx2). Subsequent blood chromosomal analysis revealed low level mosaicism (approximately 4.5%) by FISH analysis for the same inv dup (15). The bi-satellited marker was large and contained 3 extra copies of the SNRPN and 4 copies of the GABRB3 loci as shown by FISH analysis. Even the largest inv dup (15) reported has breakpoints more proximally located than the marker in this case and no reported cases with multiple copies of the SNRPN and GABRB3 probes within the marker are found. Mechanistically it has been suggested that large inv dup (15) chromosomes result from a U-type exchange involving particular sequences between homologous chromosomes or sister chromatids followed by non-disjunction.

Disclosure(s): None

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Infant with a de novo complex chromosomal rearrangement involving chromosomes 3, 5, 6, 7 10 and 18 and a minimum of ten breakpoints.

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We report a unique, de novo, complex chromosomal rearrangement in a 16-month-old Hispanic female with minimal dysmorphic features. She was the third pregnancy for her now 37 year old father and 33 year old mother. There were no problems or complications with the pregnancy. There was no history of any drug, alcohol or cigarette exposure. Family history is unremarkable. There are two older siblings whom are phenotypically normal. She was born SGA at term. This patient presented to our clinic at age 16 months with height, weight and OFC all [less]5th centile. There was severe developmental delay, microcephaly, strabismus, mid-face hypoplasia, bilateral 5th finger clinodactyly, bilateral single palmar creases, severe truncal hypotonia and bilateral overlapping toes (the 2nd and 5th over the 3rd and 4th). A head CT showed absent corpus callosum and was otherwise normal. Conventional cytogenetic analysis of a peripheral blood specimen from the patient showed a complex karyotype. Multiple structural aberrations were present involving chromosomes 3, 5, 6, 7, 10 and 18. The nature of these rearrangements was further elucidated by fluorescence in situ hybridization (FISH) of whole chromosome paint probes for all chromosomes involved. Cytogenetic evaluation of peripheral blood specimens from both parents were normal, confirming the de novo nature of this rearrangement. Although the etiology of this rearrangement is unknown, it is noteworthy that the most significant phenotypic features of this patient are small size, profound hypotonia and severe developmental delay with an absence of major dysmorphic facial features.

Disclosure(s): None

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A familial balanced reciprocal translocation t(4p;14q) resulting in complementary abnormal karyotypes in two offspring. *J McGowan-Jordan*[*1], *P Chakraborty*[1], *M Shago*[2], *S Nikkel*[1]. [1]*Department of Genetics, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada,* [2]*Department of Paediatric Laboratory Medicine, Hospital for Sick Children, Toronto, ON, Canada.*

We report a family with a balanced reciprocal translocation and two abnormal offspring with disparate abnormal phenotypes. The family was originally identified through the proband who presented with a classic Wolf-Hirshhorn phenotype. This individual was found by FISH to have a deletion of the Wolf-Hirschhorn syndrome critical region (WHSCR), at chromosome 4p16.3. Examination of parental chromosomes revealed that the father of the proband carried a balanced reciprocal translocation between the distal portion of the short arm of chromosome 4 and the distal portion of the long arm of chromosome 14. His karyotype was reported as 46,XY,t(4;14)(p16.1;q32.33). Thus, the proband had an unbalanced chromosome complement: 46,XX,der(4)t(4;14)(p16.1;q32.33). The paternal 1st cousin of the proband has been followed in a genetics clinic. She has profound mental retardation with cerebral atrophy; a prenatal karyotype was reported as normal. Her mother (the proband's aunt) had had a previous child die with multiple congenital anomalies (bilateral colobomas and cleft palate) and a normal karyotype. The proband's aunt was found to carry the same balanced reciprocal translocation as her brother. Chromosome studies were repeated and showed that the cousin of the proband has the complementary abnormal karyotype to the proband: 46,XX,der(14)t(4;14)(p16.1;q32.3). Due to the cryptic nature of the reciprocal translocation in this family, it is likely that without prior knowledge of the translocation, the unbalanced chromosome complement would have gone unrecognised.

Disclosure(s): None

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Subtelomeric FISH and karyotype analysis of 108 autistic probands. *J Morrissette*[*1], *L Medne*[1], *R McDaniell*[1], *M Souders*[2], *A Gupta*[1], *I Krantz*[1], *S Levy*[2], *E Zackai*[1], *NB Spinner*[1]. [1]*Division of Human Genetics and Molecular Biology, The Children's Hospital of Philadelphia, Philadelphia, PA,* [2]*Division of Child Development, The Children's Hospital of Philadelphia, Philadelphia, PA.*

The development of molecular probes for detection of subtelomeric chromosomal rearrangements by FISH has lead to the emergence of submicroscopic telomeric rearrangements as an important cause of human malformations and neurodevelopmental disabilities. Autistic spectrum disorder (ASD) represents a broad range of cognitive and neurobehavioral impairments that cause qualitative deficits in socialization, communication and behavior. ASD can be associated with various genetic syndromes and chromosomal abnormalities. In the vast majority of patients, ASD is non-syndromic without an identifiable genetic etiology. We performed subtelomeric FISH analysis in 108 patients with ASD in addition to standard karyotyping and Fragile X testing, with all individuals examined by a clinical geneticist to rule out a recognizable syndrome. We found 8 individuals (7.4%) with chromosome abnormalities: three individuals with unbalanced translocations (2.7%): 46,XX,der(8)t(4;8) (p14;p21); 46,XY, der(4)t(4;4)(p12q28); 46,XY.ish der(18)t(13;18)(q34;q23)pat; two with balanced translocations: one inherited from his phenotypically normal mother, 46,XY, t(3;4)(q25;q27)mat, the other inherited a Robertsonian translocation from his phenotypically normal father, 45, XY, t(13;14)pat; one individual with an acrocentric variant, 46,XY, 15p+, with normal telomeres; and one with an inversion, 46,XX,inv(7)(q22.1q34)mat; and one 47,XYY. We suspect that the etiology of autism in both the Robertsonian translocation carrier and the individual with 47,XYY is due to other factors, not related to the chromosome differences. The chromosomal abnormality was cytogenetically detectable in 7/8 patients (the der(18),t(13;18) was cytogenetically invisible). The der(18) patient also had several structural defects and dysmorphia and a family history. Based on the findings of this study, we believe subtelomeric FISH analysis is not indicated in patients with isolated ASD without additional findings, such as dysmorphia, structural malformations or family history. This work is supported in part by grants from the Foerderer Fund for Excellence at the Children's Hospital of Philadelphia (RM and JJDM), and by grants from National Institutes of Health MO1 RR-00240 (JJDM). This project was conducted as part of the Pennsylvania Center for Autism and Developmental Disabilities Research and Epidemiology (PA-CADDRE) and supported by a grant from the Centers for Disease Control and Prevention (U10/CCU3220394-01) at the University of Pennsylvania School of Nursing and The Children's Hospital of Philadelphia, Philadelphia, PA.

Disclosure(s): None

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Definition of useful cut-offs for HER2 fish analysis of clinical breast tumor samples. *R Mueller*[1], R Parkes[1], F O'Malley[2,3]. [1]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, [2]Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, [3]University of Toronto, Toronto, Canada.*

Background: HER2 (human epidermal growth factor receptor 2) is a transmembrane glycoprotein whose activation transmits signals for growth to the cell nucleus. High levels of HER2 protein expression as a result of HER2 gene amplification occur in 25% of breast tumors and are associated with high proliferation rates and poor prognosis. HER2 status is used to determine patient eligibility for Herceptin (a humanized version of a mouse monoclonal antibody that targets the molecule for therapy) and may be useful to predict responsiveness to other therapies such as tamoxifen, taxanes and anthracyclines. HER2 status in formalin fixed, paraffin-embedded tumor tissue samples is commonly assessed using immunohistochemistry (IHC) to detect protein expression. When suboptimal tissue fixation in a paraffin embedded sample has compromised HER2 IHC results, fluorescence in situ hybridization (FISH) analysis of HER2 gene copy number can be used to resolve HER2 status. In FISH analysis, 60 nonoverlapping, invasive breast tumor cells are scored for the number of HER2 and chromosome 17 (CEP 17) fluorescent signals; this ratio is 1 in cells with a normal gene copy number of HER2 and >2 when the HER2 gene is amplified. **Design:** For a group of 270 breast tumor samples submitted for clinical diagnosis, we recorded individual cell FISH scores and evaluated the contribution of tumor cell heterogeneity to the overall results for each sample. We created statistical models to compare HER2/CEP17 ratios or HER2 copy number alone to determine which model most clearly predicted both positive and negative immunohistochemistry results obtained using the antibody CB11. **Results:** The ratio of HER2/CEP17 was superior to HER2 copy number alone in predicting CB11 positive immunohistochemistry results. A HER2/CEP17 FISH ratio cutoff of 2.66-3 most sharply demarcated samples that had positive IHC results from negative IHC results. Moving the cutoff from 2 to 3 improved the overall concordance rate from 89.7% to 93.7% for this study group. For ratios ranging from 2.0-2.66, only 4/19 cases were positive by CB11, while in the range 1.8-2.2, only 1/16 was positive by CB11 and in samples with ratios ranging from 1.5-2, only 1/27 cases was positive by CB11. **Conclusions:** Breast tumors with a HER2/CEP17 ratio less than 3 and polysomy of chromosome 17 are unlikely to overexpress HER2. The consistent FISH ratios seen throughout the 60 cells counted per sample suggest that most tumors are homogeneous. In a minority of tumor samples, the ratio of HER2 to chromosome 17 signals is between 1 and 2 making HER2 status difficult to report.

Disclosure(s): None

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Constitutional ring chromosome 21: is there an association with acute myeloid leukemia?

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Acute myeloid leukemia (AML) has an incidence of 5.2/1 000 000 in the pediatric population. The risk increases to 1/100-200 for individuals with Down syndrome. Acquired rearrangements of chromosome 21, often involving the AML1 (CBFA2) locus, are not unusual in the presence of AML. We report a boy with developmental delay and unremarkable features who presented at age 12 with type M0 AML. He was CD33, CD13, CD34, and HLA-DR positive and Sudan Black and myeloperoxidase (MPO) negative. M7 AML was excluded, as CD 61, a marker of platelet/megakaryocytes, was negative. Initial karyotype analysis of the bone marrow showed 46,XY, r(21)[15]/ 45,XY, -21[3]/46,XY[2].ish r(21)(AML1+). Further analysis revealed that his constitutional karyotype had the ring chromosome,46,XY r(21)(p11.1q22).ish r(21)((D21S1219,D21S1220)-), and that the "normal (46, XY)" karyotype was associated with disease. This cell line disappeared with chemotherapy. He is now 7 months post chemotherapy and has worsening pancytopenia, which is now transfusion dependent. The clinical diagnosis at this time is myelodysplasia. The phenotypic presentation in an individual with a constitutional ring chromosome 21 is variable depending on the size of the material that is deleted or duplicated. There also does not appear to be phenotypic imprinting effects for chromosome 21. There have been three cases reported with constitutional ring 21 and AML. Two had M7 (acute megakaryoblastic leukemia) and the third had M0 with amplification of AML1. However, no increases in number of AML1 signals were seen in the cytogenetic studies of our patient. The two "normal" chromosome 21's seen in our patient's abnormal cell line are likely isodisomic as the telomeric region was present on both. Variable copy numbers of isodicentric chromosome 21's have been reported with AML with no increase in amplification of AML1. We wonder if there are other regions or genes on chromosome 21 that are responsible for AML when they are isodisomic.

Disclosure(s): None

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Phenotypic and molecular cytogenetic characterization of a child with terminal deletion of 7q and partial terminal trisomy of 8q [46,XY,der(7)t(7:8)(q36;q24.3)]. *J Pappas*[1], P Patsalis*[2]. [1]New York University, School of Medicine, Human Genetics Program, [2]The Cyprus Institute of Neurology and Genetics.*

We present a six year old boy with mental retardation, dysmorphic features and a chromosome deletion-duplication syndrome. Many cases with a deletion-duplication syndrome of various chromosomes have been published in medical literature. The phenotypes of these cases include features described separately in association with the deletion and the duplication. This is the first report of a case with 46,XY,der(7)t(7:8)(q36;q24.3). Our patient was small for gestational age and had torticollis and cryptorchidism at birth. His features at six years included short stature, poor weight gain, microcephaly, torticollis (tilt on the left), facial asymmetry, short and up slanted palpebral fissures, broad tip of the nose, malar hypoplasia, down turned corners of the mouth and cutaneous syndactyly between the third and fourth fingers and third and fourth toes bilaterally. He had generalized hypotonia. He started walking in his second year of life and his gait remained wide-based. He had recurrent episodes of otitis media but his hearing was preserved. His language consisted of a few single words. He had hypermetropia and astigmatism and mild esotropia. He demonstrated autistic features like inattention, repetitive hand flapping and hand washing like movements. Brain magnetic resonance imaging revealed hypoplasia of the corpus callosum and syringomyelia of the cervical cord. The chromosome analysis at birth was reported as normal. Fluorescent in situ hybridization (FISH) for the chromosome subtelomeric areas revealed subtelomeric deletion 7q and subtelomeric duplication 8q. Further study of the high resolution G-banded chromosomes of the patient defined the breakpoints 46,XY,der(7)t(7:8)(q36;q24.3). Chromosome testing of both parents by G-banding and FISH was normal. We compared the phenotype of this case with cases reported in the medical literature with deletion 7q (JG Pappas et al, 2002; SG Frints, 1998; RS Verma, 1992) and duplication 8q (S Stengel-Rutkowski, 1992). The phenotype of our case includes features described in both of these syndromes. Our case demonstrates the utility of FISH in the evaluation of individuals with mental retardation and dysmorphic features and introduces a new deletion-duplication syndrome.

Disclosure(s): None

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13p and Yq homology have anything to do with male fertility status: a novel familial inheritance of 13p deletion. *L Rao*[1], A Babu[1], K Murthy[1], M Deenadayal[2], L Singh[1]. [1]Centre for Cellular & Molecular Biology, [2]Infertility Institute and Research Centre.*

Answers to deviations regarding the decline in fertility rate, and altered fecundability have gradually focused on individual genetic make up. Microdeletion syndromes are a heterogeneous group of disorders caused by deletion of specific regions of chromosomal DNA that are not visible using standard chromosome analysis. These are clinical genetic syndromes that result from loss of parts of chromosomes being recognized increasingly as a cause of congenital abnormality. The natural transmission of these chromosome deletions are rare events occasionally reported in the literature. Telomeric deletions (deletions at either end of a chromosome) account for many nonspecific cases of mental retardation with dysmorphic features. We present an interesting finding in a family with four males and two females with inheritance of p arm deletion of chromosome 13 for the past four generations. This defect is predominant in males of the family, with abnormal fertility status and low intellectual abilities, whereas females with the defect are absolutely normal. The deletion was confirmed initially with G banding and later using Fluorescence in situ hybridization. This reveals that genes on p arm of chromosome 13 have association with the mental status and fertility aspects. It is important to define the pathogenetic significance of deletion of chromosome 13p in association with intellectual rates and infertility. All the males with the defect suffered from primary infertility with oligoasthenoteratozoospermia. Molecular investigations using STS markers and genes showed normal Y chromosome. Further studies are in progress to narrow down the region of the chromosome deletion breakpoint and map the candidate gene(s) for the cause in this region. This will allow understanding the possible mechanisms by which the deletion might affect meiosis in spermatogenesis and lead to infertility. Chromosome Y is the smallest one in the human chromosome set, in which there are SRY, DAZ, DFFRY and other few genes related to testicular development and spermatogenesis. In addition to the genes on the Y chromosome, many autosomal genes are also involved in testicular development and spermatogenesis. Hypothetically, the altered fertility rate observed among the males in the four generations follows the position-effect variegation (PEV) phenomenon. The sequence blast analysis of the breakpoints in deletion region of chromosome 13p in the affected individuals showed 85% homology with the Yq region. Molecular characterization is currently underway to annotate the underlying sequences with Yq region. This would further find the novel relationship between autosomal aberrations and testicular dysgenesis or spermatogenesis arrest and map the corresponding regions on each autosome in regard to the recorded aberrations accompanying these disturbances.

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Cytogenetic prenatal diagnosis in fetal fluids.

D Mayen[*1], G Razo[1], Md Zavaleta[1], JL Saucedo[1], R Garcia Cavazos[2], M Guzman[3]. [1]Genetics Department. Instituto Nacional de Perinatologia, Mexico City, [2]Biomedic Research Department. Instituto Nacional de Perinatologia, Mexico City, [3]Fetal Maternal Medicine. Instituto Nacional de Perinatologia. Mexico City.*

Introduction The invasive techniques traditionally used to obtain fetal cells for chromosome analysis are chorionic villus sampling, amniocentesis and cordocentesis. However in some cases those methods are difficult to performed, specially in the presence of structural defects in the fetus, advance gestational age or oligoamnios, so an alternative method is required. Material and Methods In those cases with fetal structural defects detected by high resolution ultrasound where traditional invasive procedures are difficult to perform, we offered to the pregnant woman somatocentesis. Results We performed fifty nine cases of somatocentesis in fetal cells from cystic hygroma, urine and pleural and ascitis fluids. The cytogenetic technique was standarized for each particular sample and results were obtained after three or four days. We had no cytogenetic results in seven samples: five from cystic hygroma and two from urine. The 13 abnormal karyotypes include: 11 from cystic hygroma [ten 45,X; one 46,XY-13+der(13)t(13;18)] Conclusion Somatocentesis is a good alternative method to obtain fetal cells in cases where traditional methods represents technical difficulties and with specific modifications in cytogenetic technique according to the cell type a karyotype with good band resolution can be obtain.

Disclosure(s): None

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Holoprosencephaly and cystic renal dysplasia associated with der(7)t(7;9)(q36;q32)mat.

H Risheg[*1], L Seaver[1], R Lebel[1], A Srivastava[1], S Menon[1], B DuPont[1]. [1]Greenwood Genetic Center, Greenwood, South Carolina.*

Holoprosencephaly (HPE) refers to developmental defects which result in malformations of the brain and face. We report a case of a 24-year-old primigravida. Ultrasonography at 21 weeks showed oligohydraminios with kidney and heart defects. Amniocentesis revealed a fetal karyotype of 46,XY,add(7)(q36). Studies on the parents showed the mother to be a carrier of a balanced reciprocal translocation 46,XX,t(7;9)(q36;q32). The father had a normal male karyotype. At autopsy, the male fetus was found to have "semilobar" HPE, median cleft lip and cleft palate, severe bilateral multicystic renal dysplasia and resultant bilateral pulmonary hypoplasia. FISH analysis was performed to investigate the possible involvement of the target gene for HPE on 7q36 region, known as the Sonic hedgehog gene locus (SHH), using the SHH probe BAC clone RP11-69O3 (Genbank Accession # AC078834). The fetus was found to be monosomic for 7q36-qter, resulting in hemizygosity for the SHH gene locus, and trisomic for 9q32-qter. The combination of cystic kidneys and HPE has been reported in other 7q deletion patients and not in patients with trisomy 9q. We believe the complex phenotype of multiple anomalies with two main features (HPE and cystic kidneys) resulted from a combination of trisomy 9q32-qter and monosomy of 7q36-qter. Other defined HPE loci are located on chromosome 2, 13, 18, and 21. These loci have been rarely associated with renal anomalies, but not with cystic changes of the kidneys. When phenotypic features include both HPE and cystic kidneys, it may be important to investigate for chromosomal aberrations in the 7q36 region.

Disclosure(s): None

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Cryptic deletions of the Wolf Hirschhorn syndrome (WHS) locus defined by all telomere FISH testing but not by routine GTG banding. *MJ Sutcliffe*[1,2], DP Dumont[1], P Callea[1], E Callahan[1], SM Gellatly[1], NR Wilbanks[1], BG Kousseff[2], T Pal[1,2], L Pollack[3], JD Ranells[2], H Rossbach[4]. [1]All Children's Hospital, St Petersburg, FL, [2]University of South Florida, Tampa, FL, [3]Orlando Regional Health Service, Orlando, FL, [4]St Joseph's Hospital, Tampa, FL.*

Patient HC, referred at age 18 mths with developmental delay and hypotonia, had a normal 46,XY male karyotype. Microdeletion FISH analysis at 2 yrs of age was negative for Angelman Syndrome. A bone marrow specimen was submitted at age 4 yrs for precursor B Acute Lymphoblastic Leukemia. When reporting the acquired clonal abnormalities involving chromosomes 20 and 21 associated with ALL, the oncologist revealed that the patient had subsequently been diagnosed with WHS that had been confirmed by WHS FISH analysis at another facility. Our follow-up with the All Telomere FISH panel and WHS probe confirmed deletion of both the 4p subtelomere and WHS loci but also revealed the presence of 12p subtelomeric material on 4p. The constitutional karyotype was revised as: 46,XY,der(4)t(4;12)(p16.2;p13.32).ish del(4)(p16.3p16.3)(TEL4P-,WHS-),der(4)t(4;12)(TEL4P-, TEL12P+;TEL12P+,TEL4P-). Patient TT was born prematurely at 34 wks. Her history included CHD and FTT and at 6 mths (adjusted for prematurity) functional age was 1-3 mths. Chromosome analysis at age 9mths was reported as normal female 46,XX. At age 11 mths, the All Telomere study was performed due to the patient's continued clinical presentation of developmental delay. Deletion of the 4p subtelomere region was noted with deletion of the WHS locus also demonstrated. The karyotype was amended to include ish del(4)(p16.3p16.3)(TELP4-,WHS-). Retrospective analysis of both original GTG banded chromosome studies failed to reveal a 4p deletion at 450-500 banding resolution, although in the first instance - where the region was masked by 12p - a barely discernible band discrepancy was noted. In the second case the deletion was submicroscopic. Of the 33 All Telomere FISH analyses performed during the past 1 yr, 4 cases (12%) were demonstrated to have a deletion. One of these cases, involving chromosome 14, was resolvable by GTG banding (although reported as normal by another facility). The other case, deletion 1p, was not discernible even at 550-650 resolution level. In summary, the two cases showing a 4p deletion by All Telomere FISH also involved a deletion of the WHS locus. Neither infant presented with typical clinical features suggesting WHS at the time they were initially referred for cytogenetics. Historically, WHS has been associated with large deletions of 4p clearly visible by routine cytogenetic analysis. Since the 4p region is reported as gene rich, the "characteristic" features may in fact be part of a contiguous gene syndrome. If so, the diagnostic features may need to be redefined for WHS microdeletions compared to those usually ascribed to the syndrome.

Disclosure(s): None

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Deletion (1)(q42) and minimal phenotype: a function of limited monosomy or of age of evaluation? *M Thangavelu*[1], M Bocian[2]. [1]Genzyme Genetics, Orange, California, [2]Deptartment of Pediatrics, University of California, Irvine, California.*

Phenotypes associated with varying degrees of terminal deletion of the long arm of chromosome 1 (1q) have been documented by many investigators. These include cases with deletion of material distal to breakpoints at 1q42, 1q43 and 1q44. However, only a handful of cases have been studied using high resolution techniques such as fluorescence in situ hybridization (FISH) to rule out presence of duplicated material from another chromosome. We present a patient with del(1q) with minimal phenotype and without involvement of any other chromosomal segment. The patient was delivered by uncomplicated C-section with Apgars of 8 and 9 (birthweight, 3140 gm; length, 48.5 cm; head circumference, 33 cm). Neonatal complications included apnea-bradycardia spells and gastroesophageal reflux. Genetic evaluation at the age of 4 weeks revealed no dysmorphic features. Spine X-rays revealed a single thoracic vertebral anomaly and fusion of the 6th and 7th ribs. Results of biochemical studies (plasma amino acids, urine ammonia, lactate pyruvate and carnitine) were normal. CT and MRI scans of the brain did not reveal any structural abnormalities. G-banded chromosomes revealed a deleted chromosome 1 with loss of material distal to band 1q42. A panel of 41 subtelomere probes were used in the FISH studies to determine the integrity of telomeres of all the chromosomes. The 1q subtelomere probe did not hybridize to the abnormal chromosome 1 or any other chromosome and no other subtelomere probe hybridized to the abnormal chromosome 1 substantiating the cytogenetic observation of a deletion without presence of material from another chromosome. Parental chromosomes were normal. Craniofacial features (micro/brachycephaly, upslanted palpebral fissures, epicanthus, low set/dysplastic ears, abnormal mouth, short webbed neck) and CNS anomalies (agenesis of the corpus callosum) reportedly associated with deletion of the distal region of the long arm of chromosome 1 were absent in the proband. The lack of significant clinical abnormalities may be due to the minimal nature of deletion. However, the possibility of an evolving phenotype with increasing age cannot be ruled out. Additional molecular investigations to further characterize extent of the deletion are being considered.

Disclosure(s): None

CYTOGENETICS POSTERS

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Phenotype associated with pure partial duplication of the long arm of chromosome 4 (q31 to q35). *M Thangavelu*[*1], *M Bocian*[2]. [1]*Genzyme Genetics, Orange, California,* [2]*Department of Pediatrics, University of California, Irvine, California.*

Karyotype-phenotype correlations in trisomies are most accurate in cases without an accompanying monosomy. We present a case of partial trisomy for the long arm of chromosome 4, for the region between q31 and q35 without additional monosomy. The patient, of African American background, was delivered at 32 weeks by normal vaginal delivery. There was alleged fetal exposure to cocaine, alcohol and marijuana. Birthweight was 3 lbs 14 ounces and length was 17 1/2 inches. There was an early history of laryngomalacia. At age 2 years the patient displayed failure to thrive, global developmental delay and speech delay. He presented for genetic evaluation at 14 years (height at 25th centile; weight at 15th centile). Features observed included microcephaly (OFC average for 5 years), small ears, epicanthal folds, low wide nasal base and bridge, short philtrum, flat malar region, marked anterior open bite, inability to flex thumbs at the interphalangeal joint (normal X-ray), flat feet and undescended testes. Vision was normal and there was no clinical evidence of cardiac abnormality. Chromosome studies revealed an insertional translocation from the long arm of chromosome 4 (4p) into the short arm of chromosome 10 (10p); 46,XY,ins(10;4)(p?12;q35.1q31.3).ish der(10)ins(10;4)(10pSUBTEL+,wcp10+,wcp4+,wcp10+) resulting in pure partial trisomy for chromosome 4q31.3q35.1. Microcephaly, short philtrum, epicanthic folds, mental retardation, and short stature have been previously observed to be associated with duplication of the long arm of chromosome 4. Duplication of the region 4q31.3 - q33 has been reported to have a normal phenotype (Maltby EL et al, 1999). Therefore the phenotype observed in this case is likely to be the result of the duplication between 4q33 - q35.1.

Disclosure(s): None

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A non-standard deletion in a patient with Prader-Willi syndrome: implications for FISH-based diagnostic strategies. *E Thorland*[*1], *J Ireland*[2], *A Adeyinka*[1], *L Courteau*[3], *D Dawson*[3], *D Babovic-Vuksanovic*[2], *S Jalal*[1]. [1]*Cytogenetics Laboratory, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester MN,* [2]*Medical Genetics, Mayo Clinic, Rochester MN,* [3]*Molecular Genetics Laboratory, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester MN.*

Prader-Willi (PWS) and Angelman syndromes (AS) are distinct neurodevelopmental disorders caused by haploinsufficiency from the paternal or maternal copy of an imprinted portion of chromosome 15q11.2-12 region. Several distinct genetic mechanisms may account for the loss of expression. Deletions of approximately 5 Mb are the most frequent cause of 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. A few non-standard deletion sizes have been reported. FISH with probes specific to the critical region are frequently used to identify these deletions. However, the location and number of probes utilized varies. We routinely analyze 10 metaphase and 100 interphase cells with two commercially available probes which hybridize to regions containing the SNRPN and D15S10 loci for PWS and AS, respectively, to rule out deletions and duplications. Methylation testing by Southern blot using the PW71 probe was performed on a 34-year-old female patient who was clinically diagnosed with PWS at one month of age. The clinical history includes phenotypic features typical for PWS patients. Methylation analysis was consistent with a diagnosis of PWS. Follow-up FISH analysis indicated that the D15S10 probe was present. However, the SNRPN probe was deleted, indicating that this patient harbored a rare deletion smaller than that typically observed for PWS patients. Using microsatellite markers across the 15q11.2-12 region, we were able to demonstrate heterozygosity at several loci, including D15S1021 and D15S1506 flanking the SNRPN gene. This analysis demonstrated that the deletion could be no larger than 500 kb, which is the smallest deletion reported to date (not including relatively small deletions that have been reported at the imprinting center). To more precisely define the deletion size, EBV-transformed lymphoblasts were used to create somatic cell hybrids containing each of the chromosome 15s. PCR primers were designed to the PAR5 and IPW loci, in addition to exon 1 of the SNRPN gene. PCR using DNA extracted from the somatic cell hybrids demonstrated that all 3 regions were deleted. Thus, the deletion includes the imprinting center and is between 160 and 500 kb in size. In conclusion, the use of multiple probes enhances detection of such rare deletions. Probes specific to both the imprinting center (deletion of which causes PWS) such as SNRPN and the UBE3A gene (the putative disease-causing gene in AS) such as D15S10 should allow the detection of many non-standard deletions.

Disclosure(s): None

CYTOGENETICS POSTERS

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Characterization of prenatally diagnosed marker chromosomes: an analysis of 185 cases from 491,596 amniocentesis samples. *F Wallace*[1], A Donnenfeld[1], S Berend[2], M Shaham[3]. [1]Genzyme Genetics, Philadelphia, PA, [2]Genzyme Genetics, Santa Fe, NM, [3]Genzyme Genetics, Yonkers, NY.*

Objective: To determine the frequency, distribution and ability to characterize prenatally diagnosed marker chromosomes using conventional cytogenetic methods and fluorescence in situ hybridization (FISH) analysis. Materials and methods: The Genzyme Genetics database was queried for marker chromosomes diagnosed by amniocentesis from January 1996 to August 2003. A total of 465 marker chromosomes were detected from 491,596 amniocentesis samples. Characterization was attempted in 239 (51%) cases, and was done with the permission of the referring physician on those cases which had sufficient material. The following properties of the marker chromosomes were evaluated: chromosomal origin, and determination if it was familial or de novo. The protocol for identification initially employed standard cytogenetic techniques followed by FISH analysis. FISH analysis included the use of centromeric probes, unique sequence probes, probes specific for ribosomal RNA (rRNA) and whole chromosome painting probes. The centromeric probe for chromosomes 13 and 21, and the centromeric probe for chromosomes 14 and 22 cross-hybridize; thus, centromeric probes cannot distinguish between chromosomes 13 and 21 and chromosomes 14 and 22. Results: Four hundred and sixty-five marker chromosomes were detected from a total of 491,596 amnios for an incidence of 0.95 per 1000. Identification of the marker chromosome was achieved in 185 of 239 cases (77.5%). One hundred and thirty-nine marker chromosomes (75%) were derived from the acrocentric chromosomes 13, 14, 15, 21 and 22. Of the one hundred and thirty-nine marker chromosomes derived from acrocentric chromosomes, 44 (23.8%) were of chromosome 15 origin, 60 (32.4%) originated from chromosome 14 or 22, 32 (17.3%) were derived from chromosome 13 or 21 and 3 (1.6%) could only be determined to be derived from an acrocentric chromosome because of the presence of rRNA sequences. Ten marker chromosomes (5.5%) were derived from the X chromosome and 1 (0.5%) was derived from the Y chromosome. Of the remaining 35 (19%) marker chromosomes, 31 (16.8%) were derived from other chromosomes, 1 (0.5%) was of multiple chromosome origin and 3 (1.6%) could only be determined to contain centromeric DNA. There were 85 cases where parental testing was informative. Fifty-five (65 %) marker chromosomes were de novo, 16 (19%) were of maternal origin and 14 (16%) were of paternal origin. Thirty-four (18.4%) of the marker chromosome specimens were mosaic. Conclusions: In this study marker chromosomes occurred with an incidence of 0.95 per 1000. They were characterized in 77% of cases, and 75% of these were derived from acrocentric chromosomes. In cases where parental testing was informative 65% of the marker chromosomes were de novo and 35% were familial. Eighteen percent of the identified marker chromosome samples were mosaic. Knowledge of the chromosomal origin, familial occurrence and mosaicism status of a marker chromosome, combined with other studies such as uniparental disomy analysis and high resolution sonography are helpful in providing prognostic information for expectant parents.

Disclosure(s): Presenter and/or authors are employed by, have employee stock options and receive full reimbursement for participation from Genzyme Genetics, which sponsors research activities relevant to this presentation.

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Spectral karyotyping (SKY™) identification of constitutional cytogenetic abnormalities: five years' experience at a national reference laboratory. *B White*[1], S Wang[1], V Sulcova[1], M Ayad[1], L Dong[1], J Neidich[1], D LaGrave[1], A Anguiano[1]. [1]Quest Diagnostics Nichols Institute, San Juan Capistrano, CA.*

SKY is an interferometer-based fluorescence in situ hybridization (FISH) method that paints each human chromosome a different color, allowing simultaneous identification of all 24 chromosomes in a single hybridization. SKY has shown utility for detection of chromosomal rearrangements as well as identification of marker chromosomes and additional material of unknown derivation. Here we present our 5-year experience with SKY for identifying constitutional abnormalities found in the course of prenatal and postnatal cytogenetic studies. All positive SKY findings were confirmed by FISH whole-chromosome painting; centromeric and locus-specific probes were applied for further definition in selected cases. Cases with satellited markers were underrepresented because of positive identification by GTG banding followed by specific FISH probes. A total of 95 cases were studied with SKY, 82 to clarify abnormal cytogenetic findings and 13 to detect rearrangements in cases with apparently normal karyotypes. Forty-nine cases (52%) had supernumerary markers; marker origin was defined in 17 of 20 prenatal and all 29 postnatal cases (94% total). In 2 postnatal cases, centromeric and acro-p-arm FISH was used to confirm the origin as chromosome 14 or 22 following equivocal SKY results. Only chromosome 8 was represented more than once in prenatal marker studies (2 cases); chromosomes 2, 8, 15, 17, and 22 were the most frequent in postnatal studies (3 each). Twenty cases had additional material of unknown derivation; SKY positively identified 3 of 4 prenatal and 18 of 20 postnatal cases (88% total). In the 3 cases not identified by SKY, the additional material was derived from heterochromatin. Only chromosomes 1, 9, 13, and 18 were involved in more than 1 case of additional material (2 each). Six postnatal cases had supernumerary rings; the SKY result was positive in all of these and identified a ring 15 chromosome in 3. In 3 other postnatal cases, SKY more precisely defined karyotypes with complex autosomal rearrangements. Of the 13 cases with apparently normal karyotypes, SKY detected a reciprocal translocation in only 1 postnatal study (8%). Overall, SKY positively identified 77 (93%) of 82 cases with abnormal cytogenetic results not fully defined by GTG banding. However, SKY detected rearrangements in only 1 of 13 cases (8%) with apparently normal karyotypes. We conclude that SKY is best applied for identification of constitutional abnormalities in both prenatal and postnatal cases with supernumerary markers and rings, additional rearranged material, or complex rearrangements. Information on corresponding clinical correlations will build the value of this method for genetic counseling of families with these abnormalities.

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What my girlfriend didn't know: developing a low-literacy bilingual fotonovela on folic acid.

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The Latina population in East Los Angeles is a high risk group for spina bifida and yet knowledge, attitudes and behaviors in this group do not support the use of daily folic acid supplementation. We formed a partnership between the USC Schools of Medicine and Pharmacy, local pharmacies and community organizations to develop a fotonovela, a soap-opera like dramatic story in captioned photographs, to increase knowledge about spina bifida and folic acid, change attitudes about and promote the use of vitamins in Latina women age 18-22. Led by Latina facilitators, focus groups of young Latinas answered questions and were encouraged to express their beliefs and concerns about vitamins, body weight, body image, health, health care providers, disease prevention, pregnancy, pregnancy planning, family history, family dynamics, spina bifida and other birth defects. We used this information to develop themes for the plot line of the fotonovela. The story addressed myths about weight gain and vitamin use, concerns about body image and weight, lack of information about family history, the importance of the mother's role in the family, pharmacists as primary sources of health information and Latina pride. Desired behaviors were modeled in the story including taking a daily multivitamin, reading nutrition information on a cereal box and using a coupon to purchase vitamins. Actors portraying the characters in the story were vetted by the focus groups as were the size of the fotonovela and other design elements. A native Spanish speaker translated the script into Spanish. We chose a dramatic color photo and title for the cover. Frequently asked questions about folic acid and coupons for vitamins were included in the centerfold. We published a single bilingual edition of 15,000 copies of the fotonovela in the summer of 2003. The fotonovelas were distributed in East Los Angeles by promotoras, bilingual trained lay health workers, and in local pharmacies from July through November 2003. During this time the participating pharmacies monitored coupon use and tracked vitamin sales. We also studied the effectiveness of the fotonovela. Knowledge, attitudes, intent and behaviors of a sample group of Latinas were measured before and after reading the fotonovela and compared to a control group who read other available Spanish language educational materials on folic acid. This data will be presented and discussed.

Disclosure(s): Good Neighbor Pharmacies sponsors research activities relevant to this presentation.

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Can genetic algorithms aid primary care physicians in decision making for genetic conditions?

SM Dobin[1], JB Saucier[1], J Whetteckey[1], M Slovak[1], MH Rajab[1]. [1]Scott and White Hospital and The Texas A&M University System Health Science Center College of Medicine, Temple, TX.*

The National Coalition for Health Professional Education in Genetics (NCHPEG) listed as one of its core competencies that all health professionals should be able to gather genetic family-history information and identify clients who would benefit from genetic services. Previous studies have shown that most primary care physicians do not have sufficient time or genetics training to take and discuss a full genetic family history and pedigree with all of their patients. Therefore, physicians need a quick method of obtaining and interpreting a family history. The objective of this study was to determine if primary care physicians can effectively use a set of genetic algorithms in conjunction with a family history form to provide genetic counseling information to their patients. We designed the algorithms based on current genetic risk literature. An example of one of the algorithms is if the patient has a first degree relative with isolated cleft lip and palate, the physician should counsel a 3-5% recurrence risk. Twenty patients seen in Genetics Clinics (prenatal, pediatric, and cancer) at Scott and White participated in the study. Subjects filled out the family history form at the beginning of their genetics appointment. Once data collection was complete, the genetic counselor made a set of recommendations based on the information from the family history form for each subject. A resident physician (OB/GYN) applied the algorithms to the information from the forms and recorded what recommendations she would have made to each patient. The overall percentage of agreement between the recommendations of the physician and the recommendations of the genetic counselor was 78.8%. This agreement rate is an excellent start. Many of the discrepancies discovered, such as a lack of information for 2nd degree relatives, can easily be corrected by adding additional information. Our results demonstrate that genetic algorithms can effectively aid primary care physicians in their daily decision making process.

Disclosure(s): None

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Can a family history form work as a genetic screening tool? *JB Saucier*[*1], *SM Dobin*[1], *J Whetteckey*[1], *MH Rajab*[1]. [1]*Scott and White Hospital and The Texas A&M University System Health Science Center College of Medicine, Temple, TX.*

In this era of advanced molecular genetics, family history is still being used by most as a genetic screening tool. The CDC recently began a multi-million dollar project investigating family history screening for public health practice. Our objective was to validate a family history form (FHF) that we locally developed as a screening tool for primary care physicians. The FHF was designed based on contributions from physicians, laboratory personnel and forms used at other institutions. Our final design was a bubble form with a list of approximately 50 genetic and common disorders for the patient and his 1st and 2nd degree relatives. Twenty patients seen in genetics clinics (prenatal, pediatric and cancer) at Scott and White participated in the study. Patients filled out the FHF at the beginning of their appointment. The genetic counselor obtained a 3 generation pedigree as part of the appointment. The FHF was considered valid if it agreed with 90% (or more) of the family history information gathered by the pedigree. Of the twenty FHF completed, four of them matched at least 90% of the pedigree information. The overall percentage of agreement for all disease entities noted on the pedigree was 56.4%. By our stated criteria, the FHF is invalid ($p>0.99$). In fourteen cases the form picked up family information that was not obtained by the pedigree. This unexpected finding is most likely due to the indication for their genetics appointment influencing the pedigree interview. As expected, the pedigree contained information not on the form such as ½ siblings, 3rd degree relatives, carrier status and ages at diagnosis. There were obvious areas of patient confusion when filling out the FHF, such as determining degree of relatedness. Only one incident, out of the 110 diseases noted on the pedigree, occurred where a patient misinterpreted the disease entity on the form (hypothyroidism for thyroid cancer). In conclusion, FHF with the bubble format is confusing for patients and limits the amount of information that can be obtained. Our next step is to study a different design, such as the "fill in the blank" methodology.

Disclosure(s): None

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Profile of an educational initiative in epidemiology for genetic counseling students. *S Dolan*[*1], *K Damus*[1], *C Lieber*[2]. [1]*Albert Einstein College of Medicine, Bronx, NY,* [2]*Human Genetics Program, Sarah Lawrence College, Bronxville, NY.*

Genetic epidemiology provides tools to assess the genetic contribution to the etiology of complex diseases, explore gene-environment interactions, and understand the epidemiology of genetic disease. Currently, there is little formal training in epidemiology for genetics professionals such as genetic counselors. In order to introduce the basic concepts of genetic epidemiology, a four-week seminar was developed and piloted in the first year curriculum of the Human Genetics Program at Sarah Lawrence College where students earn a master's degree and become certified genetic counselors. The broad objective of the course was to introduce an epidemiologic approach to genetic disease, testing, and counseling. Specifically, the course aimed to provide students with key genetic and epidemiologic concepts, introduce the basic structure of study design, and provide opportunities to evaluate examples from the literature. The course met weekly for four weeks. Each three-hour session was comprised of a one-hour lecture introducing key concepts, a one-hour case study carried out in a small group format, and a one-hour journal club in the large group setting. In order to assess knowledge, attitudes, and practice, pre and post-test surveys were distributed and filled out anonymously. Twenty students were enrolled in the class. All twenty returned the pre-test and 19 returned the post-test. All respondents were women. The average age was 25 years. Regarding knowledge, 70% of students reported "very little" knowledge and 30% reported "some" knowledge about epidemiology on the pre-test, versus 5.3% reporting "a great deal" of knowledge and 94% reporting "some" knowledge about genetic epidemiology on the post-test. In general, students demonstrated a good understanding of screening for disease with 55% correctly identifying the definition of sensitivity and 70% correctly identifying the definition of positive predictive value on the pre-test. However, knowledge of relative risk and odds ratio increased as a result of the course, with 42.1% more students correctly interpreting relative risk on the post-test and 33.4% more students correctly demonstrating a knowledge of the odds ratio. Most interesting is that interest in public health increased dramatically as a result of the course. Specifically, when asked to comment on the statement, "I am interested in public health," 20% of students said "strongly agree", 70% said "agree" and 10% said "not sure" on the pre-test, whereas on the post-test, 38.8% of students "strongly agreed" and 61% said "agreed." In conclusion, teaching genetic counseling students about genetic epidemiology can increase their interest in public health and increase their contribution to understanding the relationship between human genetic variation and various human diseases.

Disclosure(s): None

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An innovative way to compile public opinion about genetics issues – the living room forums.

D Doyle[1], A Brothers[1]. [1]WA State Dept of Health.*

Public opinion is critical to the development of sound public policy. In Washington State, attempts to compile such opinions have included telephone surveys as well as community meetings. However, in each of these, it was clear that participants didn't always fully understand the issues and frequently comments suggested they were addressing other scientific or technological advances such as cloning or stem cell research. Therefore, a model was developed that includes limited education and a discussion among friends and colleagues about genetics issues – known as the Living Room Forums. For each Forum, a host from the community is selected and asked to invite 6-15 of their neighbors or friends to their home for a two hour discussion about genetics issues. The Department of Health provides dinner, educational materials such as a fact sheet and descriptive scenarios, a facilitator to stimulate but not lead the dialogue, a genetics expert who remains silent unless a technical question is raised, and a recorder. All Forums are taped, and following the dialogue, an article summarizing the views expressed is submitted to the local newspaper along with quotes from the residents who participated. In addition, the DOH web site URL is provided allowing readers the opportunity to view the fact sheets and descriptive scenarios used in the Forums, as well as enter their views to the same questions posed to the Forum participants through an on-line survey. Three topic areas were explored: (1) genetic discrimination, (2) newborn screening, and (3) equity of genetic services. A total of 17 Living Room Forums were held between April and December 2003, including two pilot Forums. Participants ranged in age from 18-82 years of age, with ethnic backgrounds that were consistent with the demographics of the state's population. All had at least a high school degree with the majority having some college. Common themes heard in all Forums included concerns that existing state and federal; laws didn't go far enough to protect individuals from the potential misuse of their genetic information, that the current health care system is ill prepared for all of the emerging genetic tests, and that everyone should have access to testing but whether or not the test should be covered by an insurer should be determined by whether or not there is some intervention available.

Disclosure(s): None

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A survey of psychiatrists' knowledge, opinions and practice patterns regarding genetics, with implications for patient care.

CT Finn[1,2], MA Wilcox[3], BR Korf[4], DL Blacker[1], SA Racette[1], P Sklar[1,5], JW Smoller[1]. [1]Massachusetts General Hospital, Boston, MA, [2]Harvard-Partners Center for Genetics and Genomics, Boston, MA, [3]Boston Univeristy School of Medicine, [4]University of Alabama, Birmingham, AL, [5]Whitehead Institute, Boston, MA.*

Objective: Knowledge about the genetic basis of psychiatric illness is growing rapidly, and psychiatrists may be called upon to incorporate this information into clinical practice. The goal of this study was to assess psychiatrists' familiarity with, and attitudes towards genetic information. Methods: We surveyed participants attending a continuing medical education course, and measured knowledge, opinions, and current practice patterns in regard to psychiatric genetics. Results: Responses were received from 352 psychiatrists (54% of those surveyed). The majority of psychiatrists scored poorly on measures of general and psychiatric genetic knowledge. A subset of respondents expressed attitudes about potential uses of genetic information in psychiatry that vary from standards endorsed by genetics professionals. While 83% of psychiatrists said that they consider it their role to discuss genetic information with psychiatric patients and their families, only 23% felt competent to do so, and only 15% agreed that their medical training had prepared them for this role. Currently, psychiatrists refer few patients for genetics consultation. Overall, psychiatrists indicated interest in further education in genetics. Conclusion: This survey is the first step in assessing psychiatrists' preparation for the clinical application of genetic knowledge, and suggests areas where additional educational efforts and interdisciplinary collaboration may be needed.

Disclosure(s): None

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Protecting health, protecting speech: genetic testing, government oversight, and the First Amendment. *GH Javitt*[1], E Stanley[1], K Hudson[1]. [1]Genetics and Public Policy Center.*

As scientists discover an increasing number of genetic disease markers, the market for genetic testing products and services continues to grow. Many of these tests are carried out in the context of reproduction, to inform decisions about whether to initiate or continue a pregnancy, while others are used to diagnose disease or to predict an individual's own future risk of disease (e.g., BRCA1). The advent of the Internet has facilitated consumer access to genetic testing, both to "mainstream" tests and tests of dubious clinical validity and utility. Government advisory committees in the U.S. and abroad have examined the issue of consumer-directed marketing of genetic tests, and some have recommended limiting or banning consumer-oriented marketing. Concerns include the public's limited sophistication regarding genetics and the lack of comprehensive premarket review of tests or oversight of advertisement content. Some fear that consumers may be harmed if they receive testing without adequate advance counseling or if they obtain tests whose results falsely reassure or falsely alarm them. Some in the U.S. have recommended that the Federal Trade Commission (FTC) and Food and Drug Administration (FDA) work together to ensure that advertisements convey both the risks and potential benefits of testing and avoid misleading consumers. The First Amendment to the U.S. Constitution prohibits the government from suppressing speech. Historically, the First Amendment has been recognized to prohibit government censorship of political, social, or artistic expression. In recent years, the Supreme Court has increasingly extended First Amendment protections to "commercial speech," i.e., speech relating solely to the sale and promotion of products and services. The Court has concluded that such speech advances the values of consumer autonomy and choice. Even the FDA, which has traditionally been given significant latitude in regulating communications by the pharmaceutical industry, has in recent years been curtailed by the courts in such efforts because of First Amendment constraints. This presentation will explore the potential impact of recent First Amendment jurisprudence on proposals for increased government regulation of consumer-oriented advertising and promotion of genetic testing. It will illustrate the potential limitations on government's ability to regulate communications related to genetic testing promotion, and discuss the types of regulations that are more or less likely to be viewed as consistent with First Amendment protection.

Disclosure(s): None

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MCAD screening of newborns using tandem mass spectrometry as a first tier screen with 985A>G mutation analysis as a second tier screen. *B Jones* R Hermos, T Zytkovicz, A Comeau, V Shih, D Marsden, C Larson.*

Background: Universal population screening for Medium Chain Acyl CoA Dehydrogenase (MCAD) deficiency by the New England Newborn Screening Program (NENSP) has identified 16 infants who have been confirmed by biochemical testing to have MCAD, a disorder of fatty acid oxidation. Clinical circumstances for these infants at the time of screening notification include one case of a clinically symptomatic neonate, as well as 15 instances of clinically well infants. Methods: In five years, >500,000 infants have been screened by Tandem Mass spectrometry for MCAD markers, principally octanoylcarnitine (C8) at NENSP. Positive screens went on to DNA screening for the most common MCAD mutation, 985A>G. Infants found to have 1 or more copies of this mutation, and/or persistent or marked C8 elevations were referred to Metabolic Specialists for additional evaluation (including biochemical confirmatory testing). Results: Tandem Mass Spectrometry screening of the entire newborn cohort has detected 985A>G homozygosity in 60 % of screening detectable cases of MCAD, whereas clinical detection of MCAD has been associated with homozygosity for 985A>G in 80 % of cases. In one case detected by universal newborn screening (which was homozygous for 985A>G), clinical presentation with cardiopulmonary arrest and hypothermia preceded notification of screening results, with onset of symptoms at <1d of age; otherwise, screening notifications have been routinely available prior to clinical onset of symptoms for newborns with MCAD markers. Conclusion: Newborn screening for MCAD generally identifies in a sufficiently timely manner infants at risk for catastrophic clinical outcomes related to the inborn error of metabolism, MCAD. Universal population screening for MCAD appears to identify individuals at risk for early symptomatic presentations and individuals who are homozygous for the common mutation (985A>G) as well as individuals that have only one copy of the commonly assocaited MCAD mutation; thus far, only one child identified by screening (who was a homozygous for the common mutation) has died despite early notification of affected status. Further outcome studies are warranted for the additional MCAD detections by population-based newborn screening to better elucidate the genotype/phenotype association for MCAD.

Disclosure(s): None

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An evolving model for effective Ashkenazi Jewish genetic disease screening: the Philadelphia experience. *RB Keep*[1], AS Schneider, DL Dorsainville[1]. [1]Albert Einstein Medical Center, Philadelphia, PA.*

The Ashkenazi Jewish Genetic Disease Screening Program at Albert Einstein Medical Center in Philadelphia, was established in 1999. The Program provides outreach, education, genetic counseling and carrier screening to individuals of Ashkenazi Jewish descent. Despite extensive efforts to educate the public and physicians the overall interest from the Jewish Community has been disappointing. Our experiences have led us to hypothesize that several factors may impede Ashkenazi Jewish individuals from seeking genetic screening. These include ° cost of carrier testing ° lack of public awareness of when, where, why, and how to facilitate screening ° limited knowledge of the diseases that occur more frequently among Jewish individuals Based on our hypotheses we developed a model to increase participation in genetic screening. A qualitative study was conducted during the one month of the year when we offer free screening for eight Jewish genetic diseases in the Community. We promoted these events via enhanced marketing tactics (i.e. website advertisement, radio and print ads, posters, word of mouth, and letters to Rabbis) beginning eight weeks prior to the screening period. A total of 140 individuals were screened during the month and 82 completed our questionnaire. The participants were surveyed to assess the effectiveness of our strategies, to gain insight into their motivations for choosing to be tested at that time, and their knowledge of the diseases and the testing process. The questionnaire included information about demographics, such as age, gender, marital status, education, and ethnicity. Our largest referral base was word of mouth—family member or friend suggested testing. Those screened felt that newspaper, print and TV ads were the most effective way to get the "word out " about testing in the Jewish Community. Family planning (64.6%) and cost of testing (62.2%) was a major factor for all groups. At least 7 of 82 respondents knew about one of the diseases they were being tested for, usually Tay-Sachs. Over 90% understood the mode of inheritance of these diseases. We have concluded that hiring a coordinator to help focus our efforts on our most responsive groups—engaged couples, married with no children, and college students--would help to increase uptake of testing. With targeted advertising, reduced testing fees, educational seminars focusing on the lesser known diseases, participation in community events, and increasing our community contacts, we hope to make our program a Jewish-community household name.

Disclosure(s): None

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Mortality in Duchenne muscular dystrophy: an analysis of multiple cause mortality data, 1983-1997. *A Kenneson*[1], Q Yang[1], R Olney[1], S Rasmussen[1], J Friedman[2]. [1]National Center on Birth Defects and Developmental Disabilites, Centers for Disease Control and Prevention, Atlanta GA, [2]Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada.*

We analyzed population-based data from death certificates in the Multiple Cause Mortality Files compiled by the National Center for Health Statistics. From 1983 through 1997, 13,095 deaths in the United States were associated with ICD-9 code 359.1 (hereditary progressive muscular dystrophy), which includes Duchenne muscular dystrophy (DMD) and other conditions. The age at death in these individuals fell into a tri-modal distribution with peaks at 0, 17, and 62 years, representing congenital, childhood and adult onset cases. The cases in the younger group (0 to 12 years) are 37.3% females, and those in the older group (30 to 99 years) are 40.6% female, indicating a predominance of autosomal cases. The cases in the middle group (13 to 29 years) are 95.6% male, consistent with the X-linked inheritance in most cases. Males in this group were presumed to have DMD (N=4857). Underlying causes, contributing factors, and demographic characteristics were assessed in the DMD cases. DMD was more common among deaths in white males (1 in 3386) than among deaths in black males (1 in 4409) ($p=0.001$) in this age group. The distribution of age at death did not differ between racial groups. State-specific median age at death ranged from 17 years to 21 years. DMD-associated deaths occurred at a significantly earlier age in the Southeastern U.S. (median=19 years) than in other regions of the country (median=20 years, $p=0.003$). From 1983 through 1997, there was a small but statistically significant increase in the median age at death for both blacks and whites ($p=0.039$). During this time period, the overall median age at death increased from 19 to 20 years. Factors commonly listed as contributing to death included cardiomyopathy and cardiac dysrhythmias (34.2%), and pneumonia and other respiratory infections (20.8%). Cardiomyopathies and cardiac dysrhythmias were more commonly documented among black males (49%) than among white males (32.3%) ($p<0.0001$). Among blacks but not whites, the age at death was younger in individuals with cardiac involvement than in those without cardiac involvement. Based on analysis of these data, DMD appears to occur less often among deaths in blacks and is more often associated with cardiac involvement than in whites. Challenges in the interpretation of these data include the lack of an ICD-9 code specific for DMD, and potential recording biases in underlying cause of death and contributing factors.

Disclosure(s): None

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The LSU/Children's Hospital's Health Insurance Portability and Accountability Act (HIPAA) notice of privacy practices. *Y Lacassie*[1,2], *L Florez*[1]. [1]*Department of Pediatrics, Louisiana State University Health Sciences Center, New Orleans, LA,* [2]*Children's Hospital, New Orleans, LA.*

On April 14, 2003, new federal regulations regarding privacy practices became effective. This new federal legislation, called HIPAA, requires that our practice tell the patients and/or their families how we protect private health information. The patients are entitled to request a restriction on the medical information we use or disclose about their diagnosis, treatment, counseling, billing/payment, or for the evaluation of our services. Although in the Division of Genetics the medical information has always been treated as personal and private and we abide by the regulations of Children's Hospital and LSUHSC, in April 2003 we posted specific guidelines on the Children's website[http:www.chnola.org/clinical_serv_genetics.htm]. The 7 aspects we have included as specific areas of concern regarding evaluations in the field of Clinical Genetics are: 1. Detailed information. One of the most important regulations of HIPAA deals with obtaining the minimum necessary information to provide treatment, receive payment and improve operation. In Genetics, however, we need to obtain as extensive family, prenatal, natal, perinatal and postnatal histories as possible. The less information provided, the lower the accuracy of the diagnoses. Sometimes only extensive family information allows us to establish the diagnosis, pattern of inheritance or other information important for our assessment; 2. Pictures. These are important to document our findings, to facilitate the diagnostic search, to evaluate the possibility of normal familial variation, to check progression or changes in the physical appearance, etc. We frequently request that the patient or relatives bring pictures of parents and other relatives at different ages for more in-depth evaluation. 3. Copies of letters. The letter to the referring physician summarizing our evaluation includes positive antecedents and findings, diagnosis or possible diagnosis, and a diagnostic plan, including recommended tests, consultations and follow-up, and genetic counseling issues, if possible. We send copies to physicians/professionals involved in the care of the patient; 4. Disclosure of a genetic diagnosis to other family members. The establishment of some genetic diagnoses or patterns of inheritance may have implications for other members of the family; 5. Communication of diagnosis to insurance companies; 6. Communication of diagnosis or list of birth defects for public health policies; 7. Presence of medical students/residents/Ph.D.s' students/fellows. These and other aspects and comments regarding this notice will be discussed.

Disclosure(s): None

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Ethical issues arising from discovery of the ataxia syndrome associated with FMRL premutation (FXTAS). *RR Lebel*[1], *GF Guzauskas*[1]. [1]*Greenwood Genetics Center, Greenwood, SC.*

The FMR1 gene is well known for being the location of mutations causing Fragile X syndrome. The 5' untranslated region of the gene has a variable number of CGG repeats; normal individuals have fewer repeats (7-54), affected individuals more (>230). Persons in the intermediate (pre-mutation) group do not express the Fragile X phenotype, but are considered at risk for the number of repeats expanding and being expressed in the next generation. Relatives of Fragile X patients are usually notified of their possible carrier status and tested for it; they are told that the only ramification of this test will be the risk of expansion in their offspring. Recent studies have shown that some pre-mutation carriers develop a phenotype of tremor, ataxia, and cognitive loss (FXTAS) in middle age. This new information introduces a number of dilemmas, both ethical and practical in nature. Under ordinary circumstances, the duty to re-contact does not extend to patients who consulted a geneticist many years ago. However, the existence of computerized databases may provide an opportunity for reference laboratories to alert referring physicians regarding the identities of pre-mutation carriers, allowing for such re-contact. Even so, until FXTAS is better defined, and its incidence in the at-risk population established, such re-contact may be premature or even damaging. Further, FMR1 pre-mutations may play a significant etiologic role for movement disorders subject to being mistaken for Huntington disease, Parkinsonism, etc. It is essential that the genetics community address questions about who should be re-contacted with potentially life-altering information about the ultimate significance of pre-mutation carrier status, and by what methods they should be contacted. Implications for additional complexity in future counseling settings are also important: what should be told to new patients found to be carriers? to families of infants identified by newborn screening?

Disclosure(s): None

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The Genetic and Rare Diseases Information Center: a federal government resource. *J Lewis*[1], K Hagerty[1], L Lander[1], M DellaRocca[1], E Soper[1], S Weaver[1], A Cowan[1], L Lanier[2], H Hyatt-Knorr[3]. [1]Aspen Systems, Rockville, MD, [2]National Human Genome Research Institute, NIH, Bethesda, MD, [3]Office of Rare Diseases, NIH, Bethesda MD.*

The National Human Genome Research Institute (NHGRI) and the Office of Rare Diseases (ORD), National Institutes of Health, initiated the Genetic and Rare Diseases Information Center (GARD) to provide free and immediate access to accurate, reliable information about genetic and rare diseases, in English and Spanish. More than 6,000 rare diseases currently affect approximately 25 million Americans. A large percentage of these rare diseases are considered genetic. Because many of these diseases affect relatively few individuals, information about the conditions may be difficult to find; this difficulty can, in turn, add to the stress of a genetic or rare disease diagnosis. Many people are now turning to the Internet as a source of medical information. In fact, Internet health surveys have shown that approximately 50 percent of American adults search the Internet for health information. People without Internet access may ask friends and family to search for them. The value of online health information, however, can frequently be limited by inaccuracy and unreliability—which can be difficult even for experienced information seekers to assess. In addition, 25 percent of Americans do not have access to Internet health resources. Closing such a health information gap can improve the overall quality of care for health consumers. For more than 2 years, GARD has provided a bridge between information seekers and reliable materials on genetic and rare diseases, research, genetic services, literature searches, support organizations, and other relevant resources. Experienced Information Specialists are available to answer questions by toll-free telephone, TTY, e-mail, fax, and letter. The telephone provides GARD users immediate access to an Information Specialist. Written responses are provided in 5 to 10 business days. To enhance understanding, written responses can include information about relevant clinical trials and institutional expertise in the field; relevant textbook materials translated into lay language; and contact informaion for local genetic services. Electronic responses also include direct links to resources in the public domain such as NIH Information Centers and publications, Combined Health Information Database (CHID) search results, GeneTests reviews, and information from support group Web sites. GARD has responded to more than 6,000 inquiries from patients, their family members, health professionals, educators, students, journalists, researchers, and the general public. The Information Center assists patients and their family members as they navigate health information on the Internet by guiding them to the location of high-quality resources. The Information Center also helps health professionals provide their patients with lay-language disease summaries and other resources that can be revisited for ongoing information and support.

Disclosure(s): None

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The nature of families and individuals who have adopted and/or are waiting to adopt a child with Down syndrome. *H Lindh*[1], A Donnenfeld[2], R Steele[3], J Page-Steiner[3]. [1]Genzyme Genetics, Dallas, TX, [2]Genzyme Genetics, Philadelphia, PA, [3]Down Syndrome Association of Greater Cincinnati, Cincinnati, OH.*

Objective: To obtain information on the nature of, and reasons why families are interested in adopting children with Down syndrome (DS) and the understanding of DS among these prospective adoptive parents. Methods: We developed and mailed a survey to 319 individuals whom are either currently waiting to adopt or have adopted a child with Down syndrome through the Down Syndrome Association of Greater Cincinnati's Adoption Awareness Program. Questions regarding 1) demographics 2) family structure 3) exposure to individuals with DS 4) knowledge of DS 5) reasons for considering adoption 6) adoption process and 7) perspective on raising children with DS were asked. Results: There were 89 respondents out of 319 surveys mailed (27.9%). 72 (80.9%) were from those waiting to adopt a child with DS. 17 (19.1%) were from those who have already adopted a child with DS. Nearly 50% learned of the Adoption Awareness Program via the Internet, whereas only 1 respondent received this information from his/her doctor (1.1%). The primary reasons for choosing to adopt a child with DS were 1) felt equipped with resources to raise a child with DS and 2) positive past experience with individuals with DS. The majority was aware that individuals with DS have mental retardation and an increased incidence of congenital anomalies. The majority either somewhat agreed or agreed that adoption of a child with DS would make their life more stressful. When given the choice if they would prefer to adopt a child with or without DS, or no preference, none stated a preference to adopt a child without DS. Respondents were nationwide, and the majority were married, lived in a community of 50,000 or less, and had an annual income greater than or equal to $40,000. Conclusions: Many families and individuals are eager to adopt children with DS. The majority is aware of the various medical conditions associated with Down syndrome and understands that parenting a child with DS could likely make their lives more stressful. They are interested in adoption of a child with DS primarily because they have the resources to care for these children and they have had positive experiences with children with DS. The majority of expectant adoptive families obtained information on the availability of adopting a child with DS from the Internet, and not from health care providers. This study will increase awareness among health care providers regarding the option of placing children with DS for adoption. This information can be transmitted to couples prenatally diagnosed with a DS fetus so that all their options regarding pregnancy management may be explored.

Disclosure(s): None

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WITHDRAWN

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Blood phenylalanine levels pre and post a residential camp experience. *J Peipert*[1], F Rohr[2], D Johnson[3], C Phornphutkul[1,4], S Waisbren[2,5]. [1]Child Development Center, Rhode Island Hospital, Providence, RI, [2]Boston Children's Hospital, MA, [3]New England Newborn Screening Program, MA, [4]Brown Medical School, Providence, RI, [5]Harvard Medical School, MA.*

Objective: To document the change in blood phenylalanine (Phe) levels (mg/dl) as a marker for metabolic control in children ages 7-15 years with classical PKU attending a YMCA summer overnight camp. **Design:** We performed an observational cohort study of 18 children attending a 5-day summer camp. All subjects had Phe measured at the beginning of a 5-day overnight summer camp and again at the final evening of camp. **Subjects/Setting:** Our study population consisted of children ages 7-15 years with classical PKU who attended a 5-day overnight YMCA summer camp in Cape Cod (Sandwich), MA. **Intervention:** The camp was not instructional for PKU but rather has as its mission to enable children with PKU to have a camp experience in which their diet was facilitated by staff (RD's, low-pro menu, low-pro cook). The children were encouraged to make their own metabolic formula with minimal supervision, drink their metabolic formula without supervision, make their own meal choices from a PKU meal line with Phe content listed, and record their meal and daily PHE intake. On the first complete day of camp, a brief overview of PKU was given for the entire camp staff and campers with and without PKU so that all children would have some understanding of PKU. **Outcome Measure:** On the last evening of camp, blood samples were collected on filter papers for analysis by tandem mass spectroscopy. **Statistical Analyses Performed:** Mean Phe levels before and after the camp were compared using a paired t-test with $P<0.05$ considered statistically significant. **Results:** The mean Phe level at the beginning of camp was 8.8 mg/dl (standard deviation (s.d.) = 4.5) and the final level was 4.6 mg/dl (s.d. = 2.5; mean difference = 4.2). This difference is both clinically and statistically significant ($P<0.0001$). **Conclusions:** Short-term effects of the camp environment resulted in improved metabolic control. Factors speculated as attributing to this improvement are decreased barriers to dietary compliance (variety of readily available low-protein foods, reinforcement of diet through recording intake in diet records, increased acceptance of diet due to large number of other campers also with PKU) and readily available staff to assist with diet adherence.

Disclosure(s): None

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Using Infogenetics GIFT version 3.5 to access Internet resources by nurse practitioners, nurse midwives, and physician assistants as part of the Genetics Interdisciplinary Faculty Training (GIFT) program.

V Proud[*1], *N James*[2], *T Cox*[3]. [1]*Dept. Pediatrics, Eastern Virginia Medical School, Norfolk, VA,* [2]*School of Nursing, Duke University, Durham, NC,* [3]*Physician Assistant Program, EVMS, Norfolk, VA.*

Primary care providers (PCPs) want to know how to use the new genetics information to make appropriate referrals and to apply it for evaluation and testing in daily patient care. For almost 20 years clinical geneticists have used knowledge-bases such as the Mendelian Inheritance in Man, Reprotox, and GeneTests Directory of Molecular Laboratories, together with expert systems like the London Dysmorphology Database, in the daily practice of Clinical Genetics. Now, many are online including Clinical Care Guidelines by AAP and ACMG. Interestingly, it has become more difficult for the average practitioner to access and use the excellent databases available, even when they know to ask specific genetic questions. In order to find unique ways to encourage PCPs to access and use these databases, we updated and modified INFOGENETICS during the first two years of the Duke University Genetics Interdisciplinary Faculty Training (GIFT) program. Each of the 6 participating teams received a CDROM to use in clinic in order to access the key genetics databases. This project was so well received, that it was redesigned as Gift version 3.5 during year two of the project. Fifty copies of the CDROM were distributed to the 8 teams attending the second GIFT summer course. Subsequently 125 copies were distributed at the Association of Physician Assistant Programs, ~100 copies to pediatricians at the Virginia Pediatric Society meeting and 35 copies to PA clinical preceptors in rural Virginia. INFOGENETICS simply sets an icon on the computer screen that acts as a reminder to "think genetics" and facilitates access to a single page of links to 12 nationally updated databases. The software encourages PCPs to ask specific questions such as: “Could my patient's problem be genetic?” that leads to a search in OMIM; (2) “Where is my local genetics center?” that leads to a search of GeneClinics; (3) “Can I send DNA tests for this disorder?” that leads to GeneTests; (4) “What clinical care guidelines are available?” that leads to the AAP, ACMG, and Gene Reviews; (5)“How can I contact a genetic support organizations for patient information?” that leads to The Alliance; and (6) “How can I find another family with the same problem?” that leads to MUMS. Surveys of user satisfaction suggest that this approach is well-received and may encourage PCPs by supporting their reasons for referral, and guiding them to testing and clinical care guidelines useful in patient care. Funding for this project has been provided in part by Grants the Department of Health and Human Services, Genetic Services Branch, Maternal and Child Health Bureau and the NIH ELSI Program.

Disclosure(s): None

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Genetics Program for Nursing Faculty: 2001 - 2002 curriculum change outcomes.

C Prows[*1], *C Hetteberg*[1], *R Hopkin*[1], *K Latta*[1], *J Bean*[1]. [1]*Children's Hospital Medical Center, Cincinnati,OH.*

The Genetics Program for Nursing Faculty (GPNF) was the first Federally funded concentrated effort to deliver genetics instruction and educational resources specifically to nursing faculty. The primary instructional strategy was an on-site Genetics Summer Institute (GSI) that took place annually from 1997 – 2003. In January 2002, the first 18 week Web-based Genetics Institute (WBGI) was conducted and three more since then have been completed. Together they have provided genetics continuing education instruction to 246 participants (primarily nursing faculty), including 45 minority participants. GPNF participants came from 44 different states and 199 different nursing education programs / organizations. Thirty-four of the represented nursing schools have one or more of the following minority classifications: 27 Minority Serving Institutions (MSIs) 17 Hispanic Serving Institutions (HSIs); 9 Historically Black Colleges and Universities (HBCUs); 1 Tribal College / University (TCU); and 1 Native Hawaiian Serving Institution (NHSI). A GPNF specific aim is to increase the amount of genetics content in nursing curricula. 1997 - 1999 GSI curriculum change outcomes have been previously reported. To improve return rate of the baseline survey, the curriculum survey was revised following the 2000 GSI. Baseline curriculum data were collected from 55 of the 2001 and 2002 GSI participants and 16 of the January - May 2002 WBGI participants. The participants who returned baseline surveys were resurveyed during the Spring and Fall of 2003, which allowed at least one academic year between surveys for faculty to begin increasing genetics content. Thirty-five participants returned follow up surveys (49% retention rate). After attending the Institutes, all responding participants reported that they incorporated genetics content into their own courses and 64% of those who taught clinical courses, made efforts to integrate genetics into clinical experiences. In January 2003, follow up surveys will be sent to 24 participants from the August - December 2002 WBGI. Results from the 2001 - 2002 GSIs' and 2002 WBGIs' participants will be reported with a specific emphasis on the content they targeted and their curricula change strategies.

Disclosure(s): None

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Collection of family history in epidemiologic studies of coronary artery disease: can we do better? *M Scheuner*[*1,2], P Yoon[2], M Khoury[2].*
[1]UCLA School of Public Health, Department of Health Services, [2]Office of Genomics and Disease Prevention, Centers for Disease Control and Prevention.

Family history of coronary artery disease (CAD) and related disorders increases CAD risk. Stratification of familial risk into average, moderate and high-risk groups can be used to assess risk and influence prevention strategies. We reviewed family history collected in epidemiologic studies of CAD and determined whether familial CAD risk stratification could be performed. Risk factor studies of CAD were identified through the National Heart, Lung and Blood Institute website and literature review. Data collection tools for these studies were examined for 1) number and type of relatives included, 2) history of cardiovascular diseases, diabetes, and other chronic conditions, and 3) age at disease onset. Thirteen studies were identified. There was no family history collected for 2 studies and 1 collected data only on participating relatives. Family history data collection tools were available for 10 studies; 5 used self-administered surveys and 5 used structured interviews. Pedigrees could be constructed with data collected by 5 studies. The 10 studies collected data about parents; however, one study only asked whether they were alive or dead. Data about siblings were collected in 9 studies (1 did not distinguish gender). Only 4 studies collected data about children. No study collected data for second-degree or more distant relatives. The 10 studies collected information about CAD in relatives, however, one restricted this to premature CAD and another to heart attack in parents only. Most studies collected information about "other heart disease", hypertension, stroke and diabetes. Age of disease onset was collected by 8 studies. Based on our assessment of family history collected in completed and ongoing population-based studies of CAD, there is no standardization for data collection and it is inadequate for classifying CAD risk. An instrument that systematically collects family history needed to accurately identify and stratify risk could improve assessment of this important risk factor and our understanding of its role in CAD development, progression and response to therapy. The population-based data would also be useful for evaluating validity and utility of stratifying familial risk and using it to influence early detection and prevention strategies.

Disclosure(s): None

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Innovative design of a genetics curriculum for health professional students incorporating web-based needs assessment, pre-test/post-test evaluation, case-based lectures, and standardized patient OSCE. *L Sitzer*[*1], Q Edwards[2], L Freese[1], S Bathgate[1], J Larsen[1], C Macri[1]. [1]George Washington University School of Medine and Health Sciences, Washington, DC, [2]Howard University, Washington, DC.*

We propose an educational program in genetics designed for adult learners. The proposed curriculum is designed for medical and physician assistant students at the George Washington University School of Medicine and Health Sciences. Introduction: Medical genetics is an emerging field in medicine with broad implications for future health assessment and treatment. Most graduates of health professional programs have limited experience with providing genetic services because of fragmented curricula, rapidly changing knowledge, and the previous view of genetics as pertaining to rare disorders. With completion of the Human Genome Project, identification of disease causing genes has dramatically increased. While most complex disorders such as diabetes, hypertension, heart disease, stroke, and cancer are thought to be multifactorial with both genetic and environment risks, knowledge about Mendelian genetics and family history with pedigree construction will be essential for understanding future developments in this field, and in translating those developments to clinical practice. The goal of this genetics curriculum is to provide students with a strong foundation in genetics knowledge, skills, and attitudes and to reinforce these through an active experiential approach in a simulated clinical setting. The proposed curriculum incorporates an interactive computer-based needs assessment and evaluation tools. Integral to the curriculum are didatic lectures, case-based discussions and standardized patient exercises which address the different learning style of the adult learner and reinforce basic concepts in genetics at each level of learning. The curriculum will meet all of the objectives in genetics education for health professionals as set forth by the National Coalition for Health Professional Education in Genetics (NCHPEG). By incorporation of genetics education throughout the 4 year curriculum, genetic information will be applied and integrated into the understanding of all disease processes at all levels of learning. This curriculum begins in the first year of medical school with basic science of genetics, clinical applications and instruction in 3 generation pedigrees. The second year instruction covers the molecular basis of disease. The third and fourth years incorporate objective structured clinical examinations (OSCE) and standardized patients with topics pertinent to each of the clinical rotations. Web-based resources will be used throughout. The curriculum covers: 1. Genetics of normal structure and function 2. Genetics of pathological structure and function 3. Application of genetics knowledge/skills in the clinical setting: medicine, pediatrics, obstetrics/gynecology, psychiatry, primary care, and surgery 4. Special issues in genetics: current research, available resources, ethical/cultural/theological/philosophical perspectives, and policy issues

Disclosure(s): None

EDUCATION AND PUBLIC HEALTH/ETHICAL AND LEGAL ISSUES POSTERS

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Autosomal recessive microcephaly, chorioretinal dysplasia and dysmorphic features in a sibling pair. *R Sparkes*[*1], K Boycott[1], I MacDonald[2], J Friedman[3], D McLeod[1]. [1]University of Calgary, Department of Medical Genetics, [2]University of Alberta, Departments of Medical Genetics and Ophthalmology, [3]University of British Columbia, Department of Medical Genetics.*

Approximately forty cases of congenital microcephaly with chorioretinopathy have been previously reported in the literature, the majority of which have shown an autosomal dominant pattern of inheritance. Microcephaly with lymphedema is also a recognized association. Recent evidence suggests there may be single syndrome of microcephaly-lymphedema-chorioretinal dysplasia with variable expressivity. Several authors have proposed autosomal recessive inheritance for sporadic cases with more severe manifestations, but the absence of other affected family members makes the inheritance patterns difficult to determine with certainty. A few groups, however, have described affected sibling pairs with normal parents. These families came from various ethnic backgrounds. Individuals showed variable degrees of neurological impairment, ranging from normal intelligence to severe mental retardation with seizures and spasticity. Over the last decade, several detailed descriptions of the facial features associated with both the dominant and recessive forms of this syndrome have been published. Consistent findings have included bitemporal narrowing, prominent ears, a bulbous nasal tip with anteverted nares, and a pointed chin. We report two adult, opposite-sex siblings, born to healthy, nonconsanguineous parents, with congenital microcephaly, chorioretinal dysplasia, nystagmus and dysmorphic facies. Their facial features were strikingly similar to those previously described. The female proband had additional features of mild mental retardation, a history of seizures, bilateral sensorineural hearing loss, and cerebellar atrophy on cranial MRI. None of these features were present in her brother, who had normal intelligence. However, premature delivery and perinatal anoxia may have contributed to the sister's neurological problems. These siblings appear to represent additional cases of microcephaly-chorioretinopathy with autosomal recessive inheritance and variable clinical presentation. Their findings are compared to the other reported cases of this syndrome with autosomal recessive inheritance, and the issues of clinical and genetic heterogeneity are discussed.

Disclosure(s): None

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Hawaii Genetic Awareness, Implementation and Data Integration (GeneAID) project – genetics for your practice conference: effective genetics education for health care professionals.

AC Taylor[*1]. [1]Hawaii Department of Health Genetics Program, Honolulu, Hawaii.*

Genetics education is a top priority of many public health genetics programs. Targeting genetics education to health care professionals across the State of Hawaii is one of the goals of the Hawaii Department of Health Genetics Program's Genetic Awareness, Implementation and Data Integration (GeneAID) project. In order to address issues revealed in needs assessments conducted with Hawaii physicians, a full day conference, Genetics For Your Practice, took place in Honolulu in April 2003. The 156 conference participants, consisting of physicians, medical students, nurses, and allied health professionals, were asked to complete pre- and post-conference surveys in order to assess conference satisfaction and knowledge gain, as well as previous genetics education and demographic information. Results of the survey showed that the conference was very successful at increasing genetics knowledge, and at satisfying participants and interesting them in similar future conferences. Survey analysis revealed: a significant increase in knowledge scale score among participants from 3.47 (SD=1.68) to 5.02 (SD=2.15) ($t(142)=-7.34$, $p<0.001$); an average increase of 1.42 paper-based resources and 2.34 web-based resources for genetics information identified; and 92.6% of participants expressing their desire to attend another Hawaii Department of Health Genetics conference in the future. The success of this genetics education initiative highlights the importance of various key aspects of planning a successful educational event, including needs assessments, effective development, planning and operations, and meaningful evaluation.

Disclosure(s): None

GENETIC COUNSELING POSTERS

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Proposed recommendations for genetic counseling and evaluation of couples with multiple miscarriages. *R Bennett*[1], M Laurino[1], D Saraiya[1], W Raskind[1]. [1]University of Washington.*

Traditionally, genetic evaluation of couples experiencing three or more miscarriages has been limited to karyotypic analysis. Multiple genetic and non-genetic factors are now known to contribute to pregnancy loss. To develop a standard and cost-effective approach to genetic evaluation of couples experiencing multiple pregnancy loss, the Inherited Pregnancy Loss Working Group was convened through the support of a March of Dimes Grant. Members of the IPLWG have expertise in medical genetics, genetic counseling, perinatology, maternal/fetal medicine, internal medicine, infectious disease, cytogenetics, and coagulation disorders. The proposed recommendation follow the protocol developed by the National Society of Genetic Counselors and meet criteria for class three recommendations of the U.S. Preventive Task Force (based on literature review from 1983 onward and expert opinion). The IPLWG defines "recurrent miscarriage" as "three or more clinically recognized consecutive or non-consecutive pregnancy losses occurring prior to fetal viability (<24 weeks). Our recommendations consider infectious, teratogenic, thrombophilic, cytogenetic, and Mendelian causes of miscarriages. We also review professional and patient resources, consequences of assisted reproductive technologies, and psychological and cultural issues related to pregnancy loss. The proposed recommendations are being reviewed by experts both within and beyond the NSGC as well as consumer advocacy groups. The final document will be accepted through review by the NSGC attorney, Ethics Subcommittee, and the Board of Directors. IPLWG Chairs-Robin Bennett, MS and Wendy Raskind, MD, PhD; Members: Barbara Pettersen, MS, Elizabeth Varga, MS, Stefanie Uhrich, MS, Debra Lochner Doyle, MS, Robert Resta, MS, Lisa Baumeister, MD, Kathy Leppig, MD, Larry Sheilds, MD.

Disclosure(s): None

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Options for Down syndrome screening: what determines patient choice? *K Brigatti*[1], K Berentsen[1], R Denchy[1], S Brown[1], F Malone[1]. [1]Columbia University College of Physicians and Surgeons, New York, New York.*

BACKGROUND Down syndrome screening using first trimester markers alone (the "combined" test: nuchal translucency ultrasound and maternal serum for an 85% detection rate) or together with second trimester serum quad markers (the "integrated" test with a 94% detection rate) is now possible. **OBJECTIVE** To determine patient preference and utility of results from either combined (CT) or integrated screening (IT) for Down syndrome at Columbia University Medical Center. **METHOD** We examined patient choice of screening method on 282 singleton pregnancies between February 11, 2003 and November 21, 2003, as well as preferences regarding prenatal diagnosis upon receiving results. Patient choice based on advanced maternal age (AMA, ≥35 years old) and whether test risk was increased or decreased from age-based risk were also examined. **RESULTS** Most women elected IT over CT. Patients who elected CT were generally AMA and all elected CVS when the calculated risk was higher than their age-related risk. AMA patients with a lower calculated risk that that of age generally had amniocentesis. A majority of non-AMA women who elected CT had a previous aneuploidy and all had amniocentesis following screening. Patients who elected IT were generally non-AMA. Both AMA and non-AMA patients who elected CT were likely to pursue amniocentesis when their calculated risk was higher than their age related risk (AMA women) or they had a screen positive result (non-AMA women). AMA women who chose CT were unlikely to pursue amniocentesis if their calculated risk was lower than their age-related risk. **CONCLUSION** Women who elect CT are highly motivated to pursue prenatal diagnosis. When their risk following screening is lowered, women electing CT are still likely to have prenatal diagnosis, choosing amniocentesis over CVS. Thus, CT may appeal to patients who are trying to determine if their risk is sufficiently high to warrant CVS or sufficiently low to justify waiting until the second trimester for amniocentesis. Women who choose IT are not as motivated to pursue prenatal diagnosis, but are highly responsive to screen results and are likely to elect amniocentesis when their calculated risk is screen positive (women <35 yo) or elevated above their age-related risk (AMA patients). IT may appeal to AMA patients who are undecided about amniocentesis for age alone. The high detection rate of IT appears to be more important than the later time results are available. Women who desire prenatal diagnosis may choose CT to decide which prenatal diagnostic procedure to elect, while those who use screening in deciding whether or not to elect prenatal diagnosis altogether may opt for IT.

Disclosure(s): None

GENETIC COUNSELING POSTERS

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Prenatal diagnosis of familial hemophagocytic lymphohistiocytosis: implications for pregnancy and neonatal management. *S Carter*[1], D Cordero[1], S Gross[1], A Filipovich[2].*
[1]Montefiore Med Ctr, Bronx, NY, [2]Cincinnati Children's Hospital Med Ctr, Cincinnati,OH.

Background: Familial hemophagocytic lymphohistiocytosis (FHLH) is an autosomal recessive disorder characterized by nonmalignant accumulation and multivisceral infiltration of uncontrolled T-lymphocytes and activated macrophages. Primarily affecting infants and young children, it leads to pancytopenia, fevers, hepatosplenomegaly, coagulation defects, and neurological abnormalities. The estimated incidence is about 1:50,000 births. Approximately 20%-40% of FHLH cases worldwide have mutations in perforin 1 (PRF 1), which maps to 10q21-q11. More than 50 different mutations of PRF1 have been identified in patients with FHLH. We report the first prenatal diagnosis of previously identified PRF1 gene mutations in a nonconsanguineous Dominican couple. **Case Report:** The patient is a 24-year-old G3P2002 Dominican female who presented for genetic counseling at 10 weeks' gestation. Their 18-month-old daughter was diagnosed with FHLH at age 2 months. She underwent chemotherapy until a successful unrelated bone marrow transplantation at age 10 months. Molecular analysis demonstrated that the proband was a compound heterozygote for PRF 1 mutations. She had inherited a paternal 50 del T in exon 2 that leads to premature stop codon and a maternal 1442 A>C missense mutation in exon 3. Chorionic villi sampling was performed at 12 weeks' gestation. Chromosome analysis revealed a normal 46, XX female karyotype. Mutation analysis for PRF 1 mutations demonstrated that the fetus inherited both of the mutant alleles in the PRF 1 coding sequence that are associated with FLH. The family has elected to continue the pregnancy. **Conclusion:** Aside from providing couples with reproductive options, the advantages of prenatal diagnosis include increased fetal surveillance by ultrasound since an affected fetus can develop hepatosplenomegaly and hydrops. Additionally, a search for a compatible bone marrow donor can be initiated.

Disclosure(s): None

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Presymptomatic testing of children for early-onset conditions: pantothenate kinase-associated neurodegeneration, a case report.

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With the introduction of clinical genetic testing for hereditary disorders comes complex decision-making for the families involved, as well as, for the genetic health professional. The issue of presymptomatic testing of children, especially, poses a great challenge. This is well illustrated in a family with a history of Pantothenate Kinase-Associated Neurodegeneration (PKAN). PKAN is an autosomal recessive disease of the nervous system, which includes the accumulation of iron in the brain. Common features consist of progressive dystonia, muscular rigidity, sudden involuntary muscle movements, speech problems and retinitis pigmentosa. Symptoms begin in late childhood or early adolescence. They are usually progressive and eventually cause death. A married couple with a family history of PKAN presented for preconception counseling. The wife's sister died of a disease of unknown etiology, but her medical history was consistent with PKAN. The husband's 9-year-old niece was recently diagnosed with PKAN by iron deposition in the basal ganglia on brain MRI and mutation analysis. This couple requested carrier testing with the understanding that because their family was consanguineous—their grandparents are siblings—there was an increased chance for at least one member of the couple to be a carrier. Both members of the couple were found to be carriers. With this revelation, the couple inquired about prenatal testing and testing for their 2-year-old son. Benefits and limitations of testing the 2-year-old were discussed. Benefits included ability to join a research protocol; testing could yield vital information that could be utilized by other family members; and the ability to prepare for the onset of the condition. Limitations incorporated in the session were the lack of preventive measures available to ease the onset of the disease; impact of testing on the family unit, i.e. parental change in attitude toward the affected; inability for their son to make an autonomous decision; and laboratory guidelines could impede testing a minor. Laboratory policy did not allow for the testing of presymptomatic minors. Since this was the only laboratory available to perform the analysis, we had to decline to test the child. The issues that arose from this decision will be presented. With parental insistence, we located a developing research trial, in which enrollment was dependent on the positive mutation analysis of the participant. A specimen was sent on the premise that the child was a candidate for research. The laboratory approved this indication. He was found to be a carrier. The logistics of the testing process and the conflicts it poses for the ethics of genetic health professionals will be reviewed.

Disclosure(s): None

GENETIC COUNSELING POSTERS

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Isolated bilateral upper limb anomalies: prenatal vs postnatal appearance of humeroradial synostosis, ulnar aplasia and oligodactyly.

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There are very few clinical entities with isolated bilateral forearm and hand anomalies. We report a baby boy whose prenatal ultrasound findings were different than the clinical findings seen postnatally. The mother was a 25 year-old, G1P0. Her and her partner are French Canadian and non-consanguineous. There was no relevant family history aside from a maternal fifth degree relative with a "limb difference", reportedly missing digits bilaterally. This was an uncomplicated pregnancy, with no teratogenic exposures (no thalidomide, cocaine, or infections). A level II ultrasound at 21 weeks gestation revealed very short forearms (radius & ulna) bilaterally. The hands appeared abnormal with 'likely' syndactyly. The lower extremities were normal and no other anomalies were seen. A fetal echocardiogram was normal. Genetic counselling was difficult as it was impossible to predict limb function or rule out other significant differences that may be present at birth or present in childhood. Further follow-up was required to determine causality and recurrence risk. Amniocentesis was offered and declined. The baby was born at term and seen in the genetics clinic at two weeks of age. The examination revealed a healthy male, birth weight 8lbs 2½oz (75th percentile) and head circumference at the 98th percentile. His father also has a large head circumference. The rest of the physical examination was unremarkable, excluding the upper limbs. The left upper limb has shortening of the forearm and is missing the ulna and the 4th and 5th phalanges and metacarpals. The radius is curved and does not articulate normally with the distal humerus. Movement of the elbow is restricted. The right upper limb also has significant forearm shortening and both the ulna and radius are missing. The distal aspect of the humerus appears bifid. Only two digits are present, with one having two phalanges and the other three. There is no evidence of syndactyly and the digits and the hands appear functional. The diagnosis of these findings is humeroradial synostosis, ulnar aplasia and oligodactyly. This type of limb difference is usually not found with other health concerns. This case illustrates a common genetic counselling dilemma; what to tell couples whose fetus presents with anomalies detected via ultrasound. This case demonstrates the limitations of ultrasound in its ability to predict cause, severity and the impact these anomalies will have on an individual. In such cases, it is prudent to advise couples that the differential diagnosis could include a new mutation, an autosomal recessive condition, as well as the possibility of a sporadic event. Follow-up clinical examination should be offered where possible.

Disclosure(s): None

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Mosaic trisomy 16: comparison of prognostic information derived from Internet sources versus scientific publications. *M McDermet*[1], *A Donnenfeld*[2]. [1]*Genzyme Genetics, Yonkers, NY,* [2]*Genzyme Genetics, Philadelphia, PA.*

Background: A couple presented for genetic counseling following their amniocentesis result which identified a diagnosis of mosaic trisomy 16 [47,XY+16(12)/46,XY(5)]. Genetic counseling information was presented to them that had been collected from published case reports and reviews from the medical literature regarding fetuses and children with mosaic trisomy 16. The parents were informed that there was a significant likelihood of fetal demise, developmental delay/mental retardation, growth restriction and congenital anomalies, including cardiac, renal, skeletal, GI and renal abnormalities. The parents' response to this rather dismal picture of mosaic trisomy 16 was "that's not the impression we got from the website". The website to which they referred, trisomy16.org, includes parents' descriptions of their children with mosaic trisomy 16 (one stillbirth and 13 children ranging in age from 9 months to 3rd grade). All 13 children are described as having growth delays, and some have speech and motor skill delays. Eight are described as having normal cognitive development. The descriptions for these children include: "cognitively on target", "close to developmental milestones", "normal 4 year old", "no learning disabilities", etc. For the other 5, there is no reference to cognitive development. Discussion: Many individuals perform internet searches prior to their genetic counseling appointment. This is especially true if an abnormal result has been identified as the internet is a readily available source of information. The problem, as highlighted in this case, is the validity of this information. Additionally, biased information on the best possible outcomes involving surviving children with mosaic trisomy 16 may be presented. This information may not be representative of pregnancy outcomes from prenatally diagnosed cases. This case demonstrates: 1) the need for medical genetics professionals to be aware of what information is available on the internet, 2) the need for scientific information to support, or in some cases dispute, information from a website, and 3) the need to present scientifically valid genetic counseling information which is applicable to the patient's situation. Specifically, this case demonstrates a discrepancy between information in the medical literature and information available on the internet from a support group regarding mosaic trisomy 16. The observation of such conflicting information underscores the need for additional collection and publication of additional series of children with this condition.

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GENETIC COUNSELING POSTERS

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Quality of life in adults with neurofibromatosis type 1. *PZ Page*[*1], *BR Korf*[1], *GP Page*[1], *A Leplege*[2], *P Wolkenstein*[2]. [1]*University of Alabama at Birmingham, Birmingham, AL,* [2]*Hopital Henri-Mondor, Paris, Frace.*

Neurofibromatosis type 1 (NF1) impacts the quality of life (QoL) of affected people, causing both visible manifestations and, occasionally, life-threatening illness. The progressive, unpredictable nature of the condition can be a great source of anxiety for affected individuals and their families. We report a single center, cross-sectional study designed to evaluate the impact of the severity and visibility of the NF1 phenotype on QoL. Participants were primarily recruited through the National Neurofibromatosis Foundation via both an e-mail announcement and advertisement in a newsletter. A total of 176 adults with NF1 responded and filled out the study questionnaires. Severity and visibility were evaluated using, respectively, the Riccardi and Ablon scales. The Skindex and Short Form 36 health survey (SF-36) profiles were used to evaluate, respectively, both skin disease-specific and general health QoL. In a multiple regression model controlling for sex, age, and visibility, severity remained independently associated with the alteration of 2 aspects of the general health QoL (SF-36): personal perception of good health ($p<0.001$: HQ#1) and the question, "Has pain in the past 4 weeks interfered with life?" ($p<0.001$: HQ#8). After adjusting for severity, age, and gender, patients with more visible neurofibromatosis were significantly affected by the following domains of their Skindex and General Health QoL (SF-36): personal perception that the skin condition affects their interactions with people, ($p<0.001$;D#20), if they are angry about their skin condition ($p<0.001$; D#15), if they are ashamed of their condition ($p=0.016$; D#12), and if it affects life activities such as climbing stairs ($p=0.002$; HQ#3E) and their energy level ($p<0.001$; HQ#9A). In conclusion, neurofibromatosis type 1 has a significant impact on QoL through alteration of health and appearance. The consequences of visibility and severity from the viewpoint of patients can be evaluated using both the Skindex and the SF-36.

Disclosure(s): None

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Genetic counseling issues when there are abnormalities on both X chromosomes. *E Tejero*[*1], *A Donnenfeld*[2], *R Schmidt*[3], *J Knops*[4], *M Galli*[3]. [1]*Genzyme Genetics, New York, NY,* [2]*Genzyme Genetics, Philadelphia, PA,* [3]*Genzyme Genetics, Yonkers, NY,* [4]*Genzyme Genetics, Santa Fe, NM.*

Case Report: A 36 year-old G2P0020 patient was referred for genetic counseling due to two first trimester miscarriages. Her first POC karyotype had revealed an unbalanced X;autosome translocation: 46,X,der(X)t(X;1)(q28;q11). The increased risk for a parental balanced X-autosome translocation was addressed. The patient and FOB opted for peripheral blood chromosome analysis. Routine cystic fibrosis and fragile-X carrier screening were also performed at the patient's request. **Results-** The FOB's peripheral blood chromosome results were within normal limits. The patient's peripheral blood chromosome results were 46, X, t(X;1)(q28;q11). The interpretation was an apparently balanced reciprocal translocation between the long arm of one X chromosome and the long arm of one chromosome 1. In addition, the patient was found to carry a fragile-X premutation (59 CGG repeats) on the non-translocated X chromosome. A normal CGG fragile-X sequence of 20 repeats was identified on the translocated X. **Implications-** The patient was counseled regarding an increased risk for miscarriage and gonadal dysfunction due to the translocation; and premature ovarian failure as well as expansion to a full fragile-X mutation due to her fragile-X premutation. The risk to have a live born child with an unbalanced X-autosome translocation is estimated as 20-40%, with a phenotype that can range from mild defects to severe MCA/DD. The risk for a female offspring includes MCA/DD and/or gonadal dysfunction even if the female offspring is a balanced carrier due to the potential for variable X-inactivation patterns. There is little information on males born to women with X-autosome translocations. Phenotype has ranged from normal to major genital defects. If the normal X is inherited, a male fetus would be at risk to be affected with fragile-X syndrome, a female fetus would also be at risk to inherit the full mutation. Recent research has found the risk for expansion from 59 repeats to a full fragile-X mutation to be 3.7% (Nolin et al, 2003). In addition the patient is at risk for autosomal trisomies due to advanced maternal age. **Counseling-** The risks of a balanced X;1 translocation, an unbalanced X-autosome translocation, fragile-X syndrome, premature ovarian failure, multiple miscarriage and autosomal trisomies were discussed with the patient and her husband. The possibility of prenatal diagnosis, preimplantation genetic diagnosis and egg donation were also discussed with the couple. The patient opted to have her younger sister (who was not an X-autosome translocation carrier, a fragile-X carrier or of advanced maternal age) serve as an ovum donor in a future pregnancy.

Disclosure(s): Authors are employees of, serve as paid consultants to and receive honoraria and/or travel support from Genzyme Genetics. Genzyme Genetics sponsors research activities relevant to this presentation.

MOLECULAR GENETICS POSTERS

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Association of G691S polymorphism in the RET proto-oncogene with early onset of MEN 2A in an American population. *S Ahmed*[1], K Kruckeberg[1], P Lundquist[1], W Highsmith[1]. [1]Division of Molecular Genetics, Mayo College of Medicine,Rochester, MN.*

Background: Multiple endocrine neoplasia type 2A (MEN 2A) is an autosomal dominant, hereditary cancer syndrome characterized by medullary thyroid carcinoma, primary hyperparathyroidism and pheochromocytoma. There are published reports of the association of the G691S polymorphism in exon 11 of the RET proto-oncogene with early onset of disease in individuals harboring specific point mutations in the RET proto-oncogene. These studies were performed on a Spanish population {Gil.et.al, Int.J.Cancer :99, 299-304 (2002) & Robledo.et.al. Cancer Research: 63,1814-1817 (2003)} According to these studies a significantly higher proportion of patients who presented with features of MEN 2A at an age <20 years old were homozygous for the G691S polymorphism as compared to patients who presented at an age of >20 years old. The objective of our study was to verify this modifying effect of the G691S polymorphism in an American population. **Study Design & Method:** From our database, we selected 22 RET-mutation positive probands who had developed features of MEN 2A. We divided our patients into two groups, based on the age of presentation. Group 1 (14 patients) developed features of MEN 2A after 20 years of age (mean age 47 yrs.) and group 2 (8 patients) developed symptoms before 20 years of age (mean age 10 yrs). Archived genomic DNA was analyzed from these patients. A portion of exon 11 of the RET proto-oncogene was amplified by PCR. The amplified products were subsequently subjected to enzyme digestion using Ban I and analyzed on a 2% Nusieve/Agarose gel. **Results:** Of the 14 patients in group1, 4 were heterozygous and none were homozygous for the G691S mutation. Of the 8 patients in group 2 (<20 yrs), there were two heterozygotes and 2 homozygotes. **Interpretation and Discussion:** In our study, we observed that 25% of patients (2/8) with an early age of onset (<20 yrs.) of MEN 2A were homozygous for the G691S polymorphism. In contrast, none of the 14 patients with an older age of onset (>20 yrs.) were homozygous for the G691S mutation. Although the data do not yet reach statistical significance, there is a clear trend supporting the observation that homozygosity for the G691S polymorphism is associated with an earlier age of onset of MEN 2A. This effect does not appear to be limited to the Southern European or Spanish population. Analysis of additional patient samples is ongoing.

Disclosure(s): None

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Notch3 mutations in Turkish families with CADASIL syndrome. *M Apak*[1], O Uyguner[1], H Kayserili[1], A Siva[2], A Altintas[2], S Saip[2], G Akman-Deniz[3], N Isik[4], Z Yilmaz[4], B Wollnik[1]. [1]Istanbul University, Child Health Institute, Division of Medical Genetics, [2]Istanbul University, Cerrahpasa Medical Faculty, Dept. of Neurology, [3]Istanbul University, Istanbul Medical Faculty, Dept. of Neurology, [4]Goztepe Social Security Hospital, Istanbul.*

CADASIL syndrome (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) is an adult-onset neurological disorder characterized by recurrent strokes and dementia. Mutations in the Notch3 receptor gene have been identified to cause this disorder. We describe here the clinical and molecular findings of three Turkish families with CADASIL syndrome. The recently described R110C (C328T) mutation in exon 3 of the Notch3 gene was identified in two non-related Turkish CADASIL families, suggesting, that this mutation might be a common mutation in Turkish CADASIL patients. Interestingly, both families presented with different severity of the disease. The index case of "family I" had a past history of migraine without aura was admitted with a severe migraine attack and a transient brainstem syndrome at the age of 48. An MRI at that time showed bilateral white matter lesions. Years later, her sister was seen at the age of 48, who also had migraine without aura, a generalized anxiety disorder and essential tremor. The MRI was almost identical to that of her elder sister; both anterior temporal and frontal lobes were involved, as well as basal ganglia and external capsules with characteristic hyperintense lesions. In contrast, affected family members of Family II showed a much earlier onset of the disease in the early thirtieth, typical MRI findings and a severe progression.These different phenotypic expressions in the two families carrying the same Notch3 mutation suggests, that modifying factors exist that influence the onset and severity of CADASIL syndrome. In addition, we identified the novel C210R (T601C) mutation in exon 4 of the Notch3 gene in a patient with two consecutive stroke like episode at age 49 and 51, respectively, typical MRI findings, and positive family history for neurological disease. This novel mutation, that was not found in 88 normal control chromosomes, causes an odd number of cysteins in the N-terminal EGF-domain of the Notch3 protein. This finding provides further evidence, that the change of the normally even number of cysteins in this N-terminal domain of Notch3 represents the main pathophysiological mechanisms of the disease.

Disclosure(s): None

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Pseudo uniparental disomy in galactosemia.

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Loss of function of the enzyme galactose-1-phosphate uridyl transferase (GALT), results in classic galactosemia. Over 300 mutations in the GALT gene have been identified, and an autosomal recessive pattern of inheritance is confirmed from combining biochemical phenotype, molecular genotype and clinical expression of galactosemia. Biochemical phenotype is determined by erythrocyte galactose-1-phosphate (Gal-1-P) concentration and GALT enzyme activity. Molecular phenotype is determined by PCR amplification of the GALT gene from leukocyte DNA by allele specific oligonucleotide (ASO) hybridization, DNA sequencing or restriction digestion of the genomic DNA followed by Southern blot hybridization. Here we report a potential misdiagnosis of uniparental disomy using these methods in a family of Ashkenazi Jewish descent. The proband presented with classical galactosemia confirmed by high levels of Gal-1-P and no activity of erythrocyte GALT. Erythrocyte Gal-1-P and GALT activity were <1 mg% and 6.0 µmoles/gHb/hr, and <1 mg% and 12.3 µmoles/gHb /hr in mother and father respectively. Normal level of Gal-1-P is <1 mg% and GALT is 25 ± 5 µmoles/gHb /hr. The proband was screened for the 7 most common mutations in galactosemia following multiplex PCR and ASO hybridization and was thought to be homozygous for the K285N missense mutation. However, the mother appeared to be homozygous for N314D and the father's genotype was N314D/K285N. Paternal disomy for the K285N was possible but unlikely. To clarify this situation, we performed Southern blot on EcoRI digested DNA from the patient and her parents using full length GALT cDNA as a probe. The amount of the 10 Kb GALT hybridization band in the proband and her mother was half that of her father's, providing evidence for one deleted GALT allele in both the proband and her mother. We conclude that deleted GALT alleles, common in Ashkenazim with galactosemia were present in the mother whose corrected genotype is N314D/del GALT and in the proband whose corrected GALT genotype is K285N/del GALT. We recommend confirmation of GALT genotype by Southern blot analysis when PCR is used in Ashkenazim families to avoid a misdiagnosis of homozygosity for the non-deleted allele and uniparental disomy.

Disclosure(s): None

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The CRMGEN project: new reference materials for genetic testing.

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The use of appropriate Reference Materials (RMs) to validate test equipment or testing methods is an important part of any analytical testing system. Certified reference materials (CRMs) are RMs whose characteristics have been fully documented and validated. Currently, no CRMs are available for genetic testing. The CRMGEN project is a 14-center collaboration funded by the European Commission's Measurement and Testing program*. We are developing reference measurement systems and producing CRMs for molecular genetic tests. Prototype RMs will be developed for a wide range of tests. These prototype RMs, developed in one of 4 genetics centers, will be validated in 7 other centers before extensive field trials. The knowledge gained in this process will be used to develop guidelines for the production of CRMs for any genetic test. Special emphasis will be given to the commutability of the candidate RMs, i.e. their ability to perform under a wide range of test protocols and conditions. In the Dublin laboratory, we have used the polymerase chain reaction (PCR) to produce prototype RMs. RMs have been developed for hemochromatosis and hemoglobinopathy assays, and have been successfully tested in the laboratories of the other CRMGEN partners. Field trials are taking place in conjunction with the PT scheme for hemochromatosis run by the European Molecular Genetics Quality Network. Highly multiplexed tests pose particular difficulties for the development of appropriate reference materials, but synthetic RMs have the potential to overcome many of these problems. We are now turning our attention, therefore, to the development of reference materials for multiplex genetic tests, starting with cystic fibrosis and Duchenne muscular dystrophy.
*EU Contract G6RD-CT-2001-00581 www.crmgen.com

Disclosure(s): Presenting author is currently serving as a paid consultant to Synergene Technologies Ltd., Malta.

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Molecular analysis of a family with autosomal dominant atrial septal defects. *SL Bleoo*[1], JS Parboosingh[1], M Anderson[1], J Lauzon[1], PJ Bridge[1], R McLeod[1]. [1]Dept. of Medical Genetics, Alberta Children's Hospital, Calgary, Alberta, Canada.*

Atrial septal defect (ASD) is a common form of congenital cardiac defect. Both sporadic and familial forms have been described. Recently patients with non-syndromic autosomal dominant ASD have been found to have mutations in the NKX2.5 homeobox transcription factor or the transcription factor GATA4. These transcription factors likely play a critical role in heart formation as mice with mutation to either NKX2.5 or GATA4 display cardiac defects. Identification of various patients has led to the finding that mutations in NKX2.5 can have variable expressivity including ASD, progressive atrioventricular conduction delays, ventricular septal defects (VSD), tetralogy of Fallot, or tricuspid valve abnormalities. Likewise in one family pedigree, GATA4 mutations have resulted in ASD as well as other heart defects including ventricular septal defects, atrioventricular septal defects, pulmonary valve thickening, or insufficiency of cardiac valves. We present a family with multiple individuals diagnosed with isolated ASD. The proband first came to medical attention after having both daughters diagnosed with isolated ASD. Upon investigation the proband was found to have a small ostium secundum ASD; no electrophysiologic abnormalities were noted. Five additional family members have isolated non-syndromic ASD, while one other affected family member had a transposition of the great vesicles and a ventricular septal defect (VSD). Although appearing to be transmitted in an autosomal dominant pattern, the pedigree shows reduced penetrance, which appears to be rare in familial ASD. Sequence analysis of the NKX2.5 gene and GATA4 gene will be presented.

Disclosure(s): None

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Detection of C677T/A1298C "double variant" chromosomes: implications for interpretation of MTHFR genotyping results. *N Brown*[1], V Pratt[1], A Buller[2], W Sun[2], R Chen[2], B Crossley[2], M McGinniss[2], F Quan[2], CM Strom[2]. [1]Quest Diagnostics, Nichols Institute, Chantilly VA, [2]Quest Diagnostics, Nichols Institute, San Juan Capistrano CA.*

Two common variants in the methylenetetrahydrofolate reductase (MTHFR) gene (C677T and A1298C) are reported risk factors for hyperhomocysteinemia and cardiovascular disease (Rozen, Thromb Haemost 1997). C677T homozygotes and compound heterozygotes for C677T/A1298C genotypes have been implicated in decreasing enzyme activity leading to hyperhomocysteinemia (Van der Put et al., Am J Hum Genet 1998), a known risk factor for venous thrombosis. We perform MTHFR DNA analysis as a part of a thrombophilia evaluation. We have identified 12 individuals homozygous for C677T/heterozygous for A1298C and 5 individuals heterozygous for C677T/homozygous for A1298C. These genotypes demonstrate that these 17 individuals have a single allele that contains both the C677T and A1298C variations and a second allele that contains either the C677T or the A1298C variation. The genotype for one specimen was confirmed by DNA sequencing. The presence of 3 copies of variant alleles has been previously reported by Isotalo et al. (Am J Hum Genet 2000) as being present more commonly in spontaneous abortions than in the general population. Ogino and Wilson (J Hum Genet 2003) performed a meta-analysis and concluded that MTHFR individuals with these 3 variant genotypes were detected in control populations at a higher frequency than reported in two previous studies and suggested that there may be a founder effect in some parts of the United Kingdom and Canada (Skibola et al., Prod Natl Acad Sci1999; Wiemels et al., Prod Natl Acad Sci 2001). Individuals heterozygous for the double variant MTHFR allele and a wild type allele would appear to compound heterozygotes for C677T and A1298C. Since this genotype is associated with thrombophilia, such patients may be inappropriately labeled as high risk for thrombotic episodes. Until information regarding the clinical consequences of this double variant allele becomes available, caution should be used in interpreting the genotyping results of compound heterozygosity for C677T and A1298C.

Disclosure(s): None

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Analysis of cystic fibrosis mutations by multiplexed elongation of allele-specific oligonucleotides displayed on custom BeadChips™. *B Roa*[1], I Buyse[1], S McCarthy[1], Y Song[2], Y Han[2], G Hashmi[2]. [1]Medical Genetics Laboratories, Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, [2]Bioarray Solutions Ltd, Warren, NJ.*

A novel BeadChip™ array format for multiplexed mutation detection and an associated complete assay delivery system (BioArray Solutions) has been evaluated for the ACMG Cystic Fibrosis (CF) standard mutation panel (25) plus selected ethnic-specific mutations using elongation-mediated multiplexed analysis of polymorphisms (eMAP™). The eMAP™ protocol combines multiplex amplification of genomic DNA and multiplex allele detection using allele-specific oligonucleotides (ASOs) containing variable 3'-terminal sequences attached to color-encoded beads. These beads are assembled into arrays of very small footprint on semiconductor chips. After annealing and elongation, the fluorescent-labeled mutant and wild type primer extension products are simultaneously detected by instant imaging of the entire array. Comparative studies were performed on a set of 38 CF mutations in three multiplexed groups using eMAP™, and a currently implemented system for CF testing (MALDI-TOF mass spectrometry, Sequenom). A total of 270 patient samples were analyzed in batches using high throughput robotics for both PCR set-up and post-PCR processing. Analysis using the software included in the BeadChip™ assay delivery system showed 100% concordance between results produced on both systems. Allelic variants that are known to affect some assays (e.g. F508C, R117C) did not interfere with mutation detection using eMAP™. In summary, this validation study demonstrated the BeadChip™ system and eMAP™ protocol as a highly sensitive, specific and reproducible system. This highly multiplexed assay is amenable to robotic applications to maximize throughput for high-efficiency testing.

Disclosure(s): Research/work relevant to this presentation was supported by a grant from Bioarray Solutions Ltd.

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Quality evaluation of data interpretation and reporting. *E Dequeker*[1], E Girodon[2], M Schwarz[3], M Stuhrmann[4], I Lubin[5], J Cassiman[1]. [1]Department of Human Genetics, Leuven, Belgium, [2]Service de Biochemie et Genetique Moléculaire, Créteil, France, [3]Paediatric Genetics Unit, NW Regional Molecular Genetics Laboratory, Manchester, United Kingdom, [4]Human Genetics, Hannover, Germany, [5]Division of Laboratory Systems, CDC, Atlanta, USA.*

Errors in any of the steps of a molecular genetic test may affect its results, interpretation, and use. Errors in tests that are time sensitive (i.e. prenatal) or are provided to asymptomatic persons (i.e. carrier testing) can pose significant problems. Patients are usually tested only once in lifetime and an incorrect result may not become apparent for many years. Therefore, it is important that the errors be completely eliminated. Over the past eight years proficiency testing (PT) schemes for cystic fibrosis (CF) have been organised in Europe, and 135 to 240 diagnostic laboratories participated. In 2003, 33 US laboratories participated in the European PT program under a joint project supported by the European CF network, the US Centers for Disease Control and Prevention, and the Association of Molecular Pathologists. Laboratory performance to correctly genotype improved over time. In addition, since 1999, the European PT scheme included an evaluation of the report that is normally sent to the referral physician. Reports are reviewed for content and accuracy of their interpretation. The schemes demonstrated that reporting of laboratory results varied considerably. However, over the years, the scheme revealed improvements in certain aspects of reporting including how patient and technical information is presented. Unfortunately, more than 30% of the laboratories still make at least one mistake in a submitted report (administrative errors, errors in risk calculation, wrong interpretation of the results, or confusing genotype results of samples from patients and carriers). Taking into account the different reporting policies (country specific issues), this evaluation study also provided information on the large variation between laboratories in reporting genetic testing results for carrier testing of individuals with a positive family history of CF, or for genetic confirmation testing of the clinical diagnosis of CF. Overall, there is a high variation in the format, the content, and the quality of the written reports for molecular genetic tests. This can be improved by preparing and using consensus guidelines for genetic test reporting (including the availability of model laboratory reports).

Disclosure(s): None

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A combined biochemical and molecular approach to newborn screening for cystic fibrosis: results of 122,830 samples. *KC Donahue*[*,1], *DE Freer*[1]. [1]*Pediatrix Screening, Bridgeville, PA.*

Pediatrix Screening has performed CF testing for newborns on whole blood spots for several years. Since 1995, we have used delta F508 genotyping in addition to immunoreactive trypsinogen(IRT) quantitation. In January of 2003, we added a panel of 33 mutations to our protocol. The model used and the results of our approach are presented for Pennsylvania Newborn samples received between January 1st 2003 through October 31st 2003. We utilize a sandwich immunoassay from ICN for IRT for our initial screening. Samples with an IRT >90ng/mL are re-punched and the IRT repeated. If the elevated IRT is confirmed, the filter-paper is re-punched for delta F508 analysis using Light Cycler technology (Roche). Newborn samples positive for delta F508 are then analyzed using the Innogenetics Probe Assay CFTR 33, which identifies 26 mutations recommended by the ACOG/ACMG, as well as eight other mutations and 5T/7T/9T in Intron 8. Samples with no copies of delta F508, but with IRT >130 are not tested further but a repeat sample is requested. Repeat samples are retested for persistent IRT elevation. If the IRT is >90 for the second sample, the CFTR 33 panel is performed. Newborn samples are defined as first samples obtained within 7 days of delivery. Results are reported as: "presumptive positive" only when 2 copies of known mutations have been identified; "inconclusive" when the sample has one copy of a mutation; OR an IRT >130 ng/mL blood and no delta F508 mutation; "within normal limits (WNL)" for other results. Of 122,830 newborn samples, 754 (0.6%) had an IRT of >90 ng/mL. Of these, 66 were positive for delta F508 (light cycler), and 688 had no copies of delta F508. Of the 66, 11 samples had two copies of the mutation (homozygous) identified while 55 had a single copy (heterozygous). The 55 heterozygous samples were then studied using the CFTR 33 mutation panel. Fourteen of the 55 were found to be compound heterozygotes and reported as "presumptive positive", 41 were negative for the mutations detected by the panel and were reported as "inconclusive". Five hundred nineteen of the 688 samples with no copies of the delta F508 mutation had an IRT <130 ng/mL and were reported as "WNL". The remaining 169 samples with IRT >130 ng/mL were reported as "inconclusive" and a repeat sample was requested. In 142 cases (84%), repeat samples were received. Of the 142, 15 showed a persistent elevation of IRT and were then studies using the CFTR panel. Three of the 15 samples were found to be heterozygous for a non-delta F508 mutation detected by the panel and no samples were found to have two copies of the panel mutations. Details of mutation analyses and final outcome data will be presented.

Disclosure(s): None

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De novo CFTR 749 del T variant found in a teen with mild symptoms of cystic fibrosis. *LS Hammond*[*,1], *I Virella-Lowell*[1], *SB Keiles*[2], *CM Bowman*[1]. [1]*Charleston, South Carolina,* [2]*Costa Mesa, California.*

We report on a 13-year-old girl with a cystic fibrosis transmembrane regulator (CFTR) gene alteration that is not found in either parent and which is new to the CF Mutation Database www.genet.sickkids.on.ca/cftr/. In this family unit of two parents and two children, three individuals showed varying degrees of pulmonary and/or gastrointestinal symptoms, and gene sequencing established a different genotype for each member with symptoms. Proband was seen because of emesis, abdominal pain, constipation, foul smelling stools and a previous history of pneumonia at age 7 and asthma. A sweat chloride test was borderline with result of 43.5 mmol/L. CFTR full gene sequence analysis done by Ambry Genetics Laboratory detected 621 + 1G → T mutation, M470V polymorphism, 7t/9t alleles and a novel variant, 749 del T. Proband's sister who also had history of emesis, abdominal pain and foul smelling stools previously attributed to short gut syndrome, showed sweat chloride of 52 and 54.5 mmol/L. DNA sequencing showed 621 + 1G → T mutation, 5t/9t alleles, and M470V polymorphism. Their mother, who has history of maxillary polyposis, seasonal and perennial allergies, and irritable bowel syndrome, had sweat chloride of 46 mmol/L. CFTR showed 5t/7t alleles and homozygosity for the M470V polymorphism. Their father has no symptoms. His genotype was 621 + 1G → T mutation, 7t/9t alleles and M470V. The 749delT variant in our proband was not detected in mother, father or sister. That we detected the less common 621 + 1G → T mutation in father and both daughters on repeated testing makes alternate paternity or specimen mix-up unlikely. The segregation patterns of the CFTR alleles suggest that a new mutation occurred, possibly on the maternally derived allele of our proband.

Disclosure(s): None

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A high mental functioning fragile X male with a de novo 103bp deletion including entire CGG repeat region in > 50% of his peripheral leukocytes.

X Han[1], F Chehab[1], B Powell[2], J Phalin-Rague[2]. [1]Laboratory Medicine, University of California San Francisco, [2]Children's Hospital Central California.*

The presence of over 50% of fragile mental retarded protein (FMRP) expressing leukocytes has been reported to correlate with non-mental retarded status in fragile X male patients. The deletion of all CGG repeats plus flunking sequence in fmr1 gene did not abolish the gene expression. In this study, we identified a fragile X male mosaic for a full mutation, premutation, and 103bp deletion including all CGG repeats and 42 bp immediately 5' of CGG repeat region. The deletion was present in over 50% of his leukocytes. The proband was not mentally retarded and non-dysmorphic. After analyzed six relevant members of the family, we conclude that the deletion occurred as de novo event. Mother of the proband carried an allele of premutation with 109 CGG repeats that was inherited by her three sons and expanded to a full mutation. However, each of her sons has their own mutation pattern. One her sons carried a completely methylated full mutation. Her youngest son had a full mutation with methylation mosaicism. Her eldest son, the proband, was a mosaic of a full mutation, premutation, and microdeletion. It suggests that the permutation allele carried by the mother was expanded after fertilization and somatic instability caused the de novo microdeletion and mosaicism. However, we are unable to rule out the possibility of the meiotic instability present in the maternal oocytes.

Disclosure(s): None

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Validation of p53 mutational analysis using dHPLC and sequencing.

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p53 is a multifunctional transcription factor that is intimately involved in the cellular response to stressful stimuli such as DNA damage and hypoxia. Substantial evidence demonstrates that the obliteration of the normal p53 pathway is a critical step in the initiation and progression of tumors. In addition to its role in the surveillance mechanisms that arrest cell cycle progression, p53 can also trigger apoptosis in response to DNA damage. Germline mutations of the p53 gene confer a high risk of diverse malignancies. The highest frequency of inherited p53 defects have been noted in Li-Fraumeni syndrome (LFS). We have evaluated the usefulness of denaturing high performance liquid chromatography (dHPLC) as a diagnostic tool for scanning the p53 gene to identify point mutations, small deletions, and insertions. The coding region of the p53 gene (11 exons) was divided in 10 overlapping fragments to encompass the coding sequence and the splicing regions. All PCR reactions were amplified simultaneously using the same reaction conditions in a 96-well format and then analyzed by dHPLC, using empirically determined optimum temperatures for partial fragment denaturation. Following dHPLC analysis, all positive results displaying heteroduplexes were sequenced to confirm and describe the exact alteration. The assay was validated using dHPLC with 20 wild-type samples to scan for variants, which were subsequently sequenced to identify single nucleotide polymorphisms (SNPs), and a set of previously tested patient specimens containing disease-causing mutations. Our methodology allows an increase in the sensitivity and reproducibility for mutation detection as compared to the conventional methods for mutation detection and will facilitate screening of large series of patients. Screening for p53 gene mutations enables an accurate and routine determination of the p53 status of patients with cancer and may be applied in clinical oncology to cancer diagnosis, prediction of prognosis and response to treatment.

Disclosure(s): None

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Molecular analysis of a translocation in a family with dominant skeletal dysplasia: toward a novel gene/mechanism.

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Previously, our group reported a family in which five generations of skeletal defects correlated with transmission of a balanced translocation t(13;17)(q22.1;q23.3) (Stalker et al. (2001) Am J Med Genet 103:339-341). Major features include Robin-type cleft palate, pectus excavatum, rib dysplasia, and scapular hypoplasia. We hypothesize that the translocation alters expression of one or more genes, causing this condition through one of several possible mechanisms such as direct disruption or gene fusion. As a first step toward determining what gene(s) are involved, we employed the power of somatic cell hybrids to provide a method to fine map the translocation breakpoints. Mouse-human somatic cell hybrid lines were created from one patient's lymphocytes. We characterized these and found one each carrying a derivative translocation chromosome (der(13) and der(17)), and several segregating the normal chromosomes. Each cell line was assessed by PCR for the presence (or absence) of a series of chromosome-specific markers to narrow the intervals containing the breakpoints. This mapping initially used known microsatellite markers and STSs. Further resolution was accomplished using PCR primers we designed from genome sequence to further sub-divide the region between flanking positive and negative markers on both chromosomes. We have currently narrowed the breakpoint regions to less than 8 kb on each chromosome. We will present the results of ongoing work to precisely localize the translocation breakpoints and identify candidate genes within or near the breakpoints whose altered expression may be responsible for the clinical features seen in this family. There are no genes in these regions that are yet known to be involved in hereditary skeletal disorders, and thus the study of this family offers an opportunity to identify new genes and biochemical pathways that affect skeletal development and might be involved more broadly in conditions such as isolated cleft palate.

Disclosure(s): None

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Validation of the cystic fibrosis 3199del6 Invader assay for clinical use.

K Friedman[1], J Howitt[1], W McCachren[1], M Eisenberg[1]. [1] Center for Molecular Biology and Pathology, Laboratory Corporation of America, Research Triangle Park, NC.*

Background: Based on its prevalence within certain groups affected with cystic fibrosis (CF), the I148T mutation was included in the original ACOG/ACMG mutation panel for cystic fibrosis screening. Recent studies suggest that it may not be a CF-inducing mutation, but rather a polymorphism serving as a marker for the presence of a second disease-associated mutation, 3199del6. Currently available CF testing platforms do not detect or genotype the 3199del6 allele. This study describes the use of Invader technology to develop an analyte specific reagent (ASR) for use in detecting the presence of the 3199del6 mutation in genomic DNA. Methods: Invader is a homogenous, isothermal, linear signal amplification system that can detect specific genomic DNAs and/or nucleotide polymorphisms without target amplification. The technology is based on the Cleavase enzyme's ability to cleave a specific structure generated by the hybridization of two overlapping oligonucleotides on a target DNA. A set of Invader oligonucleotides probes specific for the 3199del6 mutation were designed and used in a laboratory-developed assay to detect the presence of the 3199del6 mutation. The assay was used in a multi-well plate format to test patient derived genomic DNAs previously characterized with a PCR-based reference method for the presence of specific CF-associated mutations. Results: The laboratory-developed test using the Invader CFTR 3199del6 ASR demonstrated 100% concordance with the results obtained using the reference method in detecting the presence of the 3199del6 mutation in genomic DNA and did so without prior target amplification. It was also capable of providing reliable genotyping of specimens with regard to homozygous or heterozygous status for the mutation. Further studies are underway to validate the use of the test with automated platforms for 96-well and 384-well plate set-up and detection. Conclusions: The use of the Invader CFTR 3199del6 ASR allowed the development of a reliable, easy to use, high throughput and cost effective laboratory method of genotyping genomic DNA for the 3199del6 CF mutation. It also demonstrated the flexibility afforded by the use of the Invader technology in adapting to changes in the mutational patterns required for CF screening and diagnosis. This characteristic of the technology is extremely important as future changes in the ACOG/ACMG CF mutation panel are expected and laboratory tests will have to be modified in response to those changes.

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Microarray comparative genomic hybridization in the detection of aneuploidy in clinical cytogenic specimens. *AJ Iafrate*[1], C Lee[1]. [1]Dept. Pathology, Brigham and Women's Hospital, Boston, MA.*

Microarray comparative genomic hybridization has shown great promise in clinical genetic diagnostics, and several labs have published successful early efforts in clinical validation. We are exploring the use of array CGH in the clinical setting, and have focused on establishing conditions for the use of human DNA isolated from a variety of specimen types, with a focus on those specimens from which it is difficult to obtain significant quantities of high quality DNA- including uncultured and cultured amniocytes and fixed cells (3:1 methanol: acetic acid). Using 1 megabase commercially available Spectral Genomics BAC arrays we have found a reproducible detection of aneuploidy in these specimens, including detection of trisomies 13 and 21, deletions of the Prader-Willi/Angelman's locus on 15q, and elucidation of several marker chromosomes. These array CGH results correlated with classical karyotype and FISH analysis of the same cases. We have also successfully arrayed cases with limiting amounts of DNA by first amplifying the sample with whole genome amplification methods using the phi29 DNA polymerase. These studies will aid research studies of constitutional and tumor cytogenetics, and more importantly improve the ability to analyze clinical cases with limited or borderline quality DNA.

Disclosure(s): None

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MGX™ 4d array platform: a streamlined system integrating genomics and bioinformatics for cancer research and drug discovery. *M Iyer*[1], R Philip[1], A Steel[1], E Eastman[1]. [1]MetriGenix, Inc.*

Molecular profiling by DNA microarray technology has made significant contributions to the understanding of many diseases, especially cancer. Cancer is a heterogeneous disease at the morphological and molecular levels leading to diverse clinical presentation. As many different tumors are systematically analyzed, massive amounts of gene expression data have accumulated both in public and private databases. One of the challenges is how to efficiently utilize the accumulated research data to develop new standards for cancer classifications, better prognosis and prediction of therapeutic outcomes, and to identify new molecular targets for drug discovery. Cancer-specific gene sets, or disease signatures, generated from microarray studies need to be validated using independent cancer samples and sophisticated analytical tools. The ultimate value of these molecular markers will be determined by testing in large-scale, multi-center clinical trials that require a unified and more efficient assay system. The MetriGenix 4D™ array system lends itself well to serve these needs. The MGX 4D™ System consists of a patented Flow-thru Chip™ contained within a microfluidic cartridge, automated hybridization and chemiluminescence detection stations, and data analysis software. Disease-relevant gene sets are identified through extensive data mining of comprehensive gene expression databases followed by sophisticated data analysis. Gene selection is based on expression signatures and fold changes between normal and diseased sample groups. Differential advantages of the 4D system will also be presented. In studies with our Cancer arrays, our goal is to determine biological markers for potential early detection and clinical diagnostics in the general population using a well defined data mining strategy and an easy-to-use validation platform. We have used Gene Signature Differential Analysis, Fold Change, Linear Discriminate Analysis (LDA), and Principle Component Analysis (PCA) to mine one of the world's largest human gene expression high-density array proprietary database. We will describe the use of tumor-specific 4D arrays to characterize molecular changes in lung and colon tumor samples and the results were compared with results using quantitative RT-PCR (TaqMan™). Data will be presented that describes the gene selection process including data from the patent pending genes selected that mark the signature for the diseases will be presented. Data will also be presented on expression pattern of the important signature genes using patient samples. MGX 4D Cancer arrays are aimed at understanding the molecular basis of cancers in the hope that this knowledge can be used to design improved diagnostic and therapeutic approaches for this disease.

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Interstitial deletion of chromosome 12q: genotype-phenotype correlation of two patients utilizing array comparative genomic hybridization. *KA Rauen*[1], OD Klein[1], PD Cotter[1,2,3], A Weiss[4], D Bick[4], DG Albertson[5,6], D Pinkel[5]. [1]Division of Medical Genetics, Department of Pediatrics, University of California San Francisco, San Francisco, CA, [2]Division of Medical Genetics, Children's Hospital and Research Center, Oakland, CA, [3]US Labs, Inc., Irvine, CA, [4]Genetics Center, Children's Hospital of Wisconsin, Milwaukee, WI, [5]Department of Laboratory Medicine, University of California San Francisco, San Francisco, CA, [6]Cancer Research Institute, University of California San Francisco, San Francisco, CA.*

Deletions of chromosome 12q are rare, with only 11 reported cases in the literature. We have recently described 2 cases with cytogenetically identical interstitial deletions of the long arm of chromosome 12. The first case was a girl who presented with a phenotype consistent with cardio-facio-cutaneous (CFC) syndrome including characteristic dysmorphic craniofacial features, sparse curly hair, scant eyebrows, hyperkeratosis pilaris, a muscular VSD and developmental delay. Cytogenetic analysis demonstrated a female karyotype 46,XX,del(12)(q21.2q22), implicating a possible locus for CFC syndrome (Am J Med Genet. 2000. 93:219-222). Subsequently, we reported a second patient who had the identical 12q deletion. Cytogenetic analysis demonstrated a male with a karyotype 47,XYY,del(12)(q21.2q22) (Am J Med Genet. 2002. 110: 51-56). Phenotypic features of this male proband included craniofacial anomalies, ectodermal findings, right renal anomaly, cryptorchidism, a history of a small PDA with persistent patent foramen ovale and developmental delay. Although the patient did not have the classic composite phenotype for CFC syndrome, there were many features observed in this patient that are seen in CFC syndrome. Here, we report a third patient with an identical interstitial deletion: 46,XY,del(12)(q21.2q22). Phenotypic features of this male proband included craniofacial and ectodermal anomalies, right renal anomaly, cryptorchidism, a history of a small PDA with persistent patent foramen ovale, mild ventriculomegaly on brain MRI, hyperopia and developmental delay. To further define the extent of the chromosomal aberration in the two male probands, microarray-based comparative genomic hybridization (array CGH) analysis was performed. Array CGH is a technology that measures copy number change across the entire genome. The array used in this study consisted of genomic clones covering the genome with an average resolution of 1.4 Mb, which is significantly higher than conventional GTG-banding. Although cytogenetic analysis of the two patients were concordant, molecular analysis by array CGH revealed that the patients had discordant distal breakpoints. Detailed phenotypic analysis and comparison of the molecular breakpoints in these two patients as determined by array CGH will be discussed. Array CGH, as demonstrated in this study, allows breakpoints to be localized at the molecular level and provides accurate sizing of chromosomal aberrations. This, in turn, may impact a patient's phenotype and prognosis, as well as provide finer mapping of candidate genes that may be implicated in specific malformations. Analysis by array CGH will greatly assist in refining genotype-phenotype correlations in complex chromosomal aberrations and rearrangements.

Disclosure(s): None

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Detection of 3199del6 mutation by real-time PCR using Roche LightCycler. *Q Li*[1], D Sun[1]. [1]Department of New Technology and Test Development, South Bend Medical Foundation.*

I148 mutation is one of the 25 Cystic Fibrosis mutations recommended by the American College of Medical Genetics and American College of Obstetrics and Gynecology and was believed to represent a sever (pancreatic insufficient) mutation. Researchers have recently discovered a correlation between I148T and another cystic fibrosis mutation namely 3199del6, a 6 base pair deletion at position 3199 of the messenger RNA. Approximately 6% of CF carriers are positive for I148T and approximately one of every fifty I148T carriers will be positive for 3199del6. The conventional method for detecting 3199del6 mutation is DNA sequencing which is time consuming and labor intensive. We have developed a rapid method for detection of 3199del6 mutation by real-time PCR on LightCycler which fluorometrically monitor real-time formation of PCR products during thermal cycling with fluorescein labeled probe. The amplification step was performed with a primer set coding for a region in exon 17a of CFTR gene. 3'-fluorescein labeled probe hybridizes to an internal sequence of the amplified fragment and gives an increase in fluorescence intensity, which is monitored by Lightcycler instrument. During the DNA melting curve analysis, the temperature is slowly elevated above the melting point of the PCR product and fluorescence is measured continuously. Upon denaturation (ssDNA formation), the fluorescein labeled probe is released and resulting in a decreasing fluorescence signal which provides accurate melting temperatures for amplified DNA fragment. The mean melting temperatures (Tm) of the amplified DNA fragments with 3166del6 mutation (mutant) and the DNA fragments without 3199del6 mutation (wild type) are 56.3 C and 59.9 C, respectively. The between run precision is 0.55% for 3199del6 peak and 1.06% for wild type peak. This method unambiguously identified 3199del6 positive and negative patients previously tested by ARUP reference laboratory. An analysis of 32 samples can be completed within 90 minutes by this rapid real-time PCR method. In summary, the method described is fast, reliable, cost-effective and well adapted for routine laboratory testing.

Disclosure(s): None

MOLECULAR GENETICS POSTERS

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Human germline mutation origins: estimates of the male to female sex ratio of mutation and the first observation of a mosaic individual with two different substitutions at the same base. *S Sommer*[1], X Li[1], A Halangoda[2], A Karlea[1], W Scaringe[1], K Hill[3]. [1]Department of Molecular Genetics, City of Hope National Medical Center, Duarte, CA, USA, [2]Qiagen, San Francisco, CA, USA, [3]Biology Department, The University of Western Ontario, London, Ontario, Canada.*

We previously estimated the sex ratios of germline mutation in the Factor IX gene (F9) and showed that they vary significantly with mutation type (Ketterling et al. 1999. Hum Genet 105:629). We have since doubled the sample size of F9 origins and added Factor VIII (F8) and Duchenne muscular dystrophy (DMD) origins to our database, tripling the total number of germline mutation origins available for analysis. We have confirmed a dramatic difference in the sex ratio of G>A relative to A>G transitions (1.35 vs. 8.9) and shown that F8 and DMD have sex ratios of germline mutation similar to those of F9. We have observed 6 of 10 mosaic individuals with G>A transitions not at CpG suggesting that non-CpG methylation may occur in early embryogenesis. We also identified the first case of a nucleotide bi-mutation. Two different substitutions were identified at the same base position in a hemophilia A family (395A>C carried by the mother and 395A>G carried by a maternal aunt). The maternal grandfather was determined to be the origin of these mutations and was shown to be mosaic for both mutant alleles, as well as the normal allele. The C and G mutant alleles were estimated to be present in the grandfather's blood leukocytes at levels of about 1/10 and 1/160, respectively, of the normal A allele. Nucleotide bi-mutations with one allele at such a low frequency would rarely be detected yet may be frequent. This observation is consistent with a highly mutagenic DNA adduct or lesion present in the zygote. The combined origin data from these X-linked genes allow a robust appraisal of the circumstances in the human germline under which certain mutational events occur.

Disclosure(s): None

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Identification of galt mutations using specific assays and gene scanning. *J McKinney*[1], M Campbell[1], S Dobrowolski[1], W Zhao[2], C Saunders[2]. [1]Idaho Technology, Inc., Salt Lake City, UT, [2]Dept. of Pathology, Children's Mercy Hospital, Kansas City, MO.*

Galactosemia (OMIM #230400) most frequently results from mutations in the GALT gene (9p13) and is a target of newborn screening efforts in most states. Classic galactosemia occurs in approximately 1 in 30,000 in the general U.S. population, while milder variants occur in up to 1 in 10,000. Over 170 mutations have been identified in the GALT gene, 4 of which account for approximately 70% of mutant GALT alleles. The common Duarte variant (N314D) results in partial enzyme deficiency (25% in heterozygotes, 50% in homozygotes) and is present in approximately 7% of the heterogeneous American population. When present in trans with a classical G allele, the Duarte variant causes a milder "D/G" disease that can mimic classical disease in newborn screening. The overlapping ranges observed in patients with D/G, G/N, and D/D genotypes complicate interpretation of abnormal GALT enzyme assays. Genotype analysis is profoundly useful for differentiation of D/G compound heterozygotes from truly affected patients. Data is presented illustrating genotyping of the 4 most common mutations (S135L, Q188R, L195P, and K285N) and the Duarte variant using SimpleProbe assays on the LightCycler. Detection probes were designed as perfect matches for the mutation to assure that any other variant that may occur within the region covered by the probe would cause a destabilization and result in a lower probe melting temperature. Samples were initially genotyped on the LightCycler for the 4 common mutations and the Duarte variant. DNA samples from patients with known abnormal enzyme activity, who tested negative for the above mutations, were selected for further analysis. Gene scanning to identify rare mutations in exons 5-10 was performed using a novel platform based on dye binding and high-resolution thermal denaturation of whole amplicon by fluorescent melting curve analysis. A number of samples were observed to harbor sequence variants indicated by melting curve analysis and subsequently defined by direct sequencing. This combination of genotype analysis for common mutations followed by reflex scanning using high-resolution melting curve analysis is a time- and cost-effective method for rapid, comprehensive genotype analysis of the GALT gene.

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Validation of a comprehensive MCAD scanning assay. *J McKinney*[*1], *S Dobrowolski*[1], *J Winters*[2], *S Hahn*[2]. [1]*Idaho Technology, Inc., Salt Lake City, UT,* [2]*Mayo Clinics, Rochester, MN.*

Medium-chain acyl-CoA dehydrogenase (MCAD, OMIM #607008) catalyzes the initial reaction in the beta-oxidation of C4-C12 straight-chain acyl-CoA. MCAD deficiency is the most frequently observed inborn error of fatty acid oxidation. Inherited deficiency of MCAD is characterized by intolerance to prolonged fasting, recurrent episodes of hypoglycemic coma, impaired ketogenesis, and low plasma and tissue carnitine levels. The disorder can be severe, and even fatal in young patients. Newborn screening for abnormal acylcarnitine profiles is common, however these profiles can differ greatly between MCAD deficient patients and there appears to be no straightforward genotype/phenotype relationship. The most frequent genetic cause of MCAD deficiency is the common A985G mutation, which results in a Lys-304-Glu substitution in the mature protein. Rapid, homogeneous detection at the A985G locus, in addition to the next most common MCAD variant (T199C) is described. Comprehensive analysis of the MCAD gene has traditionally relied on slow, costly, and labor-intensive techniques. A rapid, homogenous method of scanning the coding regions of the MCAD gene (12 exons) has recently been described and is presented herein. PCR product size ranged from 129 to 223 base pairs, with exon 11 assayed as 2 overlapping fragments of 198 and 197 base pairs due to its size. Fragments are amplified by rapid cycle PCR in the presence of LCGreen™ I, a new dsDNA specific fluorescent binding dye (Idaho Technology). Fluorescent melting curve analysis is performed from data obtained by high-resolution thermal denaturation (melting) on the new HR-1 instrument (Idaho Technology). A total of 37 DNA specimens, including several with abnormal acylcarnitine profiles, were comprehensively screened for sequence variants. Over half of the samples contained 2 or more mutations in the coding regions of the MCAD gene. All fragments where sequence variants were indicated by fluorescent melting curve analysis were prepared for sequencing. Results confirmed the presence of mutations in the heterozygous state in all samples, while those that appeared negative by melting curve analysis were confirmed negative by sequencing. The combination of HR-1 and LCGreen I provide a rapid, sensitive, and cost effective alternative for traditional scanning methods, with the added advantage of a homogenous reaction.

Disclosure(s): Presenter/authors are receiving honoraria and/or travel support from Idaho Technology.

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WITHDRAWN

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Coexistent mutations in the androgen receptor and SRY genes in a case of X,Y sex reversal: which one defines the phenotype? *O Mueller*[*1], *G Jervis*[2,3], *B Kousseff*[3]. [1]*Molecular Genetics Laboratory, All Children's Hospital, St. Petersburg, Florida,* [2]*Genzyme Laboratory, Tampa, Florida,* [3]*Regional Genetics Program, Department of Pediatrics, University of South Florida, Tampa, Florida.*

X,Y Sex reversal are caused by disruptions of a number of biochemical processes, including testicular unresponsiveness to human chorionic gonadotropin or luteinizing hormone, inborn errors of testosterone biosynthesis, defects in androgen-dependent target tissues, defects in synthesis, secretion, or response to anti-Mullerian hormone, or maternal ingestion of estrogen and progestogens. Mutations in the androgen receptor gene are relatively common causes of X,Y sex reversal associated with androgen insensitivity syndrome (AIS). Over 250 different mutations have been described that cause the three recognized variants of AIS. Complete AIS is associated with a completely female phenotype with normal external secondary sexual development, although they have a blind vagina, absent uterus, female adnexa, and abdominal or inguinal testes. Partial AIS is associated with varying degrees of ambiguous genitalia, including hypospadias, and mild AIS which often presents only with undermasculinization. Mutations in the SRY gene, which is the first gene in the testicular determination pathway, also cause X, Y sex reversal, but, unlike AIS, usually have a total absence of male secondary sexual development. We describe a patient who presented with a female phenotype, adequate pubic hair and breast development, blind vagina, complete absence of male secondary sex characteristics without axillary hair and ambiguity of genitals. Anthropometrics were within the normal range. DNA was extracted from a peripheral blood specimen and analyzed for androgen receptor and SRY gene mutations by automated DNA sequencing. The SRY gene was found to have a g to c base change which causes a threonine substitution for arginine at codon 84 (R84T). This mutation has not been previously described in X,Y sex reversal patients, although similar amino acid changes near this codon have been reported. The androgen receptor gene was found to have a deletion of two adenosine bases in codon 854 within the androgen-binding domain. This deletion resulted in a frame shift that altered all amino acids up to codon 881, where an out-of-frame termination codon was revealed. This particular deletion has also not been previously reported, but similar frame shift mutations in this region have been described with typical signs of complete AIS. The phenotype is as in complete AIS and it does not show signs that are a result of the SRY mutation. As expected, the mother has the same AIS mutation and there was history of maternal aunt with a similar 46,XY female phenotype. The mutations in this family have not been previously reported but similar mutations are in the Human Gene Mutation Database. There was no evidence of synergism of the two mutations within the phenotype.

Disclosure(s): None

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Clinical validation of neurofibromatosis 1 mutations by direct DNA sequencing.

JJ Mulvihill[*1], SM Purandare[1], D Pei[1], S Li[1]. [1]Department of Pediatrics, the University of Oklahoma Health Sciences Center.*

Neurofibromatosis 1(NF1) is one of the most common of the dominant neurogenetic disorders, affecting about 1 in every 3500 individuals worldwide. It is characterized by multiple café-au-lait spots, peripheral neurofibromas, Lisch nodules and distinctive bony lesions. It is fully penetrant by adulthood. The NF1 gene spans 350 kb of genomic DNA, containing 60 exons and encoding 12 kb of mRNA. Detection of NF1 gene mutations has presented a considerable challenge because of its diversity of genetic mutations, the absence of any obvious mutational clustering, the size of the gene, and the existence of numerous psuedogenes. Currently, detection of NF1 gene mutations is approached by FISH, protein truncation test or DHPLC followed by DNA sequencing. We have used a whole NF1 cDNA screening methodology to study 94 individuals; these included definite clinical patients (both familial and sporadic by NIH criteria), normal individuals as controls and "problematic" patients with some clinical suspicion of NF1. After informed consent, RNA from peripheral blood was obtained from each individual using the total RNA purification kit (Qiagen). The RNA was reverse transcribed using Superscript II reverse transcriptase (Gibco, BRL). The entire NF1 cDNA was amplified in 10 overlapping fragments, ranging from 634 to 1262 bps. The size of the PCR products was verified by electrophoresis in 2% agarose gels before sequencing. So far, different types of known and novel mutations have been identified in the patient group, a deletion occurred in one individual of the "problematic" group, and as expected, no mutation has been found in the normal controls. Advantages and disadvantages of direct NF1 cDNA sequencing will be presented.

Disclosure(s): None

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Notch 3 mutations in patients presenting with abnormal white matter.

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Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a rare genetic disorder suspected in patients with a positive family history and who have signs and symptoms that include stroke, migraine, dementia and white matter abnormalities seen on MRI. Mutations in the Notch 3 gene, which is involved in signaling cascades that control cell fate during development, are detected in approximately 90% of cases. In about one third of CADASIL patients, the earliest manifestation is migraine, frequently with aura, occurring in the fourth decade. In some patients cognitive decline is the first symptom but both the severity and progression of impairment is highly variable. Patients go on to experience transient ischemic attacks, strokes, and multiple subcortical infarcts. CADASIL is likely to be underdiagnosed as the signs and symptoms are non-specific and variable. In patients presenting with brain white matter abnormalities and migraine with aura, CADASIL is rarely included in the differential diagnosis. This study will evaluate whether CADASIL should be investigated in patients with white matter abnormalities presenting with either migraine with aura or strokes. Preliminary sequence analysis of exons 3 and 4 of the Notch 3 gene (studies indicate that approximately 65% of CADASIL mutations are present in exons 3 and 4) identified 3 mutations in 4 unrelated individuals out of 19 individuals tested. Mutation analysis of the remaining Notch 3 exons is ongoing.

Disclosure(s): None

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Reproducibility and performance of vysis GSA300 platform for pre- and post-natal array-based CGH diagnostics. *E Pestova*[*1], *T Ruffalo*[1], *T Marble*[1], *M Lucas*[1], *A Wong*[2], *D Ledbetter*[2], *K Wilber*[1], *W King*[1]. [1]*Vysis, subsidiary of Abbott,* [2]*Emory University.*

Genosensor™ Array 300 (GSA300) is a multiplex platform for array-based comparative genomic hybridization that detects unbalanced genomic aberrations including whole chromosome gains/losses, microdeletions, duplication, and unbalanced sub-telomeric rearrangements. In pre- and post- natal diagnostics, multiplex technologies such as CGH microarrays permit the simultaneous measurement of copy number changes across many genomic loci in a single experiment and require relatively small amount of specimen for analysis. Because of its greater resolution over conventional cytogenetics, it is ideally suited for the detection of small chromosomal aberrations that are associated with multiple disorders ranging from idiopathic mental retardation to micro-deletions syndromes. In the present study, we assessed the analytical performance, reproducibility, and utility of GSA300 arrays for potential application in pre- and post-natal diagnostics. We evaluated the performance of the arrays using DNA extracted from 1 microdeletion cell line (46,XX,del(22)(q11.2q11.2) Di-George) and 4 trisomic cell lines (47, XY, +13, 47, XY, +21, 47,XY,+18, and 47,XY,+2), blood samples from eight apparently normal donors, and amniotic cells obtained from apparently normal male and female pregnancies. In our studies, using DNA isolated from cell lines and normal reference DNA we were able to reliably detect each of the known genomic abnormalities (analytical sensitivity of >90%, specificity of >99% on a per clone basis) with low user-to-user variation. Using blood from normal clinical specimens and normal blood donors, sex of the test DNA was correctly identified in all cases (analytical sensitivity >90.9% and specificity of >99% on a per clone basis), and an average CV for the replicate chips for the same specimen was <10%. Amniotic cells grown in culture allowed for accurate and reproducible identification of fetal gender. We have also demonstrated the ability to detect a 14qtel microduplication in one of the amniotic specimens.

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SNP genotyping by high resolution melting of small amplicons. *R Pryor*[*1], *M Liew*[2], *R Palais*[3], *C Wittwer*[1,2]. [1]*Dept. of Pathology, University of Utah School of Medicine, Salt Lake City, UT,* [2]*Institute for Clinical and Experimental Pathology, ARUP, Salt Lake City UT,* [3]*Dept. of Mathematics, University of Utah, Salt Lake City, UT.*

Homogeneous PCR methods for genotyping single nucleotide polymorphisms (SNP) usually require fluorescently labeled oligonucleotide probes or allele specific amplification. High-resolution melting of amplicons with the DNA dye LCGreen I (Idaho Technology) is a homogeneous, closed-tube method of heteroduplex detection that does not require probes or real-time PCR. We adapted this system to genotype SNPs after rapid PCR (12 min) of small amplicons (<50 bp). All possible SNPs were systematically studied with engineered plasmids. In addition, the clinical SNP targets, factor V (Leiden) G1691A, HFE C187G, beta globin (HbS) A17T, MTHFR A1298C, and prothrombin G20210A were studied. In all cases, heterozygotes were easily identified because the heteroduplexes produced changed the shape of the melting curve. In most cases, homozygous polymorphisms were also distinguishable from each other by melting temperature (Tm) shifts. When the amplicon size is small, these differences are large enough that they can usually be seen on regular (low-resolution) real-time instruments. However, about 15-20% of SNPs are A/T or G/C exchanges with very small Tm differences between homozygotes. These differences require high-resolution instrumentation (HR-1, Idaho Technology) for complete genotyping. Even with high-resolution analysis, one-quarter of A/T and G/C SNPs show nearest neighbor symmetry, and, as predicted, the homozygotes cannot be resolved. In these rare cases, adding 15-20% of a known homozygous genotype to unknown samples produces different amounts of heteroduplexes and clustering of the melting curves according to genotype. The method is simple, rapid, and inexpensive, requiring only PCR, a DNA dye, and melting instrumentation.

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The PTPN11 gene and nonsyndromic isolated hypertrophic cardiomyopathy. *A Roberts*[*1], H Rehm[1,2,3], B McDonough[4,5], S Barr[4,5], C Seidman[4,5], J Seidman[4,5], R Kucherlapati[1,2,5]. [1]Harvard Medical School Genetics Training Program, Boston, MA, [2]Harvard Medical School-Partners Healthcare Center for Genetics and Genomics, Laboratory for Molecular Medicine, Cambridge, MA, [3]Department of Pathology, Brigham and Women's Hospital, Boston, MA, [4]Division of Cardiology, Brigham and Women's Hospital, Boston, MA, [5]Department of Genetics, Harvard Medical School, Boston, MA.*

Familial hypertrophic cardiomyopathy (HCM) is inherited as an autosomal dominant disease. HCM has an estimated prevalence of 1 in 500 and is the most common heritable cardiovascular disease (Maron et al 1995). Mutations in beta-cardiac myosin heavy chain, cardiac actin, cardiac troponin T, alpha-tropomyosin, cardiac troponin I, cardiac myosin binding protein C, and the myosin light chains have all been shown to cause HCM (CardioGenomics.org, 2003). It has been estimated that 50-80% of patients with Noonan syndrome have an abnormal echocardiogram (Allanson, 1987). Pulmonary valve stenosis is the most common cardiac anomaly. HCM is found in 20-30% of affected individuals (Ishizawa 1996). Tartaglia (2002), published a study of PTPN11 gene mutations in 119 unrelated patients with the clinical diagnosis of Noonan syndrome. Mutations were found in 45% of these individuals. Of twenty probands with Noonan syndrome and HCM, fifteen percent had a detectable PTPN1I mutation. Three of eleven LEOPARD syndrome patients with PTPN11 mutations also had HCM (Legius 2002 and Digilio 2002). It is not uncommon for a gene responsible for a multiple anomaly syndrome to be associated with one of the sentinel defects occurring in isolation. Sarkozy et al (2003) screened twenty patients with nonsyndromic pulmonary valve stenosis for PTPN11 gene mutations by PCR amplification and single strand conformation polymorphism. No fragments with aberrant migration patterns were identified by this method. To date, there has been no evaluation of the role of the PTPN11 gene mutations in nonsyndromic isolated HCM. The Cardio Genomics Sarcomere Gene Mutation Screening Project, in an effort to define the complete set of disease-causing sarcomere gene mutations, has screened the genes encoding sarcomere proteins in adult HCM patients. Of 64 probands, 40 had a detectable mutation in one of eight sarcomere genes sequenced: MYH7, MYBPC3, TNNT2, TNNI3, TPM1, ACTC, MYL2, or MYL3. We received anonymized DNA samples from the remaining 24 probands that did not have a detectable sarcomere gene mutation for the purpose of screening for PTPN11 gene mutations. The PTPN11 gene contains fifteen exons. Primer pairs were designed to amplify exons, exon/intron boundaries, and short intron flanking stretches. Purified PCR product was sequenced in the forward and reverse direction and sequences were reviewed using Mutation Surveyor software (SoftGenetics, Inc) and compared to normal control sequences. To date, we have sequenced 357 of the 24 patients' 360 exons. There were no detectable mutations found in the coding regions. It would be important to repeat this analysis in a larger, pediatric population before concluding that the PTPN11 gene is not involved in the pathogenesis of nonsyndromic isolated HCM.

Disclosure(s): None

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Role of macrophage DNASE (Dnase1L3) in systemic lupus erythematosus (SLE). *MC Schneider*[*3], ML Lu[2], A Wilber[1]. [1]University of Minnesota, Mol., Cell., Dev. Bio, and Genetics Dept., Minneapolis, MN, [2]Division of Urological Research, Department of Urology, Brigham and Women's Hospital, Boston MA, [3]Division Genetics, Department Pediatrics, Southern Illinois University, Springfield IL.*

Systemic lupus erythematosus (SLE) is characterized by autoantibodies to nucleosomal components, including DNA. While deficiency of DNASE1 (D1) predisposes to SLE in both mice and men, DI mutations are uncommon in polygenic human and murine SLE; however, both MRL-lpr and (NZB x NZW) F1 SLE models have a mutation in the macrophage-DNASE, Dnase1L3 (D3), leading to the non-conservative T89I substitution. Dnase1 and Dnase1l3 are both found in the serum and highly homologous, they differ in one specific assay. When media conditioned with D3 is added to HeLa cells, liposomal gene transfection is blocked, a capacity absent for D1. In eukaryotic and prokaryotic in vitro expression studies, the mutation present in SLE mice decreases D3's nuclease activity against naked or free DNA by only ~2 fold; however, the mutant enzyme's ability to block liposomal DNA transfection of HeLa cells is decreased by 8-fold. Induced cellular levels of D3 protein are present in splenocytes and bone-marrow-derived macrophages from SLE mice, yet parallel increases in secreted free DNA-nuclease activity are absent. Media conditioned by C57BL/6 macrophages confers an interferon-gamma (Ifn-g)-inducible barrier to transfection to HeLa cells. This inducible activity parallels cellular D3 levels and DNASE activity in conditioned media, strongly suggesting D3 is the agent of this macrophage-secreted function. Macrophages from both MRL-lpr and (NZB x NZW) F1 mice are defective in the capacity to block liposomal transfection. We proceeded to analyze the genomic sequence of DNASE1L3 in 29 independent patients with SLE. African-Americans from the LMRR (lupus multiplex registry and repository) were chosen for analysis, since they had previously shown linkage to a marker near DNASE1L3. Here we report a heterozygous complete loss of function mutation present in a patient with human SLE. In conclusion, loss of function mutations in this macrophage DNASE are associated with human and murine SLE.

Disclosure(s): None

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MECP2 variants in psychiatric diseases: possible association with autism. *S Sommer*[*1], J Feng[1], A Shibayama[1], C Glanzmann[1], J Yan[1], E Cook[2], N Craddock[3], I Jones[3], D Goldman[4], LL Heston[5]. [1]Department of Molecular Genetics, City of Hope National Medical Center, Duarte, CA, USA, [2]Department of Psychiatry, University of Chicago, Chicago, IL, USA, [3]Division of Neuroscience, University of Birmingham, Queen Elizabeth Psychiatric Hospital, Birmingham, UK, [4]Department of Psychiatry, NIAAA, NIH, Bethesda, MD, [5]Department of Psychiatry, University of Washington, Seattle, WA, USA.*

Mutations in the gene coding methyl-CpG-binding protein 2 (MECP2) cause Rett syndrome (RTT) and have also been reported in a number of X-linked mental retardation syndromes. Putative mutations have recently been described in a few autistic patients and a boy with language disorder and schizophrenia. In this study, DNA samples from individuals with schizophrenia and other psychiatric diseases were scanned in order to explore whether phenotypic spectrum of mutations in the MECP2 gene extends beyond the traditional diagnosis of RTT and X-linked mental retardation syndromes. The coding regions, adjacent splicing junctions and highly conserved segments of the 3'-untranslated region (3'-UTR) were examined in 214 patients including 106 with schizophrenia, 24 with autism and 84 patients with other psychiatric diseases by DOVAM-S. To our knowledge, this is the first analysis of variants in highly conserved regions of the 3' UTR of this gene. A total of 1.5 megabases was scanned (5.2 kb per haploid gene). Higher frequencies of missense and 3'-UTR variants were found in autism. One missense and two 3'-UTR variants were found in 24 patients with autism versus one patient with a missense change in 144 ethnically similar patients without autism (p=0.009). These mutations suggest a possible association between the MECP2 gene and autism, warranting further study.

Disclosure(s): None

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Detection of extremely rare alleles by bidirectional-pyrophosphorolysis activated polymerization allele-specific amplification (Bi-PAP-A): measurement of mutation load in mammalian tissues. *S Sommer*[*1], Q Liu[1]. [1]Department of Molecular Genetics, City of Hope National Medical Center, Duarte, CA, USA.*

Pyrophosphorolysis activated polymerization (PAP) has been developed to detect extremely rare mutations in complex genomes. In theory, PAP can detect a copy of single base mutation present in $3x10^{11}$ copies of the wild type allele. In practice, the selectivity of detection is limited by polymerase extension errors, a bypass reaction, from the unblocked oligonucleotide annealed to the opposing strand. Bi-directional PAP allele-specific amplification (Bi-PAP-A) is a novel method that uses two opposing 3' terminal blocked Pyrophosphorolysis activatable oligonucleotides (P*) with one nucleotide overlap at their 3' termini. This eliminates the problematic bypass reaction. The selectivity of Bi-PAP-A was examined using Λ phage DNA as a model system. Bi-PAP-A selectively detected two copies of a rare mutated allele in the presence of at least $2x10^{9}$ copies of the wild type ë phage DNA. Bi-PAP-A then was applied to direct detection of spontaneous somatic mutations in the mouse genome at a frequency as low as $3x10^{-9}$. A 370-fold variation in the frequency of a specific somatic mutation among different mouse samples was found, suggesting clonal expansion of mutation occurring during early development and a hyper-Poisson variance. Bi-PAP-A is a rapid, general and automatable method for detection of rare mutations.

Disclosure(s): None

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Rescue of amniotic fluid cells for rapid prenatal DNA diagnosis. *MD Speevak*[*1], *J Dolling*[1], *C Murray*[1], *H Bruyere*[2], *SA Farrell*[1]. [1]*Genetics, The Credit Valley Hospital, Mississauga, Ontario, Canada,* [2]*Cytogenetics Laboratory, Vancouver Hospital and Health Sciences Centre, Vancouver, British Columbia, Canada.*

Rapid molecular diagnoses from uncultured amniotic fluid cells are often hampered by insufficient sample volume and poor yield, leading to long waits for results because the cultured back-up sample must be used. In addition, sometimes the amniotic fluid sample must be split with the cytogenetics laboratory, further compounding the problem of insufficient sample volume and leading to a tug-of-war between otherwise co-operative laboratory personnel. We devised a method to retrieve floaters from the initial amniotic fluid cell culture, on the day of first feed. Floaters consist of debris, dead cells and living cells which have lifted from the culture during mitotic division. The floater DNA was analyzed for quality and quantity. The analysis indicates we obtain high quality, high molecular weight fetal DNA and with no evidence of maternal cell contamination. This method is robust and suitable for all types of PCR analyses.

Disclosure(s): None

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CFTR mutation distribution among US Hispanic and African American individuals: evaluation in known affected and carrier screening populations.
EA Sugarman[*1], *EM Rohlfs*[1], *BA Allitto*[1]. [1]*Genzyme Genetics, Molecular Diagnostics Laboratory, Westborough, MA.*

While CF is reported to occur in approximately 1/10,000 Hispanic and 1/15,000 African American births, there have been limited data on the frequency of mutations in carriers and CF patients. We report mutation distribution for Hispanic and African American individuals referred from across the United States for analysis of 86 CFTR mutations from January 2001 - September 2003. Patient ethnicities and indications for testing were provided by referring physicians. The Hispanic population included 161 individuals with a CF diagnosis and 15,335 individuals referred for carrier screening with no family history. In the affected population, 30 different mutations were identified; the most prevalent include: ΔF508, G542X, R334W, 3120+1G>A, W1089X, 3876delA, and R1066C. An additional 7 mutations were identified on 2 or 3 chromosomes while 16 mutations were identified once. In the Hispanic carrier screening population, 33 different mutations were found among the 287 carriers identified. The most prevalent were: ΔF508, D1152H, R117H, G542X, L206W, I148T (3199del6 status unknown), ΔI507, R1066C and R553X. An additional 12 mutations were identified on 2 to 6 chromosomes, and 12 others were identified once. By comparing results from affected and carrier screening individuals tested with the same mutation panel, the variable phenotypes associated with R117H and D1152H are confirmed by their relatively increased frequency among carriers. Individuals who were I148T positive but not tested for 3199del6 were included as carriers; those who were I148T positive and 3199del6 negative were not. Among African Americans, mutation distribution was studied in 108 patients with a CF diagnosis and 8976 individuals referred for carrier screening. In the CF population, 21 different mutations were identified. Of the 9 mutations identified more than once, the most prevalent were: ΔF508, 3120+1G>A, 2307insA, and A559T. In the carrier screening population, 23 different mutations were identified among 94 carriers. Of the 9 mutations identified more than once, the most prevalent were: ΔF508, 3120+1G>A, A559T and R117H. Based on these and previously reported data (Heim et al., 2001), we have identified 38 mutations among Hispanic and 27 among African American individuals with CF. This study demonstrates that a diverse group of mutations is most appropriate for diagnostic and carrier screening in these populations. A standard 25 mutation panel is not sufficient for the ~30% of our CF carrier screening referrals that come from minority or mixed ethnic populations. To best serve the increasingly diverse U.S. population, ethnic specific mutations should be included in mutation panels.

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In vitro functional analysis of a connexin 26 protein bearing an F142L amino acid substitution. *RL Alford*[*1], *H Tang*[1], *CW Brown*[2], *G Richard*[3]. [1]*The Bobby R. Alford Department of Otorhinolaryngology and Communicative Sciences, Baylor College of Medicine, Houston, Texas,* [2]*Departments of Molecular and Human Genetics and Pediatrics, Baylor College of Medicine, Houston, Texas,* [3]*Department of Dermatology and Cutaneous Biology, Thomas Jefferson University, Philadelphia, Pennsylvania.*

In vitro functional analysis of a 424T>C mutation in the gene GJB2, encoding Connexin 26 (Cx26), was performed. This mutation is predicted to cause incorporation of a leucine (L) amino acid residue in place of phenylalanine (F) at position 142 of Cx26. This mutation was initially reported as a novel, apparently de novo, heterozygous amino acid substitution in a child with sensorineural hearing loss, psoriasiform skin lesions, and involvement of mucous membranes and teeth (Brown et al, J Invest Dermatol 2003;121(5):1221-1223). To analyze the functional consequences of the F142L amino acid substitution, wild type and mutant GJB2 coding sequences were cloned into the vector pEGFP-N1 to express Cx26 as a carboxyterminal fusion protein with enhanced green fluorescent protein (EGFP). Wild type and mutant GJB2-EGFP constructs were transfected into gap junction communication incompetent HeLa cells. Tranfected HeLa cells were fixed, counterstained with DAPI and Texas Red-X phalloidin (for F-actin), and visualized by fluorescent microscopy. HeLa cells expressing wild type Cx26-EGFP demonstrated strong EGFP fluorescence in the perinuclear areas as well as at points of cell-cell contact, indicative of formation of gap junction plaques. In contrast, HeLa cells transfected with the GJB2(F142L)-EGFP constructs demonstrated a dramatically reduced level of fluorescence despite apparently adequate transcription of the transfected GJB2 gene as measured by RT-PCR. Further, the Cx26(F142L) protein failed to localize to the plasma membrane and form gap junction plaques. These data suggest that the F142L mutation is a functionally important mutation that alters the sub-cellular localization of Cx26 and thereby affects the ability of the mutant protein to form gap junction plaques.

Disclosure(s): None

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Feasibility of single cell DNA methylation testing for Angelman and Prader-Willi syndromes: potential use for pre-implantation diagnosis.

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Three patients with Angelman syndrome –an imprinted gene disorder, were recently reported in association with intracellular sperm injection (ICSI) technique, used for in vitro fertilization (Orstavik et al., Am J Hum Genet., 2003, 72:218-9). The affected individuals that were conceived using ICSI belong to a rare sub-type of Angelman syndrome that consists of an imprinting defect without an imprinting center mutation. They would be successfully identified using DNA methylation analysis of sequences on the proximal chromosome 15 region that is used for diagnostic evaluations of Angelman syndrome patients. We attempted minaturization of two PCR-based methylation testing protocols in order to determine the feasibility of single cell diagnosis for Angeman syndrome which may be potentially usefull for preimplantation diagnosis while performing ICSI. Such testing would also identify patients with Pader-Willi syndrome, another imprinting gene disorder that to-date was not reported in association with ICSI. We used nested, touch-down PCR protocols to amplify serial dilutions of bisulfite-treated genomic DNA in order to determine limiting template amounts that may be successfully amplified with the two methods studied. For template amounts lower than 800 pg the nested bisulfite restriction analysis method produced more consistent results compared to the nested methylation-specific PCR method. This work was supported in part by the New York State Office of Mental Retardation.

Disclosure(s): None

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Interleukin (IL)-13 haplotype association studies of C-1055T and ARG130GLN polymorphisms in African American asthma and atopy patients.

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IL-13 gene, which resides in Chromosome 5q31 region, has been implicated as a good candidate gene in the etiology of Th-2 dependent respiratory diseases such as Asthma. Earlier, for the first time in literature, we have shown that the mutant –1055T allele in IL-13 promoter polymorphism was a risk factor in the development of asthma and atopy in African Americans. Subsequently, we have done association studies also with the IL-13 coding polymorphism Arg130Gln (exon 4) in the African American asthmatic patients and found that the mutant 130Gln allele was a risk factor in the development of asthma. In the present investigation we have analyzed both the IL-13 promoter and the coding polymorphisms in African American asthmatics. We have genotyped 47 asthmatic patients and 148 control individuals without asthma. IL-13 double marker analysis revealed a combined mutant 1055T allele and 130Gln allele frequency of 39.1% among asthmatic patients as compared to 20.3% in controls. Our haplotype analysis revealed a significant association of IL-13 markers with the asthma phenotype as compared to the controls, Chi square 5.7, pValue <0.015, odds ratio of 2.5, with the 95%CI of 1-5.5. Based on our data we believe that IL-13 haplotype has a significant association with asthmatic phenotype in the African Americans.

Disclosure(s): None

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Association of IL-13 GLN130 genotype with asthma and atopy in African Americans.

K Yanamandra[*1], *D Napper*[1], *S Ursin*[1], *H Chen*[1], *T Thurmon*[1], *J Bocchini Jr*[1], *J Moissidis*[1], *S Bahna*[1]. [1]*Pediatrics Department, LSU Health Sciences Center, Shreveport, LA .*

Earlier findings have suggested a strong genetic influence of the chromosome 5q31 region in the development of Th2-mediated diseases including allergic asthma and Atopy. Several interleukins (IL) such as IL-3, 4, 5, 13 etc, are residents in this region. IL-13 seems to be a good candidate gene in the etiology of Asthma and Atopy. In 1999, van der Pouw Kraan et al identified a single nucleotide polymorphism (SNP) in the promoter region of IL-13 gene, a transition C to T at position –1055 (C-1055T). They found this SNP to be associated with increased risk of allergic asthma in the Dutch Caucasians. In 2000, they also associated C-1055T polymorphism with chronic obstructive pulmonary disease (COPD). Subsequently several reports supported the initial findings of van der Pouw Kraan et.al. in the Caucasians. There is however, very limited data on the association of this polymorphism with asthma or atopy in Africans or African-Americans. Thus, we have evaluated IL-13 C-1055T SNP genotyping in over 40 patients and compared the genotypes from 100 control infants. Our pilot data showed 24% -1055TT homozygotes in patients compared to 12% in controls (p=<0.03, odds ratio 4.8). Mutant alleles also were significantly higher in patients than in controls 54.6% vs 37.9% (p=<0.03, odds ratio 2). For the first time in literature we showed that the –1055T mutant allele as a risk factor in the etiology of asthma and atopy in African-Americans. Because we obtained interesting association results from IL-13 C-1055T polymorphism studies, in the present investigation we extended our association studies to another polymorphism, IL-13 Arg130Gln in African Americans. We have performed IL-13 Arg110Gln genotypes on 79 asthmatic patients and 200 control subjects. We found a significant difference in the mutant Gln130 allele frequency in asthmatics as compared to controls, 27% vs 18% respectively, with a Chi.square >5, pValue <0.03, odds ratio of 1.7, 95% CI 1-2.7. Based on our data we believe that the IL-13 mutant Gln130 genotype is a risk factor in the etiology of asthma and atopy in African-Americans. As this is the first report on this association in African-Americans, studies from other researchers are required for verification. Detailed data will be presented.

Disclosure(s): None

PERINATAL GENETICS POSTERS

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Prenatal diagnosis of de novo X;autosome translocations. *PD Cotter*[1,2,3]*. L Abrams*[1]*. [1]Division of Medical Genetics, Children's Hospital Oakland, Oakland, CA, [2]Department of Pathology, Children's Hospital Oakland, Oakland, CA, [3]Division of Genetics, US Labs Inc, Irvine, CA.*

The identification of a de novo apparently balanced structural chromosome rearrangement at prenatal diagnosis can be problematic and raises unique genetic counseling issues. Two breakpoint rearrangements such as reciprocal translocations or inversions have a 6.7% empirical risk of phenotypic abnormality. Abnormal phenotypes are thought to result from gene disruption, position effect or deletion at one of the breakpoints. Prenatal diagnosis of de novo X;autosome translocations is rare, and presents additional unique risks due to the effects of X inactivation and the possibility of disruption of the single active copy of an X-linked gene. We report the identification of a de novo apparently balanced t(X;6)(q26;q23) ascertained after amniocentesis for advanced maternal age. The parents were counseled regarding the risk of a de novo apparently balanced translocation, including the potential risk of an X-linked Mendelian disorder resulting from disruption of a gene at the Xq26 breakpoint. While the normal X was late replicating in all metaphase no conclusions from this data could be drawn as the X-inactivation ratio in amniocytes might not be representative of other tissues. The possibility of future premature ovarian failure was also noted due to the position of the breakpoint at Xq26, although no specific risk could be ascribed. The parents elected to continue the pregnancy, and at 17 months of age the proband was phenotypically and developmentally normal. Long term follow up will be required to assess development delay and any fertility issues. Based on review of the few cases reported to date and excluding any risk for later reproductive abnormalities, we estimated the risk of phenotypic abnormality or developmental delay in a prenatally ascertained de novo X;autosome carrier to be as high as 50%. This case illustrates the complexities in counseling for prenatally ascertained de novo X;autosome translocations and the need for additional cases to be reported.

Disclosure(s): None

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Perinatologist experience with support people during invasive prenatal diagnostic procedures. *EH Blaise*[1]*, AE Donnenfeld*[1,2]*. [1]Genzyme Genetics Philadelphia, PA, [2]Drexel University College of Medicine Philadelphia, PA.*

Objective: To examine perinatologists' policies and experiences regarding the value and safety of support people at amniocentesis and CVS procedures. Materials and Methods: A 10 question survey was administered to 16 MFM specialists in the Philadelphia area regarding their experiences involving support people at amniocentesis and CVS procedures. Data on numbers of procedures these perinatologists have performed, their policies regarding support people, their perceptions regarding the value of support people, and untoward experiences they have encountered were obtained. Results: Among the 16 perinatologists, an estimated 99,700 invasive prenatal diagnostic procedures had been performed. Syncopal episodes were reported among 41 support people 0.04%). Of these 41 individuals, 18 were standing while observing the procedure. No serious injuries were reported for those who lost consciousness. There were 22 instances of support people being disruptive during a procedure (0.02%). Types of disruption included abusive behavior toward the patient, argumentative behavior and incessant questioning before and during a procedure. All MFM physicians permitted support people in the room. Eight MFM specialists did not limit the number of support people. Two MFM's permitted only one support person, three allowed two support people, one allowed three support people and two did not specify their limit. Twelve of 16 MFM physicians permitted children in the room (dependent on their age, behavior, and whether there was a supervising adult). Four indicated they would never allow children in the room during an invasive procedure. Eleven of 16 MFM specialists insisted that all support people remain seated during the procedure. The remaining five preferred the support person to sit, but did not insist on it. Fourteen of 16 perinatologists (88%) believed support people are a calming influence on the patient prior to and during a procedure. One indicated that support people have no effect on a patient's anxiety and one indicated that support people are not helpful to a patient at amniocentesis or CVS. The majority of respondents indicated that a support person is often a parent of the fetus and therefore has a right to be involved with the prenatal diagnostic process. Conclusions: The presence of a support person at an amniocentesis or CVS procedure is overwhelmingly viewed by MFM physicians as beneficial to the patient. Support people are rarely disruptive or problematic. Syncopal episodes among support people are unusual but as a precaution, all support people should remain seated throughout the procedure.

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Relationship between first trimester free-β HCG and newborn weight. *J DeLeon*[1], J Santolaya-Forgas[1]. [1]Division Reproductive Genetics. Texas Tech University. Amarillo.TX.*

Our objective was to determine if first trimester free-β hCG correlated with newborn weight. MATERIAL AND METHODS: We evaluated 200 patients who had consented to donate blood for biochemical research during pregnancy. All gestations were dated sonographically. We excluded 56 patients because they had preterm labor (N:19), IUGR (N:10), hypertension (N:5), diabetes (N:8) or fetal loss (N:14). 144 patients reached term and delivered appropriate for gestational age neonates. 123 of these women had donated blood between 10+1 and 14+6 weeks gestation. Their serum was thawed and maternal serum free-β hCG determined by microparticle enzyme immunoassay in 97 of the samples. Results were expressed as MoMs for gestational age. Pearson's correlation coefficient was used to determine possible trends between maternal serum free-βhCG and newborn weight. RESULTS: A significant inverse correlation was noted between first trimester freeβ hCG and newborn weight (MoM free- βhCG = 3.53 – 0.0006 newborn weight, N: 97; p ‹ 0.03). CONCLUSION: These results obtained from normal pregnancies delivering at term suggest that first trimester free-β hCG inversely correlates with newborn weight. Further studies are required to confirm this finding and to determine if petite mothers having petite but appropriate for gestational age newborns are more likely to be screened positive using first trimester maternal serum markers.

Disclosure(s): None

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A sensitive molecular method (Invader® assay) for detection of genomic copy number changes: novel application for the detection of chromosomal aneuploidy. *RK Iyer*[1], KS Park[1], JL Pelkey[1], PN Rao[1]. [1]Department of Pathology and Lab Medicine, David Geffen UCLA School of Medicine, Los Angeles, CA.*

Rapid detection of aneuploidy (chromosomes 13, 18, 21, X and Y) by FISH in prenatal samples is the standard of care in medical practice. The Invader® method (Third Wave Technologies, Madison WI) method relies on a combination of sequence-specific hybridization and enzymatic recognition to generate a target-dependent signal, without target amplification. This signal is generated in a linear fashion allowing for direct quantification of a DNA sequence. Thus the Invader® platform can represent an alternative technology for the detection of nucleic acid copy number changes. In addition to its established utility for detecting mutations (i.e. SNPs, insertions, and deletions) in the target sequence, this methodology is highly quantitative. We conducted a retrospective "proof-of-principle" study to test the suitability of the Invader® method for determining chromosome copy number. Invader® assay oligonucleotides were designed using a proprietary algorithm, to specific sequences on chromosomes X (bands p11.21,q28), Y (band p11.2), 13 (band q14.2), 18 (p11.2) and 21 (Down Syndrome critical region;q22) respectively. 46 blinded, anonymized DNA samples from residual cultured amniocytes or fixed cell pellets were tested. Our results showed that this method rapidly and reproducibly differentiates between samples containing 1, 2 or 3 copies of the tested chromosome. There was a 100% concordance with the karyotype. The assay proved to be robust, requiring small amounts of genomic DNA (<25 ng), simple to set-up and perform, and required <6 hours from set-up to final results. In addition, this versatile technology could also be applicable for the detection of segmental aneusomies (deletions and duplications), as well for uniplex and multiplex detection of mutations (single base and small deletions). The Invader® platform, could therefore provide a common methodology for the development of novel assays that can be established in both cytogenetic and molecular diagnostic laboratory settings.

Disclosure(s): Third Wave Technologies, Madison WI provided probes for evaluation for this presentation.

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WITHDRAWN

PERINATAL GENETICS POSTERS

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Using First PAGE to screen for genetic risk in primary prenatal care: updating and expanding a successful strategy. *E Kloza*[*1], *S Ellingwood*[1]. [1]*Foundation for Blood Research, Scarborough, ME.*

Ongoing debate regarding the appropriateness of inclusion of primary care physicians (PCPs) as providers of genetic services suggests that PCPs will be unwilling or unable to act in that capacity. In 1996 we created and distributed to all Maine prenatal PCPs, a pre-pregnancy and prenatal risk assessment, management, and education tool known as ProgramME. This office-based approach consisted of a 15-element Genetic History Questionnaire and a companion Office Guide. Evaluation of ProgramME showed that 85% of respondents used the questionnaire on all of their prenatal patients. There was no significant increase in genetics-related telephone calls or genetics referrals. Respondents rated ProgramME 8.7 out of 10 points for satisfaction and usefulness. Concerned that the content of this tool had become outdated and that interest may have waned, we applied for and were awarded in 2002 a Mission Opportunity Investment Program (MIOP) grant from the March of Dimes to update, improve, and distribute a current version. A survey of the PCPs who had the original ProgramME indicated that 75% of respondents continued to use ProgramME. Survey results and comments of the respondents were used to update the Genetic History Questionnaire and the Office Guide so that it would be more user-friendly. Renamed First PAGE, the resource was also updated to include current clinical genetic information and support organization information. Following distribution of First PAGE to all Maine and New Hampshire prenatal PCPs, we conducted formative and summative evaluations. Sixteen percent of surveys were returned, respondents representing 219 PCPs. About 79% of Maine respondents had used ProgramME previously. A majority of respondents (85%) said that they plan to use the First PAGE questionnaire with all or most new prenatal patients. Nearly half preferred the First PAGE questionnaire to ACOG, Hollister®, or other questionnaires. Most respondents did not perceive that First PAGE would have an effect on the number of referrals made to genetics. Nearly 5 times as many respondents thought that First PAGE would decrease the number of telephone calls for genetic information compared to the number who thought their calls might increase. Almost 75% of respondents thought that First PAGE made them more confident when addressing genetic risk with patients and that it simplified genetic risk assessment. Ninety percent reported that it assisted them in addressing genetic risk early in pregnancy. We conclude that First PAGE meets the needs of PCPs for genetic education and risk assessment/management.

Disclosure(s): None

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Patient awareness of population-specific genetic screening availability in the prenatal/preconception setting. *JS Moldenhauer*[*1], *EM Weber*[2], *MS Verp*[2]. [1]*Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI,* [2]*Department of Obstetrics and Gynecology, University of Chicago, Chicago, IL.*

Objective: To assess patient awareness of population-specific genetic screening availability in the prenatal and preconception genetic counseling setting. Study Design: Patients presenting for prenatal or preconception genetic counseling over a two month period were asked whether they were offered and accepted genetic screening by their referring physicians, as well as their general awareness of genetic screening based on their ethnic background. Ethnic background was self-designated by patients. In situations where the patient was unaware of screening, documentation of screening test results and documentation of acceptance or decline of screening tests was extracted from patient records made available by referring physicians for the purpose of consultation. The population specific screening that was evaluated was sickle cell trait by hemoglobin electrophoresis in the African-American population, cystic fibrosis by mutation analysis in the Caucasian population, α- and β-thalassemia by mean corpuscular volume in the Asian, Hispanic and Mediterranean populations, and cystic fibrosis, Tay-Sachs and Canavan screening in the Ashkenazi Jewish population. Results: A total of 102 patients presented for genetic counseling in this time period, including 54 African-Americans, 26 Caucasians, 15 Hispanics, 4 Asians, 2 Asian Indians, and 1 Ashkenazi Jew. Questions were posed to 99/102 patients. The backgrounds of the patients not questioned were one Caucasian and two Asian Indian patients. Within the African-American population (54), 49/54 (90.7%) were offered screening, 25/49 (51%) of those who were screened were unaware that they were tested and 27/54 (50%) patients were aware of sickle cell screening. Of the 25 Caucasian patients, 9/25 (36%) were offered screening; 2/9 (22%) declined screening and 7/9 (78%)accepted screening. The overall awareness of screening availability was 9/25 (36%) in the Caucasian population. Of the Hispanic patients seen (15), 11/15 (73%) were screened, 10/11 (91%) of those were unaware that they had been screened and the overall awareness of thalassemia screening was 0/15. The 4 Asian patients seen were all screened for thalassemia, 2/4 (50%) were aware that they had been screened and the overall awareness of thalassemia in this population was 2/4 (50%). The one Ashkenazi Jewish patient was aware of and had accepted screening. Across all populations, the overall awareness of genetic screening availability was 39/99 (39.4%). Conclusion: Patient awareness of population-specific genetic screening is poor. Overall patient, and physician, education regarding the appropriateness of genetic screening in certain populations needs to be improved.

Disclosure(s): None

PERINATAL GENETICS POSTERS

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First trimester ultrasound detection of diastrophic dysplasia associated with increased nuchal translucency. *N Quercia*[*1], G Ryan[2], J Kingdom[2], L Bonafe[3], A Superti-Furga[3], S Unger[1]. [1]Division of Clinical and Metabolic Genetics, Hospital for Sick Children, Toronto, Canada, [2]Department of Obstetrics and Gynecology, Mount Sinai Hospital, Toronto, Canada, [3]Division of Molecular Pediatrics, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland.*

Diastrophic dysplasia (DD) is an autosomal recessive skeletal dysplasia resulting in severe short-limbed dwarfism but it is generally non-lethal. Other findings include joint limitation, clubfeet, proximally set and radially deviated thumbs ("hitch-hiker thumb"), cystic masses of the external ear, cleft palate, and progressive kyphoscoliosis. Mutations in the diastrophic dysplasia sulfate transporter (DTDST) gene are responsible for DD. The DTDST gene encodes a transmembrane protein that transports sulfate into chondrocytes. Reduced sulfate transport into chondrocytes results in the under-sulfation of proteoglycans, which in turn leads to abnormal cartilage formation. Early prenatal diagnosis of DD is possible through chorionic villus sampling or amniocentesis, for those families in whom the DTDST mutations have been previously identified. Non-invasive prenatal diagnosis may be possible but DD is usually not detected until the second trimester ultrasound around 18 weeks gestation. We report the prenatal diagnosis of DD at 12 weeks gestation using real-time ultrasonography. In the couple's first pregnancy, a skeletal dysplasia was suspected at 18 weeks gestation after the finding of short limbs on ultrasound. A follow-up ultrasound at 22 weeks gestation revealed micromelia with bowing of the tibia and ulna, bilateral severe talipes, bilateral hyperabducted thumbs, marked cervical kyphosis, increased sacral lordosis, and cleft palate. Following pregnancy termination, mutation analysis of the DTDST gene revealed that the fetus was compound heterozygous for the DTDST mutations R279W and R178X (known pathogenic mutations). During this couple's second pregnancy, an ultrasound was done at 12 weeks gestation at which time there was evidence of increased nuchal translucency (6mm) and a suspicion of short limbs and micrognathia was raised. Several days later, combined transabdominal and transvaginal ultrasound scans confirmed a multi-septated nuchal translucency and abnormalities of the fetal limbs including bilateral hyperextended thumbs, crossed lower limbs with hypomobility, marked talipes of the right foot and a "ballerina" pirouette position of the left foot. Chorionic villus sampling was performed and molecular mutation analysis confirmed a recurrence of DD with the fetus being compound heterozygous for the same DTDST mutations identified in the first pregnancy. This represents the first time that increased nuchal translucency has been reported as a finding in DD and also confirms that first trimester ultrasound can detect DD.

Disclosure(s): None

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Prenatal diagnosis of paternal uniparental disomy 14. *P Rush*[*1], M Quigg[1], J Refuerzo[1,2], J Roberson[1], S Das[3], D Van Dyke[1]. [1]Henry Ford Hospital, Detroit, MI, [2]Wayne State University, Detroit, MI, [3]University of Chicago, Chicago, IL.*

There is a well-documented phenotype for infants with paternal uniparental disomy of chromosome 14 [UPD14pat]. This phenotype includes a small, bell-shaped chest, joint contractures, small ears, protruding philtrum, short palpebral fissures, and respiratory compromise leading to early demise. Retrospectively, intrauterine growth retardation and polyhydramnios have been noted during the pregnancies with these infants. To our knowledge, the case presented here is the first reported prenatal diagnosis of UPD14pat. The 28 year old G3P1Ab1 patient presented at 24 weeks gestation with mild polyhydramnios, small fetal chest, and soft tissue swelling around the chest and at the back of the neck. Although the fetal chest was small on ultrasound, there were no other signs of a skeletal dysplasia. Amniocentesis was performed because of the ultrasound findings. The amniotic fluid cell karyotype was 45,XY,der(13;14)(q10;q10). Parental bloods were drawn for chromosome analysis and the father of the baby was found to have the same translocation. Because the ultrasound findings were suggestive of paternal uniparental disomy of chromosome 14, DNA microsatellite analysis was performed confirming the presence of UPD14pat. As the pregnancy has progressed, joint contractures of the fetal hands developed, in addition to progressively increasing polyhydramnios and soft tissue swelling. A small, bell-shaped chest with abnormal heart to chest circumference was also present and highly concerning for neonatal respiratory insufficiency. Delivery of this fetus is pending. The prenatal diagnosis of UPD14pat and its associated poor outcome has allowed the parents to discuss with the neonatologists their wishes for the care of their child after delivery. This case illustrates the need to pursue chromosome analysis whenever fetal abnormalities are noted on ultrasound because the information gained from these studies may greatly impact maternal and fetal care.

Disclosure(s): None

PERINATAL GENETICS POSTERS

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First and second trimester biochemical prenatal screening for Down syndrome by newborn gender. *J Santolaya-Forgas*[*1], *J DeLeon*[1]. [1]*Division Reproductive Genetics. Texas Tech University. Amarillo.TX.*

Our objective was to determine if male and female fetuses had different first trimester (free-βhCG and PAPP-A) or second trimester (AFP, hCG and uE3) maternal serum markers. MATERIAL AND METHODS: We evaluated 200 pregnant women who had consented to donate blood for biochemical research early in pregnancy. All gestations were dated sonographically. We excluded 65 patient because they developed preterm labor, intrauterine growth restriction, hypertension, diabetes, had a fetal loss or the newborn gender was unknown. The remaining 135 patients had term deliveries with appropriate for gestational age (GA) neonates. Of these, 123 (Group 1) had donated blood between 10+1 and 14+6 weeks gestation to determine free-βhCG (microparticle enzyme immunoassay) and PAPP-A (ELISA)and, 129 (Group 2) donated blood after the 15th week of pregnancy to determine AFP (microparticle enzyme immuno assay), hCG (microparticle enzyme immuno assay) and uE3 (ELISA). All results were expressed as MoMs for gestational age (GA). Student t-test with Bonferroni correction was used for comparison of studied variables. RESULTS: MatAge(years), Group 1 GA at sampling (Weeks), Group 2 GA at sampling (Weeks), GA-delivery(Weeks), Neonatal Weight(gm), 5'APGAR for Males (N:72)were:25;11.9;17;38.9; 3438 and 9. The respective values for females(N:63)were: 23.5; 12; 16.5; 39.3; 3252; 9.(p-value n.s.for all variables) Mean maternal serum MOM values for hCG(N120); uE3(N120); AFP(N123); FreeβhCG(N103)and PAPP-A(N64) for male newborns were: 1.26; 1.19; 1.23; 1.10; 1.12 and for female newborns were: 1.37; 0.91; 0.99; 1.15; 1.22 (uE3 $p<0.03$ and AFP $p<0.001$). CONCLUSION: These results, obtained from normal pregnancies delivering at term appropriate for gestational age newborns, suggest that fetal gender may have an effect on maternal serum screening for chromosomal abnormalities. Further larger studies are recommended.

Disclosure(s): None

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Prenatal diagnosis of an FGFR2 mutation causing a less severe presentation of Pfeiffer syndrome based on sonographic evidence of craniosynostosis. *J Sawyer*[*1], *T Feng*[2], *A Patterson-Barnett*[2], *K Kupke*[3,1]. [1]*Genzyme Genetics, Atlanta, GA,* [2]*Maternal-Fetal Specialists at Gwinnett, Lawrenceville, GA,* [3]*Neonatology Associates, Atlanta, GA.*

We report on the prenatal diagnosis of craniosynostosis of the Pfeiffer type with a confirmed mutation in the FGFR2 gene. The patient, a 38year old G1P0 Hispanic female, was referred for genetic counseling and targeted prenatal ultrasound examination at 24 weeks' gestation due to suspicion of craniosynostosis. Targeted ultrasound supported this diagnosis by identifying a significant cloverleaf malformation of the fetal head and significant proptosis. Amniocentesis testing revealed normal female chromosomes and normal AFAFP levels. Further prenatal testing of the amniotic fluid for Crouzon, Apert, Pfeiffer and Jackson-Weiss syndromes (associated with FGFR2 mutations), Non-syndromic Craniosynostosis Syndrome (FGFR3 mutations) and Seathre-Chotzen syndrome (TWIST gene mutations) was performed in attempt to further characterize the condition and to provide prognostic information for the parents. A mutation in exon 7 of the FGFR2 gene was identified. This G-->C mutation results in a Cysteine residue being substituted for Tryptophan at codon 290 (Trp290Cys). This amino acid change has been reported in other patients diagnosed with severe Pfeiffer syndrome, Type 2. In previously reported cases of prenatally diagnosed Pfeiffer syndrome with the same amino acid mutation, neonatal survival was limited to a matter of days. Other mutations have also been reported involving the same codon in the FGFR2 gene. These mutations cause different amino acid substitutions and have been associated with less severe outcomes, such as Crouzon syndrome. In the case presented, the child was delivered at 39 weeks gestation by Cesarean section and, with treatment, has survived past six months of age which is significantly longer than previously reported cases. In addition to the craniosynostosis and proptosis identified prenatally, midfacial hypoplasia, low set ears, ankylosis of the ankles, adducted broad thumbs and broad great toes were noted at delivery. These features are similar to those noted in previous case reports involving the same amino acid substitution causing severe Pfeiffer syndrome, Type 2. The infant underwent successful surgical correction for the craniosynostosis. She subsequently, however, developed hydrocephalous and feeding difficulties and shunt placement and gastrostomy feeding were necessary. By the age of 6 months, she was noted to have growth retardation and developmental delays. The current prognosis is poor due to the development of an upper airway obstruction. This case demonstrates the variability in outcome for infants prenatally diagnosed with craniosynostosis, even in cases with identifed DNA and amino acid mutations that have been previously characcterized. Despite these prognostic limitations which must be recognized when counseling parents regarding prenatal DNA diagnosis of craniosynostosis the DNA mutation information provided valuable information for the couple regarding the prognosis of this prenatally diagnosed condition.

Disclosure(s): Presenting author is an employee and stockholder of Genzyme Genetics.

PERINATAL GENETICS POSTERS

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The effect of maternal serum screening programs on trisomy 21 prenatal and postnatal diagnosis in southeast Michigan. *D Van Dyke*[1], S Ebrahim[2], A Al Saadi[3], S Powell[4], J Zenger-Hain[5]. [1]Henry Ford Health System, Detroit, MI, [2]Wayne State University, Detroit, MI, [3]William Beaumont Hospital, Royal Oak, MI, [4]St. John Hospital, Detroit, MI, [5]Oakwood Hospital, Dearborn, MI.*

Since the 1980s there have been dramatic improvements in the efficiency of maternal serum screening to identify pregnancies at risk for trisomy 21. The proportion of pregnancies screened has also increased. These events should have increased the number of prenatally diagnosed trisomy 21 cases, with a concomitant decrease in postnatal diagnoses. To evaluate the effect of maternal serum screening programs and prenatal diagnosis on trisomy 21 in the Detroit metropolitan area, we examined the change over time in percentage of trisomy 21 cases diagnosed prenatally. We compiled the number of prenatal and postnatal trisomy 21 diagnoses (696 and 811 total +21 cases, respectively) for our laboratories for each year from 1988 to 2003. Our laboratories process the vast majority of amniotic fluid and blood karyotype studies that are ordered in southeast Michigan, so while ascertainment is not complete, bias from samples sent to commercial laboratories should be minimal. In this preliminary assessment, we excluded most blood karyotypes done to confirm a prenatal diagnosis. To focus on the impact of maternal serum screening programs, we also excluded CVS cases. From 1988-1993, 33% of the trisomy 21 cases were identified prenatally. In contrast, from 1994-2003, 49% of the trisomy 21 cases were identified prenatally. The change in frequency from 1993 to 1994 was abrupt, and no other trend was apparent. This change is co-incident with the time when multiple marker serum screening programs were implemented and gained wide clinical use in our region. Our observations are comparable to those in Maine when multiple serum marker screening replaced AFP serum screening - their percentage of trisomy 21s identified prenatally increased from 49% in 1986-1990 to 57% in 1991-1993 (Palomaki et al., NEJM 1996;334:1409-10). It seems likely that the increase in trisomy 21 prenatal diagnoses relates in large part to the success of maternal serum screening programs. The percentage of trisomy 21 prenatal diagnoses should continue to rise with additional 1st and 2nd trimester serum screening markers, and the increased use of ultrasound examinations for fetal markers of aneuploidy. However, many cases of trisomy 21 will never be diagnosed prenatally because of maternal decisions to forego screening or amniocentesis, and other factors such as late presentation for prenatal care.

Disclosure(s): None

ABSTRACTS OF INVITED TALKS

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Fanconi anemia: lessons from a long-term "knowledgebase" of genotype correlations with clinical outcome. *A Auerbach*[*1], O Levran[1], D Kutler[1,2], J Satagopan[2], H Hanenberg[3]. [1]The Rockefeller University, New York, NY, [2]Memorial Sloan-Kettering Cancer Center, New York, NY, [3]Heinrich Heine University, Düsseldorf, Germany.*

Fanconi anemia (FA) is an autosomal recessive disorder characterized by cellular hypersensitivity to DNA cross-linking agents, birth defects and cancer predisposition. Recent studies demonstrate interactions among ATM, BRCA1, NBS1, BLM and FA proteins, suggesting a function for the FA proteins in DNA repair/recombination and cell cycle control. The International Fanconi Anemia Registry (IFAR) was established by our laboratory at The Rockefeller University in 1982 to collect clinical and genetic information from a large number of FA patients. Registration into the IFAR is usually at the time of diagnosis, and information is collected initially on hematologic and congenital abnormalities. Comprehensive attempts to obtain follow-up data are made on a regular basis. Our laboratory also serves as a reference center for FA diagnosis, performing diepoxybutane (DEB)-testing on over 400 specimens per year from patients with phenotypic features suggestive of FA. Cells from FA patients are screened with retroviral vectors containing cDNAs for various FA genes to identify the FA complementation group, and genomic DNA is studied for mutations in the appropriate FA gene. Of the 906 FA patients currently in the IFAR, 482 patients have been successfully classified as follows: FA-A (273), FA-C (93), FA-D1/BRCA2 (14), FA-D2 (7), FA-E (5), FA-F (10), FA-G (54) and nonFA-A/C/D1/D2/E/F/G (26). The median survival time for all IFAR patients was 24 years. FA-C was associated with a significantly poorer survival and earlier onset of acute leukemia than FA-A or FA-G. FA-C patients with at least one intron 4 (common in Ashkenazi Jews) or at least one exon 14 mutation had a more severe phenotype compared to patients with at least exon 1 mutation and no mutations in exon 14 or intron 4. The congenital malformations in the BRCA2 patients were similar to those seen in Fanconi anemia patients, ranging from only intrauterine growth retardation, failure-to thrive, microcephaly and café au lait spots, to the full spectrum of FA-associated birth defects, including hypoplastic thumbs, bilateral hip dysplasia, micropenis, ear abnormalities, imperforate anus and other gastrointestinal abnormalities. Of the 14 patients with biallelic BRCA2 mutations in the IFAR database, 5 died of early-onset brain tumors, primarily medulloblastomas and 3 developed Wilms tumors, including a 7.5 month old sibling of a patient who died of AML at 19 months. Brain tumors and Wilms tumors were not found in any other IFAR patients with known complementation group. Notably, we identified six children in 5 BRCA2 kindreds exhibiting early onset acute leukemia. Leukemia occurred at a median of 2.2 years of age in the BRCA2 patients in contrast to a median onset of 13.4 years in all other FA patients in the IFAR ($p<0.0001$). An increased incidence of early onset breast cancer was noted in several of the BRCA2 kindreds, but not in IFAR kindreds of other complementation groups. We conclude that the spectrum of malignancies in BRCA2 patients is unique, suggesting that the pathogenesis of cancer in these patients may be via a different pathway from other FA patients.

Disclosure(s): None

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Effects of embryo culture on imprinted gene expression and methylation in mice. *M Bartolomei*[*1], M Mann[1], R Verona[1], S Lee[1], A Doherty[1,2], L Nolen[1], R Schultz[2]. [1]Howard Hughes Medical Institute and University of Pennsylvania School of Medicine, Philadelphia, PA, [2]Department of Biology, University of Pennsylvania, Philadelphia, PA.*

Preimplantation development is a period of dynamic epigenetic change that begins with remodeling of the egg and sperm genomes, and ends with implantation. During this time, parental-specific imprinting marks must be established and/or maintained. These marks direct the parental-allele-specific expression patterns of a small number of genes in mammals, including the maternally-expressed H19 gene and the paternally-expressed Snrpn gene. We and others have previously demonstrated that H19 imprinting can be perturbed during preimplantation development. To define the lability of genomic imprints during this dynamic period and to determine whether loss of imprinting continues at later stages of development, imprinted gene expression and methylation were examined after in vitro preimplantation culture. Following culture of 2-cell embryos to the blastocyst stage in Whitten's medium, the normally silent paternal H19 allele was aberrantly expressed. However, only a subset of individual, cultured embryos exhibited biallelic expression while others maintained imprinted H19 expression. Loss of imprinting persisted in midgestation conceptuses. Placental tissue displayed loss of imprinting for multiple imprinted genes while imprinting was generally preserved in the corresponding embryos. Loss of imprinted expression was associated with loss of methylation at the H19 and Snrpn imprinting controls regions. These results indicate that genomic imprints are labile in tissues of trophectoderm origin and can be disturbed during preimplantation development. These results also provide a strong cautionary note for the culture of human embryos and cells since the effect of suboptimal culture on epigenetic information in humans is not known.

Disclosure(s): None

ABSTRACTS OF INVITED TALKS

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An overview of epigenetics. *AL Beaudet*. Baylor College of Medicine.*

Epigenetics can be defined as the study of changes in gene function that are stable and heritable (or potentially heritable, as in terminally differentiated neurons) and do not entail a change in DNA sequence. All genes are subject to epigenetic regulation, and these are the mechanisms that make a neuron in vivo or in cell culture stably different from a hepatocyte in vivo or in cell culture. Genomic imprinting is a small component of epigenetics in which the activity of the gene is reversibly modified depending on the sex of the parent that transmits it. Genomic imprinting affects only a small number of genes as far as is known at present. The molecular biology and biochemistry of epigenetic regulation involve the study of chromatin. Important aspects include DNA methylation, covalent modification of histones, and many non-histone chromatin proteins that are stably associated with DNA. The "histone code" describes the important epigenetic information that is stably encoded in chromatin. From a disease perspective, a genetic etiology involves an aberration in nucleotide sequence causing a disease phenotype, while an epigenetic etiology involves an aberration in epigenotype (stable/heritable change in gene expression), causing a disease phenotype in the absence of a nucleotide aberration. Both genetic disease and epigenetic disease are the result of altered gene expression. Phenotypes due to uniparental disomy provide clear examples of epigenetic diseases. Disorders such as Angelman syndrome have a mixed epigenetic/genetic and mixed de novo/inherited etiology giving rise to a single phenotype. There are Mendelian disorders of the epigenetic machinery (e.g., Rett and ICF syndromes) and others that have secondary epigenetic abnormalities (e.g., fragile X syndrome). It is possible that epigenetic abnormalities are an important factor in some complex disease traits.

Disclosure(s): None

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A laboratory diagnostic approach to hypotonia. *BA Bejjani*[1,2]. [1]Sacred Heart Medical Center, Spokane, WA, [2]Washington State University Spokane, Spokane, WA.*

Advances in understanding the molecular pathogenesis of the causes of hypotonia led to the development of improved and powerful genetic diagnostic methods. These advances helped define a new and still evolving genetic classification of neuromuscular and metabolic disorders. The introduction of these novel and informative genetic tests has also impacted the laboratory diagnostic approach to the evaluation of hypotonic infants and children. This talk will concentrate on the laboratory investigation of hypotonia and will emphasize appropriate, practical and cost effective investigations to establish a specific diagnosis. A systematic approach based on the tests currently available will be suggested.

Disclosure(s): Presenter is Medical Director and co-founder of Signature Genomic Laboratories.

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Tetrahydrobiopterin (BH4) therapy for phenylketonuria. *N Blau*. University Children's Hospital, Zurich, Switzerland.*

Exactly 50 years after Bickel and coworkers initiated dietary treatment by phenylalanine restriction clinicians and researchers worldwide are still searching for an alternative approach to the treatment of phenylketonuria (PKU). Today we know that maintaining the restricted diet is beneficial if not essential to prevent brain damage, but there are still disagreements as to how long this diet should be continued. A number of nutritional products with improved quality are available in most countries, but many adolescents and young adults generally do not comply with the recommendations for monitoring and control of phenylalanine concentrations, and two thirds of pregnant women in the United States did not follow the diet before becoming pregnant. While still facing technical difficulties to replace the defective gene and/or enzyme, one new approach to treat at least some PKU patients seems to be a close reality. A relative high percentage of patients with mild PKU may benefit from substitution with tetrahydrobiopterin (BH4) in that oral administration of the natural cofactor for phenylalanine hydroxylase (PAH) reduce their plasma phenylalanine levels. BH4 can obviously activate the specific mutated PAH by either increasing the affinity for BH4, by three-dimensional structure stabilization, or by its chaperon-like activity. It has been shown that a number of DNA mutations correlate with BH4 responsiveness and a number patients with mild PKU or hyperphenylalaninemia are presently on BH4 treatment without the low-phenylalanine diet. The main disadvantage of this approach is the relative high costs of BH4 and so far the lack of regulations in some Societies. A BIOPKU database (www.bh4.org/biopku.html) provide an extensive information on BH4-responsive HPA/PKU.

Disclosure(s): None

ABSTRACTS OF INVITED TALKS

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Utilization of cytogenetics in Children's Oncology Group (COG) acute lymphoblastic leukemia trials.
A Carroll. Univ of Alabama at Birmingham.*

Founded in 2000, the COG is an NCI-supported clinical trials cooperative group with 238 member institutions in the U.S., Canada, Europe, and Australia. In North America, approximately 95% of children with cancer are treated at a COG member institution. The karyotype of the leukemic cells in children with ALL, especially those with B-precursor ALL, is widely recognized to be of diagnostic and prognostic importance. The COG has instituted a "multidisciplinary" diagnosis and classification schema for childhood ALL taking into account blast cell morphology, cytochemical features, immunophenotype, cytogenetic findings, and molecular genetic characteristics. For children with B-precursor ALL who are older than 1 year, induction therapy depends upon the NCI (consensus) risk group that best fits the disease; each group is based on age and white blood cell (WBC) count. Children who are 1.00 to 9.99 years old and have a WBC count <50,000/µl receive standard-risk induction therapy, whereas all others receive high-risk induction therapy. The karyotype is a major part in assignment to risk groups for consolidation and continuation therapy. Patients with favorable-risk features such as trisomies of chromosomes 4, 10, and 17 or t(12;21)(TEL-AML1) are promoted from standard-risk to low-risk group or, if in the high-risk group, non-randomly assigned to the least intensive treatment arm. Patients with an unfavorable karyotype feature such as an MLL (11q23) rearrangement are assigned to the most intensive arm within their induction risk group and those patients with a very unfavorable karyotypic feature, e.g., hypodiploidy (≤44 chromosomes) or t(9;22)(BCR-ABL) are eligible to receive continuation therapy on the very high-risk protocol, regardless of the risk group to which they were assigned during induction. Given the frequencies of these karyotypic subgroups, 40 to 45% of children with B-precursor ALL will probably have their continuation therapy altered because of a karyotypic feature. This makes the detection and accurate characterization of karyotypically abnormal clones extremely important. The literature suggests that as many as 80 to 90% of children with ALL have a cytogenetic abnormality detectable by G-banding. Two of the COG's legacy groups, the Children's Cancer Group (CCG) and the Pediatric Oncology Group (POG), have gathered cytogenetics data for many years, but neither group were able to post abnormality detection rates >65%; the rate on currently open studies is 30% to 45%. Within CCG and POG, the abnormality detection rate for individual institutions ranged from <10% to >80%. Possible explanations of this wide variation and solutions for this problem will be discussed.

Disclosure(s): None

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Dissecting syndromic causes of hypotonia in infancy. *S Cassidy*. University of California, Irvine, Orange, California.*

The physician asked to determine the cause of hypotonia in an infant is faced with a myriad of possible disorders to consider. Once physical anomalies or malformations have been identified, it is appropriate to evaluate for a possible syndromic cause. There are dozens if not hundreds of possible syndromes associated with infantile hypotonia, and many with patterns of anomalies that are not yet recognizable. This presentation will provide an approach to the more common syndromic causes. As with any patient in whom a genetic disorder is being considered, careful prenatal, perinatal and family histories are essential as a first step. Physical exam should include not only a careful evaluation for dysmorphic features, but also a thorough neurologic evaluation. All patients with hypotonia in whom a diagnosis is not evident deserve an MRI scan and a chromosome analysis, since these two studies identify a significant proportion of possible diagnoses (e.g., holoprosencephaly syndromes, lissencephaly syndromes, Down syndrome, other chromosome disorders). Those disorders associated with a pattern of malformations are sometimes identifiable by specific tests and/or searches for internal malformations (e.g., Smith-Lemli-Opitz syndrome, FG syndrome, dwarfing conditions such as achondroplasia, Walker-Warburg syndrome). Specifically, ophthalmologic evaluation can be very useful (e.g., Kabuki syndrome, Marinesco-Sjogren syndrome). The presence of joint contractures can also be helpful (e.g., akinesia syndromes, Zellweger syndrome). Among the more common syndromes associated with infantile hypotonia are the overgrowth disorders, particularly Sotos, Weaver and Simpson-Golabi-Behmel syndromes. Many other disorders include growth deficiency or microcephaly. Therefore, assessment of growth parameters is essential. The most difficult diagnostic category is that of disorders in which the physical findings are subtle, non-specific, or develop over time. Several of these are the most common syndromic causes of infantile hypotonia, and so should be considered early in the diagnostic process. Included in this group are Prader-Willi (probably the most common syndromic cause of infantile hypotonia after Down syndrome), Angelman, Williams, Fragile X, Rett, and Smith-Magenis syndromes. These are now all diagnosable by specific genetic testing, at least in the majority of affected individuals. Another important condition in this category to consider is alpha-thalassemia-mental retardation. Although there is no easy protocol for arriving at a syndromic etiology of infantile hypotonia, a logical approach is possible. The availability of testing for an increasing number of such disorders is helping to facilitate this sometimes daunting task.

Disclosure(s): None

ABSTRACTS OF INVITED TALKS

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Down syndrome: caring for the person and the family. *WI Cohen*. Down Syndrome Center, Children's Hospital of Pittsburgh, PA.*

Health care providers play an important role in assuring optimal medical care for individuals with Down syndrome by early identification of congenital malformations and by the timely detection of those medical problems which occur more frequently in this population. In addition, primary care and specialty practitioners play an important role in helping families deal with the strong emotions often evoked by the unexpected birth of a child with Down syndrome. This discussion will describe the current screening protocols and the clinical approach to the family at the time of diagnosis and thereafter.

Disclosure(s): None

267
The challenge of collecting mutations that cause inherited disease. *R Cotton*. Melbourne, Victoria, Australia.*

Single gene disorders affect around 1% of all births and mutations have been estimated to affect 60% of all humans in their lifetime. Despite this there has been no systematic attempt to collect all mutations. Some collection has occurred in general databases but it has been limited due to funding or commercial restraints. This collection has, by necessity, been centralized without the involvement of experts in genes. In 1994, an initiative began to attempt to form a federation of curators of lists of mutations in genes (locus specific databases or LSDBs) to attempt to collect and curate all mutations in all genes in an expert manner. Since that time, problems to be faced have been solved and procedures have been put in place to such an extent that in 2000 all that was required was funding. Funding has been elusive but considerable progress has been made (see this and earlier progress on www.hgvs.org). Progress includes the planning of the HGVbase to receive this information; the design of a central database to serve the LSDB curators; the design and commissioning of an intake point in July 2003, i.e. the WayStation – see www.centralmutations.org ; the start of construction of a new generation of software for LSDB curators to easily use; and the design of software for use in clinics to facilitate their function as well as contribute mutations. The consortium is currently in the midst of an intensive campaign to raise funds for this worldwide initiative, which promises to improve genetic healthcare as well as research.

Disclosure(s): None

268
Clinical evidence supporting the association between assisted reproductive technology and congenital malformation syndromes. *MR DeBaun*. Washington University School of Medicine, St. Louis, Missouri.*

Recent data in human and large farm animals suggest that assisted reproductive technology (ART) is associated with overgrowth syndromes, namely Beckwith Wiedemann syndrome (BWS) and large offspring syndrome (LOS), respectively. Based on the evidence thus far, any of the following factors may be involved in the association: in-vitro technology, ovarian stimulatory drugs, progesterone administration, genotype of the parent (mother or father), and idiopathic infertility. Epidemiologic and experimental studies will be evaluated as to the strength of the association and potential study designs will be discussed to support or refute current hypotheses.

Disclosure(s): None

ABSTRACTS OF INVITED TALKS

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Second trimester troubles following first trimester screening: should the patient be offered screening again? *A Donnenfeld*. Drexel University College of Medicine and Genzyme Genetics, Phila, PA.*

Second trimester screening for Down syndrome (DS) is the current standard of care. Using four analytes in the second trimester (AFP, estriol, hCG and inhibin A), a DS detection rate of 75% at a screen positive rate of 5% has been consistently reported. However, first trimester screening using nuchal translucency, free beta hCG, and PAPP-A has consistently been reported to achieve an 80% DS detection rate at the same 5% screen positive rate. First trimester screening also has the advantage of being performed significantly earlier in pregnancy (11-14 weeks). Although sequential screening (both first and second trimester screening) can be performed and an overall DS risk can be constructed using likelihood ratios derived from the two independent risk estimates, there is an increased cumulative probability of having a screen positive result with this combined approach. This will result in an increase in false positives, an increase in the number of anxious patients requiring genetic counseling, an increase in the number of amnio procedures being offered and an increase in iatrogenic pregnancy losses. Patient and physician confusion as to how to handle discrepancies in screening test results (screen negative in the first trimester and positive in the second and visa versa) will invariably occur. If both first and second trimester DS screening are elected, the second trimester results can not be interpreted as if the first trimester screen had never happened. The results must be combined. Patients should be informed that the detection rate of second trimester DS screening is slightly less than first trimester screening, that having two separate screening tests may result in confusion regarding interpretation of results, and that their false positive rate will increase using a sequential screening approach. Patients should be informed that they have the option to decline second trimester screening after having undergone first trimester testing. A potential solution would be to recommend that the standard of care be changed to screening for Down syndrome with the first trimester, second trimester or integrated approach. As further experience and data on the performance of first trimester and integrated testing becomes available, this may occur. Until (or if) this change occurs, second trimester screening should continue to be offered to all obstetric patients as this will help insulate health care providers from medical-legal liability. If a patient accepts first trimester screening and declines second trimester screening, this should be documented in the patient's medical record. First trimester screening does not test for neural tube defects. Therefore, MSAFP screening and/or high resolution ultrasound should be offered.

Disclosure(s): Presenter is an employee of/paid consultant to, has investments with and receives travel support from Genzyme Genetics.

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The floppy infant revisited. *V Dubowitz*. Dubowitz Neuromuscular Unit, Imperial College London, UK.*

"Navigating the etiologic complexities of hypotonia" will perturb both the geneticist and the clinician. "The floppy infant" poses no such threat. It presents an important clinical problem requiring accurate diagnosis and prognosis, to ensure appropriate management. It may be accompanied by variable respiratory and feeding difficulty. Concomitant muscle weakness suggests a neuromuscular disorder; associated clinical features pinpoint individual conditions. Non-muscle disorders cover a wide range. After initial clinical diagnosis, one should proceed to the key investigation, usually needle muscle biopsy in a muscle disorder. Major molecular genetics advances now provide another powerful tool for diagnostic confirmation and in Spinal Muscular Atrophy or Congenital Myotonic Dystrophy, one may proceed direct to DNA studies for confirmation. I shall illustrate the cardinal clinical features with four well-recognised conditions. **1. Severe spinal muscular atrophy (SMA type 1)** has marked truncal and limb weakness, affecting legs more than arms and proximal muscles more than distal. Intercostal weakness with diaphragmatic sparing is unique to SMA. Confident clinical diagnosis can be confirmed SMN exon 8 deletion. **2. Congenital myotonic dystrophy** presents as a floppy infant with variable respiratory and swallowing difficulties and associated facial weakness. The diagnosis is supported by myotonia and mild facial weakness in the mother and confirmed by CTG triplet expansion in the mother and infant. Congenital myotubular myopathy has an identical clinical presentation. **3. Congenital muscular dystrophy** presents with hypotonia and weakness and a tendency to associated contractures and may have severe respiratory problems. The classical form has no associated brain malformation or significant mental retardation, which are features of Fukuyama CMD, Muscle-Eye-Brain Disease and Walker-Warburg syndrome. Merosin deficiency in about half the classical cases provided a major diagnostic advance, separating this genetically distinct group. A further major advance was the discovery that the three brain-associated disorders are abnormalities of glycosylation. Alpha-dystroglycan deficiency in the muscle provides a clue to this. Homozygous mutations in collagen 6 gene account for Ullrich syndrome, a CMD with proximal contractures and distal joint laxity. **4. Prader-Willi syndrome** is an easy diagnosis to make if you think of it and extremely difficult if you don't! Many cases have endless unnecessary investigations. Classical presenting features are profound hypotonia but good muscle power with anti-gravity limb movement. There is swallowing difficulty but no respiratory deficit and also a characteristic facies. Diagnosis can be confirmed by the deletion on paternal chromosome 15q. I have enjoyed rereading my 1980 Floppy Infant monograph and feel reassured that clinical medicine has not changed but molecular genetics has opened completely new vistas.

Disclosure(s): None

ABSTRACTS OF INVITED TALKS

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Presidential address: genetic testing. *CJ Epstein*. Department of Pediatrics, University of California, San Francisco, CA.*

Although genetic testing has long been an important component of medical genetics, the recent sequencing of the human genome and the accompanying identification of single nucleotide polymorphisms and other types of genetic variation have fueled an intensive search in both the public and private research sectors for variants that confer susceptibility to the development of many common diseases and influence how we respond to therapeutic agents. This search has led to a general perception, which has been fostered by both the professional and lay press, that a vast expansion of genetic testing will usher in an era of "personalized medicine" in which DNA testing will permit the prevention, diagnosis, and treatment of disease to be tailored to each individual. Regardless of how much of this actually turns out to be possible, it is clear that new genetic tests will be devised, that there will be pressure from both providers and consumers to use them, and that DNA-based genetic testing will be done. However, it is less clear who will be responsible for ordering these tests and for interpreting and communicating the results. Experience with genetic testing of various types, but especially with the presymptomatic identification of mutations causing or predisposing to late onset diseases of high penetrance, has already taught us that the interpretation and communication of genetic risk data are often complex and not generally within the scope of practice of most primary care physicians and medical specialists. This complexity is likely to be greater for tests directed at variants that confer relatively low changes in risk. I believe, therefore, that the active participation of both medical geneticists and genetic counselors in this process would greatly benefit other medical professionals and the public at large. However, for this to happen, it will be necessary for the training programs in medical genetics and genetic counseling to provide the required knowledge and skills. There is no question that genetics will play a major role in the medicine and health care of the future. The question is whether geneticists will be involved!

Disclosure(s): None

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Using family therapy based techniques and interventions to enhance genetic counseling. *D Eunpu*[1], L Kessler[2]. [1]Arcadia University, Glenside, PA, [2]University of Pennsylvania Health Systems, Philadelphia, PA.*

As clinical genetics has become technically more complex and as the variety of patient populations has grown, there is a significant need to understand the patient in his or her family context. Improved understanding of the family context is likely to improve one's ability to work with patients on several levels including contracting, decision-making and patient management. In addition, use of these techniques and approaches is likely to improve one's efficiency and effectiveness in clinical work. This session will provide an introduction to systems family therapy theory and techniques as applied in genetic counseling. Topics presented will include the use of genograms as well as assessment and interpretation of family communication, attitudes and beliefs. Theoretical grounding and techniques will be illustrated with clinical material and applications in practice will be outlined.

Disclosure(s): None

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Revision of evaluation and management codes for CPT: implications for clinical geneticists' billing and reimbursement. *D Flannery*. Medical College of Georgia, Augusta, GA.*

This presentation will report on the current status of the ongoing process of revision of the documentation guidelines for E&M services. The key issue of development of work-equivalent specialty-specific Clinical Examples for different levels of service will be discussed. Potential opportunities for development of new Evaluation & Assessment (E&A) codes for genetic counseling by genetic counselors will be discussed as well.

Disclosure(s): None

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HIPAA is here : now what? *L Fleisher*. Sidley Austin Brown & Wood LLC, Chicago, IL.*

On April 14, 2003, regulations promulgated under the Health Insurance Portability and Accountability Act of 1996 ("HIPAA") went into effect, creating for the first time a uniform federal "floor" of privacy protection for individually identifiable health information ("protected health information" or "PHI"). So, how are we doing? Is it as HIPAArendous as many feared? This presentation will review briefly the current state of understanding, compliance and problems with the HIPAA Privacy Rules. It will address such issues as: provider compliance rates (undercompliant v. overboard); frequently asked questions and responses; duty to warn v. HIPAA constraints; effects on research and the role of IRBs; incidental v. prohibited uses/disclosures; preemption of state laws; and most commonly filed complaints. In short, it will assess how we are handling HIPAA one year out.

Disclosure(s): None

ABSTRACTS OF INVITED TALKS

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Ataxia-telangiectasia. *R Gatti*. UCLA School of Medicine, Los Angeles, CA.*

Ataxia-Telangiectasia (A-T) is an autosomal recessive disorder that includes: early-onset ataxia, oculomotor apraxia, oculocutaneous telangiectasia, immunodeficiency, radiosensitivity, and cancer susceptibility. After Friedreich's ataxia, it is the most common recessive form of childhood ataxia in most countries. A definitive clinical diagnosis is often difficult in very young children, before the classical syndrome is fully manifested. Telangiectasia and cerebellar atrophy (as observed by MRI) usually follow the onset of neurological symptoms by several years. These factors complicate genetic counseling and family planning. Laboratory findings include: an elevated serum alphafetoprotein, trace or undetectable levels of ATM protein by immunoblotting, and radiosensitivity, as demonstrated by markedly reduced survival of lymphoblastoid cells after exposure to ionizing radiation (1 Gray). The serine/threonine kinase function of the protein can also be evaluated, using substrates like p53. Identifying mutations in the ATM gene is challenging due to the large size of the gene (150 kb; 66 exons) and the cDNA (9168 nt). Over 400 unique mutations have been defined across the entire gene; none is more frequent than 3%. Many polymorphisms are present. Founder effect mutations have been identified in A-T families from many countries; however, recent studies using SNP haplotypes suggest that most of these represent ancestral mutations rather than independent events. Haplotyping with short tandem repeat (STR) markers, using standarized allele sizes, is useful in identifying these ancestral mutations, especially within ethnically defined populations. Most mutations create nonsense codons or frame shifts with downstream premature termination codons (PTCs). Some frame shifts result from splicing defects that delete complete (type I) or partial (type III and IV) exons, or insert intronic material (pseudoexons) into the mRNA (type II). Only about 10% of ATM mutations in A-T patients are of the missense type. ATM mutations have also been detected in breast cancer patients with no family history of A-T; however, these are about 85% missense and 15% nonsense mutations and represent a different mutation spectrum than that seen in A-T families. Despite this, the risk of breast cancer in ATM heterozygotes from A-T families also appears to be increased. Recent experiments suggest that certain aminoglycosides increase the readthrough of certain ATM mutations that involve primary PTCs. Thus, defining the specific mutations and genetic mechanisms that block production of normal protein may play a prominent role in designing therapy for A-T patients. This may prove true for other genetic disorders as well.

Disclosure(s): None

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Hypotonia—when to suspect a metabolic or mitochondrial disorder. *A Gropman*[1,2]. [1]Department of Pediatrics and Neurology, Georgetown University, Washington, D.C., [2]Center for Functional and Molecular Imaging, Georgetown University, Washington, D.C.*

Hypotonia, clinically defined as impaired passive resistive to movement, is generally an indication of nervous system impairment in the infant or young child who otherwise manifests a limited repertoire of responses by which to judge neurological well being. Aberrances of tone may reveal something about the timing and type of event that may have caused the abnormal tone. However, the etiologies of hypotonia are broad, ranging from infection, chromosomal anomalies, metabolic dysfunction, and primary disorders of the central (ie structural abnormalities) and peripheral nervous systems (muscle, nerve, neuromuscular junction defects). The hypotonic infant poses a diagnostic challenge to the clinician. Although individually rare, mitochondrial and metabolic disorders remain an important part of the differential diagnosis of hypotonia in the newborn period. The window of opportunity to intervene in many of these disorders is limited, thus an integrated approach to the suspicion and detection of these disorders is mandatory. Clues from the history, pedigree, physical examination, and ancillary studies (ie MRI, EEG, BAER, EMG, pathology, biochemical and molecular laboratory studies) may help to delineate those infants who should be evaluated for these entities. This talk will focus on the approach to the infant suspected of having hypotonia due to an underlying metabolic/mitochondrial disorder.

Disclosure(s): None

ABSTRACTS OF INVITED TALKS

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Recombination and aneuploidy in humans.

T Hassold[1,2]. [1]Department of Genetics, Case Western Reserve University, Cleveland, OH, [2]Center for Human Genetics, University Hospitals of Cleveland, Cleveland, OH.*

Trisomy is the most commonly identified chromosome abnormality in humans, occurring in at least 4% of all clinically recognized pregnancies; it is the leading known cause of pregnancy loss and of mental retardation. Despite its clinical importance, we have been ignorant of the causes of meiotic nondisjunction, the process that gives rise to trisomic progeny. Over the past decade, however, genetic mapping studies have led to the identification of the first molecular correlate of human nondisjunction; i.e., altered levels and positioning of meiotic recombinational events. Specifically, studies of trisomies 13, 14, 15, 16, 18, 21, 22 and sex chromosome trisomies have shown increases in 0 cross-over events or in distal-only or pericentromeric cross-overs in meioses that lead to trisomy. These observations have led to the idea that human meiotic nondisjunction requires "two hits": first, the establishment in prophase I of a "vulnerable" bivalent and second, abnormal processing of the bivalent at metaphase I or II. The presentation will summarize the data that have led to this model. These observations on human trisomies have led us to initiate two other related sets of experiments on recombination and meiotic chromosome segregation. In the first, we have been interested in developing murine models of human nondisjunction, using situations in which pairing and/or recombination are disturbed. We will present preliminary results of two such situations, one involving paracentric inversion carriers and the other matings between genetically distant strains of mouse. We have identified significant increases in nondisjunction in both cases and in the letter situation the effect appears to increase with maternal age; thus, we are hopeful that we may have generated a useful animal model of human nondisjunction. In the second set of experiments, we have been interested in utilizing cytological methodolgy to examine recombination in individual human germ cells; i.e, using standard immunofluorescence to examine the localization patterns of the mismatch repair protein MLH1 --- thought to localize to sites of meiotic cross-overs --- in meiosis I spermatocytes. Our results confirm that MLH1 foci do, indeed, "mark" cross-overs, providing a powerful new tool to analyze recombination as it is happening. Further, our initial results suggest that abnormalities in recombination contribute to infertility, as we have already identified infertile males with meiosis I arrest phenotypes linked to recombination machinery defects.

Disclosure(s): None

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Cytogenetics of childhood acute lymphoblastic leukemia (ALL): past discoveries, new frontiers.

NA Heerema. The Ohio State University.*

Although childhood ALL has an overall event-free survival rate approaching 80%, some patients have a much greater risk for treatment failure than others. In many cases the cytogenetic profile of the leukemic cells predicts response to treatment. Historically and in recent analyses, ploidy is a significant prognostic indicator. Patients with high hyperdiploidy (HH; >50 chromosomes) generally have the best outcome, and those with hypodiploidy, particularly with < 44 chromosomes, have the poorest outcome. Additionally, as shown by the Children's Oncology Group, patients with trisomies of all three chromosomes, 4, 10, and 17, have a better outcome than patients lacking these trisomies, indicating that HH per se may not be the significant prognostic factor. Specific structural aberrations also are prognostic. An indicator of good prognosis is the cryptic t(12;21), which results in rearrangement of the TEL(ETV6) and AML1(CBFA2, RUNX1) genes. Some aberrations, e.g., deletions of 6q and aberrations of 12p, appear to have no prognostic significance, and others, e.g., t(1;19)(q23;p13.3), have less prognostic significance when specific treatment regimens are administered. Other aberrations are associated with worse outcome. MLL (11q23) aberrations predict a poor outcome, particularly for infants. An especially poor prognosis is associated with Philadelphia (Ph) chromosome–positive ALL, i.e., t(9;22)(q34;q11.2). A recent international collaborative effort showed that Ph+ childhood ALL patients who received a bone marrow transplant from a matched sibling had a better outcome than did those who received only chemotherapy. Importantly, the secondary chromosome aberrations in these patients affected outcome: patients with HH and/or a second Ph chromosome with no losses of chromosome 7, 7p, or 9p had an improved outcome. In contrast, those with losses of chromosome 7, 7p, or 9p without the above gains had a worse outcome. We recently showed that patients with losses of chromosome 7 or 7p have a worse outcome than those with normal chromosomes 7. The poor outcome was unaffected by the presence of a Ph chromosome, del(9p), ploidy, or NCI risk group. Thus, the cytogenetic profile of childhood ALL continues to be a significant predictor of response to treatment, and previously unknown relationships of cytogenetic aberrations and outcome continue to be described. New, exciting challenges to cytogenetics of childhood ALL include investigation of when, how, and why cytogenetic aberrations occur. Some aberrations originate in utero; why do some infants with aberrations develop leukemia and others do not? What determines the latency period? How do aberrations occur? How does a cell become near-haploid or HH with specific trisomies? Cytogenetics is moving from a descriptive mode in childhood ALL to one of investigation of causes and mechanisms.

Disclosure(s): None

ABSTRACTS OF INVITED TALKS

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Diagnostic and counseling considerations in a case of craniosynostosis. *EW Jabs*. Institute of Genetic Medicine, Johns Hopkins University, Baltimore, Maryland.*

Craniosynostosis, the premature fusion of one or more sutures of the skull, is one of the most common malformations at birth. It leads to an abnormal skull shape, and in moderate to severe cases, timely clinical and surgical intervention is required to prevent neurological morbidity and mortality. The birth prevalence is 1 in 2000 to 3000. Craniosynostosis can be secondary to both environmental and genetic factors. Non-syndromic craniosynostosis accounts for 85% of all cases and usually involves the sagittal suture. Craniosynostosis is a feature of over 100 syndromes which usually are autosomal dominant and involve the coronal sutures. Crouzon, Jackson-Weiss, and Pfeiffer syndromes are characterized by brachycephaly, ocular proptosis, and midface hypoplasia. The former syndrome is not associated with limb abnormalities, while the latter two have syndactyly and broad great toes. Pfeiffer syndrome can be associated with broad thumbs and radiohumeral synostosis. These conditions are due to missense and in-frame small deletion, insertion, and splicing mutations in the ligand binding, extracellular domain of fibroblast growth factor receptor 2 (FGFR2). In some cases, the same mutations can cause all three phenotypes suggesting that these conditions represent a spectrum of FGFR2 craniosynostosis syndromes. Pfeiffer syndrome can be caused by mutations in FGFR1, -2, and-3 demonstrating genetic heterogeneity. Apert syndrome is clinically distinctive with severe syndactyly and is caused by two recurrent missense mutations in FGFR2, Ser252Trp and Pro253Arg, and splicing mutations. Beare-Stevenson cutis gyrata and Crouzonodermoskeletal syndromes are associated with dermatologic abnormalities and mutations in or near the transmembrane regions of FGFR2 and FGFR3, respectively. Saethre-Chotzen syndrome is characterized by ptosis of the eyelids, facial asymmetry, low frontal hairline, and dysplastic ears. It is caused by loss of function mutations, including microdeletions, in the transcription factor gene, TWIST. Some patients with this latter syndrome can resemble patients with a FGFR3 Pro250Arg mutation. The latter mutation is associated with variable phenotypic expression of non-syndromic coronal synostosis as well as several clinical syndromes. Boston type craniosynostosis has a disease causing Pro148His mutation in a homeobox containing transcription factor, MSX2. Antley-Bixler syndrome, an autosomal recessive condition, resembles severe Pfeiffer syndrome, but also can be associated with femoral bowing and fractures and genitalia abnormalities. It is due to mutations in still another gene of another gene family! A clinical case of Antley-Bixler syndrome will be discussed with regards to: 1) overlapping features with other syndromes, 2) the phenocopy secondary to teratogenic exposure with fluconazole, and 3) the newly reported disease gene. DNA diagnostic issues will be discussed.

Disclosure(s): None

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Dysmorphism in inborn errors of metabolism. *P Kaplan*[1,2]. [1]Section of Metabolic Diseases, Children's Hospital of Philadelphia,Philadelphia,PA, [2]University of Pennsylvania School of Medicine, Philadelphia,PA.*

There is an increasing awareness that children with some hereditary inborn errors of metabolism have dysmorphism. In many cases, the dysmorphic features are subtle and/or non-specific without a pattern of features that constitute an easily recognizable syndrome. This presentation will focus on syndromes manifesting at birth and infancy, when prompt diagnosis is critical. The recognition of dysmorphic features in this context is important for several reasons: [1] Recognition of dysmorphism may aid in diagnosing the underlying disorder. Earlier diagnosis and institution of appropriate treatment may be life saving and/or improve outcomes. [2] A specific diagnosis is important for accurate genetic counseling and the planning of prenatal strategies and testing for future pregnancies.The identification of dysmorphic features may aid in this effort. [3] The recognition and inclusion of dysmorphic features in the phenotype of patients with inborn errors of metabolism will provide a more complete understanding of the pathophysiology of these disorders. Already there are several well-documented examples of the roles of intermediary metabolism or energy production in embryogenesis. For example, cholesterol is linked to the N-terminal signaling domain of Hedgehog protein, which interacts with other proteins controlling patterning and morphogenesis. Perturbations in cholesterol biosynthesis, therefore, affect not only a spectrum of membrane functions and signaling pathways postnatally, but also the embryonic development of the brain, peripheral nerves, skeleton, kidneys, gastrointestinal tract, lungs, and male sexual system. For this discussion, inborn disorders of metabolism presenting with dysmorphism are divided into two groups according to common clinical features. **Group A**: Common manifestations are hypotonia, long (often narrow) facies, high forehead, widespaced eyes, long featureless philtrum, and, in some syndromes, hypospadias. [1] Energy metabolism: Krebs tricarboxylic cycle (TCA) (a) Pyruvate dehydrogenase (PDH) deficiency (b) Alpha ketoglutarate dehydrogenase (KGD) deficiency [DOOR syndrome] Electron transport chain (ETC) (a) Cytochrome c oxidase (COX) deficiency (b) Copper transport: Menkes syndrome [2]Peroxisomal biogenesis : Zellweger syndrome - infantile adrenoleukodystrophy -infantile Refsum syndrome spectrum [3]Cholesterol synthesis (pre-squalene): Mevalonic acidopathy is included under this rubric because the dysmorphic features resemble those of inborn errors of energy metabolism. [4] Purine synthesis: Adenylosuccinate lyase deficiency **Group B**: Common manifestations are skeletal dysplasia (mainly affecting limbs), craniofacial abnormalities (especially of the mid-face), and abnormal genitalia. This category includes many inborn errors of cholesterol synthesis: (a) 7-dehydro cholesterol reductase deficiency: Smith Lemli Opitz (SLO) syndrome. (b) Desmosterolosis. (c) Lathosterolosis. (d) Congenital hemidysplasia, ichthyosiform erythroderma /nevus and limb defects (CHILD syndrome). (e) XD chondrodysplasia punctata (Conradi Hunermann Happle syndrome). (f) HEM (hemidysplasia –"moth-eaten" skeletal dysplasia): Greenberg skeletal dysplasia. (g) XR chondrodysplasia punctata

Disclosure(s): None

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Social and behavioral aspects of craniofacial anomalies. *ML Marazita*. Center for Craniofacial and Dental Genetics, University of Pittsburgh, Pittsburgh, PA, U.S.A.*

Craniofacial anomalies are among the most common visible birth defects, occurring in 1 of every 500-1000 births worldwide. Orofacial clefts such as cleft lip and cleft palate are the most common craniofacial anomalies. The severity of such anomalies varies widely, but in most cases multidisciplinary treatment is necessary. This may include craniofacial surgery, specialized dental and orthognathic treatment, speech and hearing intervention, and educational, psychological, and social assessment and/or intervention. The physical anomalies, plus these extensive interventions, pose a number of challenges to the individual, to their families, and to society. The purpose of this talk is to review our current understanding of social and behavioral aspects of craniofacial anomalies, primarily orofacial clefts. Psychosocial aspects include health beliefs and behaviors, social competence, and social support. The possibly stigmatizing nature of craniofacial anomalies (or indeed any facial differences) further contributes to possible social and behavioral implications of such anomalies. Finally, we will review particular behaviorial implications of specific craniofacial anomalies.

Disclosure(s): None

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Acylcarnitine analysis in clinical practice.
D Matern. Biochemical Genetics Laboratory, Mayo Clinic College of Medicine, Rochester, MN.*

Carnitine and its esters are physiologically present in all biological fluids. For the diagnosis of organic acidemias and particularly of fatty acid oxidation (FAO) disorders, quantitative acylcarnitine profiling plays an increasingly prominent role in all venues of clinical biochemical genetics: prenatal diagnosis, newborn screening, evaluation of symptomatic patients, and postmortem screening. Almost exclusively performed by tandem mass spectrometry (MS/MS), plasma/serum is the preferred specimen type in diagnostic settings. However, urine analysis could be valuable in the investigation of patients with organic acidemias but inconclusive/borderline urine organic acid and plasma acylcarnitine profiles. Blood dried on filter paper is analyzed for newborn screening and together with bile in the postmortem evaluation of cases of sudden and unexpected death. Cell-free supernatant of amniotic fluid is used for the prenatal diagnosis of selected inborn errors of metabolism. Acylcarnitine analysis using stable isotope-labeled internal standards provides quantitative data for acylcarnitines with chain length of 2 to 18 carbons. As many other examples of complex metabolic profiles, it is critical to complement analytical proficiency with in-depth interpretation of results and informative reporting. From 1/2001 through 10/2003, 8006 quantitative plasma acylcarnitine analyses were performed in the Biochemical Genetics Laboratory at Mayo Clinic. In 351 samples (4.4%; 1 in 22 samples) the analysis was consistent with a biochemical diagnosis of a specific disorder. Among FAO disorders (60% of all abnormal results), MCAD deficiency (n=118) was the most common diagnosis followed by VLCAD deficiency (n=27) and LCHAD/TFP deficiency (n=26). Defects in propionate metabolism (propionic acidemia and methylmalonic acidemia) were the most commonly diagnosed organic acidemias (n=66), followed by 3-methylcrotonylglycinuria (n=32) and isovaleric acidemia (n=26). While MS/MS allows for unequivocal identification of most metabolites, there are a few exceptions. In particular, the acylcarnitine species with 4 and 5 carbons represent multiple isomers. Elevations of these analytes are best followed up by urine acylglycine analysis. Furthermore, the patients' metabolic condition (anabolism vs. catabolism), treatment, and carnitine status at the time of sample collection are critical factors to be taken into consideration in the interpretation of acylcarnitine results.

Disclosure(s): None

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Regulatory approaches in genetic testing: concepts and consequences. *E McCabe*[1,2,3]. [1]Department of Pediatrics, David Geffen School of Medicine at UCLA, Los Angeles, CA, [2]Department of Human Genetics, David Geffen School of Medicine at UCLA, Los Angeles, CA, [3]UCLA Center for Society, the Individual and Genetics, Los Angeles, CA.*

The public desires access to safe and effective genetic testing to improve their health decision-making. Regulation of genetic testing is one approach to quality assurance, but does impose barriers and costs. There are a variety of regulatory approaches to genetic testing that have been implemented or proposed. These include oversight of laboratories by federal or professional groups, e.g., under the Clinical Laboratory Improvement Amendments (CLIA) or College of American Pathologists (CAP), respectively. The Secretary's Advisory Committee on Genetic Testing (SACGT) recommended oversight of genetic tests by the US Food and Drug Administration (FDA), but expressed concern that FDA regulation might have a "chilling" effect on new test development. The FDA, working with leaders from professional organizations, has refined the approach and is pursuing implementation. The majority of genetic tests are carried out with reagents prepared in the testing laboratory ("home brews"), and it is unclear whether these will come under FDA regulation. Another approach that the FDA could utilize would be labeling of genetic tests by establishing uniform standards and providing oversight for test labeling. This would provide the public and practitioners information about test performance in various settings, e.g., when symptoms are manifested, in an asymptomatic individual with a positive family history, or as part of population-based screening of asymptomatic individuals. There are also web-based educational resources available to the public and healthcare professionals, e.g., Gene Tests. The consequences of these various strategies, the burdens they impose and the potential benefits they may provide will be discussed.

Disclosure(s): Presenter serves as a paid consultant to and has investments with GeneFluidics, Monterey Park, CA. Presenter also serves on the Genzyme Advisory Board, Boston, MA.

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The neurobiology of Down syndrome: insights from studies on segmental trisomy 16 mice. *WC Mobley*. Stanford University School of Medicine.*

Cognitive deficits in learning, memory and language are characteristic of Down syndrome (DS) and are present throughout the lifespan. Significantly, all individuals with DS show the neuropathology of Alzheimer's disease (AD) by age 40. Remarkably few studies address brain structure and function in DS. Nevertheless, existing data give evidence that abnormal central nervous system (CNS) function may be linked to structural abnormalities, including the structure and function of synapses. Because studies of DS brain structure are hampered by the unavailability of brain tissue, we have studied the Ts65Dn mouse, which is trisomic for the portion of mouse chromosome 16 (Ts65Dn) that contains genes orthologous to those on the long arm of human chromosome 21 in the DS critical region. Ts65Dn mice show both developmental and age-related changes in brain function; the latter includes the presence in aged mice of progressive atrophy and apparent loss of basal forebrain cholinergic neurons, changes also found in elderly adults with DS and in AD patients. Our research is intended to define: 1) changes in synaptic structure and function that are characteristic of DS; 2) the gene(s) that produce these abnormalities; and 3) how an extra copy of this gene(s) induces the changes. The work is guided by the hypothesis that a specific gene(s), present in an extra copy, will be responsible for cognitive phenotypes. Comparing Ts65Dn and 2N (i.e. control) mice, in studies on hippocampus we have found marked changes in both the structure and function of synapses. We found widespread changes in size and shape of pre- and postsynaptic elements and in the pattern of innervation of GABAergic inputs to the dendrites of dentate granule cells. The changes were evident by the end of the development period and extended into old age. Complementing these findings, LTP was absent in the dentate gyrus and this change was due to excessive inhibition of dentate granule cells by GABAergic neurons. Finally, we have further elucidated the cause of the degeneration of basal forebrain cholinergic neurons, showing that this is due to failed retrograde transport of the neurotrophic factor NGF. Ongoing studies have pointed to a specific gene as necessary for the interruption of normal transport in these neurons. We will discuss this work and suggest how through future studies we expect to better understand the neurobiology of DS and eventually to define effective treatments for these individuals.

Disclosure(s): None

ABSTRACTS OF INVITED TALKS

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Gene-enviroment interactions in holoprosencephaly. *M Muenke*. Medical Genetics Branch, NHGRI, NIH, DHHS, Bethesda, Maryland.*

Holoprosencephaly (HPE) is the most common anomaly of the developing forebrain with a prevalence of 1:10.000 live-born and 1:250 during early embryogenesis. The spectrum of clinical findings is variable even within the same family with severe CNS anomalies in some, HPE "microsigns" in others, and completely normal phenotype in obligate carriers. The etiology of HPE is extremely heterogenous with known teratogens and genetic causes including non-random chromosome anomalies in HPE in 25-50% of newborns with HPE, familial occurrence of HPE, and known genetic syndromes or associations with HPE. Cytogenetic anomalies consist of deletions or duplications of at least eleven chromosomal regions many of which have been shown to carry genes crucial for normal forebrain development. Human HPE genes have been identified in two major signaling pathways: Sonic Hedgehog (SHH, PTCH, GLI2, ZIC2) and Nodal (TDGF1, FAST1, TGIF) pathways, and other genes including SIX3. Mutations in these genes have been identified in only 15-20% of chromosomally normal HPE patients. Although it appears likely that multiple genes contribute to the phenotype of Human HPE, evidence from both human and animal models implicate environmental factors in the pathogenesis as well. Several lines of evidence link defective cholesterol biosynthesis and low maternal cholesterol to HPE. We hypothesize that even relatively low doses of some teratogens, which by themselves may not be sufficient to cause HPE, may act in concert with other environmental or genetic variables to generate the hPE phenotype.

Disclosure(s): None

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Single gene contributions to isolated cleft lip and/or palate. *JC Murray*[1], M Marazita[2]. [1]Dept Pediatrics, University of Iowa, Iowa City, IA, [2]Dentistry and Public Health, University of Pittsburgh, Pittsburgh, PA.*

While isolated cleft lip and/or palate (CLP) is a complex trait with multiple gene and environmental causes, several single gene disorders can present as phenocopies of the isolated forms without obvious associated anomalies that would provide clues to the underlying diagnosis or molecular mechanisms. Chief among these are mutations in MSX1 (causing CLP and hypodontia), IRF6 (Van der Woude syndrome), FGFR1 (Kallmann syndrome) and P63 (EEC syndrome). We will discuss how molecular diagnostics can assist in the process of syndrome classification for these and related disorders. In addition recent evidence suggests that the IRF6 gene has underlying variation that contributes to isolated clefting in the absence of any features of Van der Woude syndrome and holds promise for identifying common variation that contributes to CLP. This variation is found in a panethnic collection of cases representing more than 8000 individuals studied worldwide. We will discuss current clinical variability in these disorders, new finding relevant to gene and gene environment interaction causes of the conditions and raise discussion points for consideration in changes in how diagnoses are considered.

Disclosure(s): Presenter serves as an unpaid consultant for GeneDx.

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Unraveling the cognitive deficits in Down syndrome. *L Nadel*. Dept. of Psychology, University of Arizona, Tucson, Arizona.*

Down syndrome is characterized by mental retardation that ranges from mild to severe. Although it manifests itself in most cognitive domains, some capacities are more impaired than others, suggesting in turn that some brain regions are more disrupted than others. Neuroscience offers two kinds of approaches to unraveling these defects: animal models and careful neuropsychological dissection of the human condition. My colleagues and I have recently studied several groups of individuals with Down syndrome, using batteries of neuropsychological tasks aimed at determining which brain structures are most at risk in this syndrome, and how these defects play out over development. Based on earlier neuroanatomical work we focused on tasks diagnostic for the functions of the cerebellum, prefrontal cortex and hippocampal formation. We used mental age matched controls so that any pervasive across-the-board deficits would be factored out. Our results to date suggest disproportionate impairment in the functions of the hippocampal formation, with some hint of more selective impairments in cerebellar and prefrontal cortical capacities. The implications of these findings, and prospects for future research and translational impacts will be discussed.

Disclosure(s): None

ABSTRACTS OF INVITED TALKS

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OHRP: past, present, and future. *M Pelias*. GenELSI Consulting, Inc., New Orleans, LA.*

OHRP was organized in 2000 under the Secretary of DHHS, as a restructuring of OPRR at NIH, for the purpose of improving and increasing protections for human research subjects. The office established the National Human Research Protections Advisory Committee to study and submit recommendations on a variety of issues related to research with human subjects. These topics included financial interests, international research, the interests of children and other vulnerable populations, research in human and medical genetics, the status of third parties as human subjects, research in the social sciences, informed consent, and decisional capacity. NHRPAC was disbanded when its charter lapsed in 2002. A second committee, the Secretary's Advisory Committee on Human Research Protections, met in July, 2003, to establish priorities, based on the platform created by NHRPAC. The committee anticipates further work on the interests of prisoners and children, with deferral of difficult IRB reviews to a DHHS-OHRP/FDA panel for final decisions on research that is otherwise not approved. The committee also anticipates study of liability issues related to IRBs, accreditation of human research protection programs by non-Federal accrediting bodies, requirements for adverse event reporting (AER), and the role of Data Safety Monitoring Boards (DSMBs).

Disclosure(s): None

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Rothmund-Thomson syndrome and the RECQ helicase disorders. *SE Plon*[1,2], LL Wang[1]. [1]Texas Children's Cancer Center, Baylor College of Medicine, Houston, TX, [2]Department of Molecular and Human Genetics, Baylor College of Medicine.*

Bloom Syndrome, Werner Syndrome and Rothmund-Thomson Syndrome (RTS) are rare autosomal recessive disorders that result in overlapping but distinct clinical phenotypes including cancer predisposition. In each case homozygous or compound heterozygous mutations in a RECQ helicase gene have been identified with little initial evidence for genetic heterogeneity. However, recent studies demonstrate locus heterogeneity, including dominant inheritance, and conversely disparate clinical phenotypes resulting from mutations in a single RECQ helicase. For example, Chen et al. (2003) have demonstrated that patients with atypical Werner Syndrome carry single heterozygous mutations in the LMNA gene encoding Lamin A/C. RTS was originally described by Rothmund, an ophthalmologist, who noted affected children of consanguineous matings with unusual skin rash (later termed poikiloderma) and juvenile onset bilateral cataracts. Clinical description of a contemporary cohort of RTS patients revealed skeletal dysplasia, radial ray anomalies, sparse hair, short stature, and osteosarcoma (OS) with infrequent cataracts. Poikiloderma was the most important diagnostic feature. Molecular analysis revealed that OS in RTS patients was associated with deleterious mutations in RECQL4. Conversely, one third of patients were negative for deleterious mutations with evidence for genetic heterogeneity. Statistical analysis confirmed a significant genotype: phenotype relationship with RECQL4 mutations and cancer risk. Siitonen et al (2003) have now described homozygous or compound heterozygous mutations in RECQL4 in patients with RAPADILINO syndrome who do not demonstrate poikiloderma and may have a lower cancer incidence. The most common mutation is a small in-frame deletion that spares the helicase domain. Conversely, we have performed RECQL4 mutation analysis on patients with other poikilodermatous disorders. Poikiloderma with neutropenia (PN) is an autosomal recessive disorder first described in Navajo patients but also seen in European families. Clinically significant neutropenia distinguishes PN from RTS, and RECQL4 mutations are absent in these patients. We have also identified an Israeli kindred with apparent X-linked inheritance of poikiloderma (significant skin findings in the males and only patchy affected areas in the females). Genotyping reveals that the RECQL4 locus is not shared between the affected males, but all affecteds share markers on Xp between DXS8051 and DXS993. Lastly, we have evaluated three adult RTS patients of Swiss Mennonite ancestry with the original Rothmund phenotype of bilateral cataracts (onset at age five years) and poikiloderma but no osteosarcoma. Mutation analysis of one proband is negative for RECQL4 mutations suggesting genetic heterogeneity that can be further studied by homozygosity mapping. Thus, truncating mutations in RECQL4 are associated with OS risk in RTS, but no simple relationship exists between the presence of poikiloderma, mode of inheritance or RECQL4 mutations.

Disclosure(s): None

ABSTRACTS OF INVITED TALKS

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The present and future landscape of direct-to-consumer marketing of genetic testing. *BW Popovich*. Xenon Genetics Inc., Burnaby, B.C., Canada.*

Genetic tests in the US are presently ordered and reported via a physician referral/follow-up process. This traditional model of medical laboratory service delivery was the norm for all clinical laboratory tests in the past, but it has become supplemented in recent years by Direct to Consumer (DTC) access to laboratory tests for a wide variety of medical conditions. Many states now allow patients to access a diverse menu of laboratory services outside of the traditional process of medical laboratory test referral, and advertising for these services are increasingly being aimed directly at the consumer. State regulations allowing DTC access to medical testing, in parallel with the increasing globalization of laboratory services caused by the internet, will likely lead to an increasing number of genetic tests being marketed DTC in the near future. This talk will review both present and future models for DTC genetic laboratory services, and review possible roles for guidance and/or oversight by professional organizations such as the American College of Medical Genetics.

Disclosure(s): None

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Development of disease specific codes for genetic disorders. *S Puck*[1], M Blitzer[2], H Toriello[3], D VanDyke[4], M Watson[5], M Williams[6]. [1]Genzyme Genetics, Santa Fe, NM, [2]U. of Maryland School of Medicine, Baltimore, MD, [3]Spectrum Healths Genetic Services, Grand Rapids, MI, [4]Henry Ford Hospital, Detroit, MI, [5]American College of Medical Genetics, Bethesda, MD, [6]Gundersen Lutheran Medical Center, LaCrosse, WI.*

A worldwide clinical coding system developed by the World Health Organization is used for epidemiological tracking of symptoms or disease diagnoses, and in the US is also the basis for third party billing, with codes serving as clinical justification for the medical service(s) provided. Our current system, ICD-9-CM, contains significant gaps with regard to genetic disorders. The Economics Committee of the ACMG appointed a subgroup to submit proposals for changes to ICD-9, to be considered for the 2003-2004 revision. The rest of the world already uses the next generation coding system, ICD-10-CM, which has greater flexibility in both the numbering and indexing functions. It also contains more material relevant to medical genetics, particularly in metabolics, thanks to the efforts of Susan Winter and Neil Buist. However, this system is not expected to be available in the US until at least 2007. We had a 6-month window in which to propose and justify changes to ICD-9; if accepted they will be in effect in 2005 and will automatically carry over into ICD-10. Our decision-making principles included: 1) choosing diseases not covered in ICD-10; 2) choosing diseases or disease categories of higher frequency; and 3) wherever possible, fitting our changes into existing codes rather than creating new ones. We proposed changes in these areas: metabolic (including newborn screening), chromosome anomalies, other and unspecified anomalies, teratogens, genetic disease carrier screening, and family history. About half of our proposals are under active consideration; the rest may be considered in April 2004.

Disclosure(s): Presenter is an employee of Genzyme Genetics and receives honorarium and/or travel support and has investments with Genzyme Genetics.

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Importance of genetic lesions detected by conventional and molecular methods in acute myeloid leukemia. *SC Raimondi*. St Jude Children's Research Hospital.*

Acute myeloid leukemia (AML) is associated with heterogeneous subgroups of specific chromosomal aberrations. Moreover, leukemic cells in as many as 85% of pediatric patients with AML have an abnormal karyotype. Because such abnormalities are often indicators of prognosis, results of cytogenetic investigation are usually required for enrollment on AML protocols; however, in only a few studies have cytogenetic results been obtained for at least 90% of cases. The inability to collect cytogenetic information could compromise treatment assignment. The World Health Organization (WHO) has proposed 4 major categories of AML, some of which are based on chromosomal abnormalities: 1) AML with recurrent genetic abnormalities such as t(8;21), t(15;17), inv(16), and 11q23 abnormalities; 2) AML with multilineage dysplasia; 3) therapy-related AML; and 4) AML not otherwise categorized (including FAB subtypes M0 and M7). In children and adults with AML, prognostic factors include initial clinical features, t(8;21), t(15;17), inv(16), Down syndrome (in AML M7 only), and response to treatment. Factors indicating an unfavorable prognosis include monosomy 7 and complex chromosomal aberrations. Because of increased intensity of chemotherapy and improved supportive care, AML in 80% to 90% of pediatric patients enters remission, and their probability of 5-year event-free survival is about 50%. Knowledge of the molecular pathogenesis of hematologic malignancies has been gained through the characterization of breakpoints and the cloning of genes involved in these translocations and, in some instances, causally implicated in pathogenesis. Many of these rearranged genes encode tyrosine kinases, transcription factors, or other proteins that have relevant activities during the cell cycle and apoptosis. Point mutations in hematologic transcription factors and tyrosine kinases (e.g., CBFA2, C/EBP, FLT3, and c-KIT) also contribute to leukemogenesis. The ultimate phenotypic consequence of many of these rearrangements and mutations is the impairment of hematopoietic development. Approximately 40% of all known fusion transcripts can be detected in pediatric AML patients by RT-PCR at initial diagnosis. Although FISH and RT-PCR have improved the detection of subtle chromosomal aberrations that elude conventional cytogenetics, new molecular methods are being developed to comprehensively identify chromosomal abnormalities that lead to genomic instability that causes cancer. The integration of the functional genome profile with traditional molecular cytogenetic observations could lead to the identification of additional genes that play crucial roles in the development and progression of cancer and to the discovery of agents that selectively inhibit proteins or molecular pathways required for evolution of the malignancy.

Disclosure(s): None

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Epidemiology of craniofacial anomalies.
S Rasmussen. National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, GA.*

Craniofacial anomalies are among the most common birth defects, but their etiology is not well understood. In most cases, both genetic and environmental factors are believed to be involved in their etiology. Epidemiological studies have provided information on a wide range of topics, including birth prevalence; geographic, racial/ethnic, and gender variation; and genetic and environmental risk factors. Results from some of these studies will be presented, including results from the ongoing National Birth Defects Prevention Study. The discussion will primarily focus on three defects: orofacial clefts, holoprosencephaly, and craniosynostosis.

Disclosure(s): None

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Current practice in diagnostic DNA sequencing.
CS Richards[1,2]. [1]Dept. Molecular & Human Genetics, Baylor College of Medicine, Houston, TX, [2]Dept. Molecular & Medical Genetics, Oregon Health Science Univ., Portland, OR.*

Three years ago the Diagnostic Sequencing Laboratory (DSL) emerged as a new clinical laboratory initiative by the Department of Molecular and Human Genetics at Baylor College of Medicine. Major goals for the DSL included: development of a combinatorial approach to challenging diagnostic dilemmas using state-of-the-art technologies which were sequence-based; development of a cancer genetic testing program at Baylor; development of clinical research program that interfaces with the clinical laboratory; and development of a good quality assurance program for a clinical sequencing laboratory. For the testing platform, we chose to combine denaturing high performance liquid chromatography (dHPLC) to scan large genes to identify mutations, followed by an automated 16-channel capillary sequencer to confirm exact sequence alterations, using Sequencher software for sequence analysis. Our repertoire of cancer tests developed include the APC gene (15 exons) for familial adenomatous polyposis (FAP) (and more recently, MYH with 16 exons), MLH1, MSH2, and MSH6 genes (51 exons) for hereditary non-polyposis colon cancer (HNPCC), the RECQL4 gene (21 exons) for Rothmund-Thomson Syndrome (RTS), and p53 which is still in development. Among other interesting challenges was the development of testing for X-linked disorders, including ocular albinism (the OA1 gene, 9 exons) and at the other end of the spectrum, the dystrophin gene (79 exons), which we undertook as a research project. We present our cumulative experience, including our vision of the challenges for sequence-based diagnostics.

Disclosure(s): None

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Use of family history to identify adults at increased risk for chronic diseases and mendelian disorders. *MT Scheuner*[1,2], PW Yoon[2], MJ Khoury[2]. [1]UCLA School of Public Health, Department of Health Services, [2]Office of Genomics and Disease Prevention, Centers for Disease Control and Prevention.*

Family history is a risk factor for many common chronic disorders of adulthood, including coronary heart disease, stroke, diabetes and cancer. Family history reflects genetic susceptibilities as well as shared environmental, cultural and behavioral risk factors. Stratification of familial risk into different risk categories (e.g., average, moderate, high) is possible by considering: the number of affected relatives and their degree of relationship, the ages of disease onset, and the occurrence of associated conditions. Individuals with increased familial disease risk might benefit from personalized prevention recommendations specific to their familial risk, which could include assessment and modification of risk factors, lifestyle changes, alternative screening strategies for early detection and chemopreventive therapies. Individuals with the highest familial risk might have Mendelian disorders associated with a large magnitude of risk for a spectrum of diseases and usually with early ages of onset. In these cases, referral for genetic evaluation should be considered, including pedigree analysis, risk assessment, genetic counseling and education, discussion of available genetic testing, and recommendations for risk-appropriate screening and preventive interventions.

Disclosure(s): None

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ART techniques, their indications and relationship to epigenetic fetal effects. *JL Simpson*. Baylor College of Medicine, Houston, Texas.*

Approximately 10% of U.S. couples experience infertility, nearly equally apportioned between male and female infertility. Etiology is extremely heterogeneous, with treatment options concomitantly varied. For most male infertility, ART is the only option; for female infertility, ART is often either the only or by far the best option. Discerning whether ART is associated with fetal consequences due to perturbations of imprinting is especially important because the percentage of births due to ART continues to increase. Pivotal to addressing this issue is appreciating heterogeneity, the technique used. In the year 2000, 71,556 new ART cycles were performed in the U.S. in 383 clinics, 47% utilizing ICSI. Indications vary. Infertility often has a genetic basis (e.g., DAZ, FRAX permutation), and many causative genes have pleiotropic effects. It follows that comparing neonatal outcome of ART pregnancies to outcome in the general population is a serious pitfall in experimental design. Yet without ART, infertile couples cannot have offspring; thus, no true control is possible. To date only minimal stratification of data has been possible, usually simply considering ICSI with IVF v IVF alone. Precise indications for ART need to be taken into account, in particular the genetic basis for azoospermia. Precise ICSI technique is relevant, and a host of confounding factors considered: culture media, light source, technician health status (smoking), osmolality, temperature. Failure to take into account these variables is likely to result in disparate results, once larger population-based studies are reported.

Disclosure(s): None

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Quintessential indicators in the diagnosis of hypopigmentation disorders. *RA Spritz*. Human Medical Genetics Program, University of Colorado Health Sciences Center, Denver, CO.*

Disorders of pigmentation were among the first genetic diseases ever recognized because of their visually striking clinical phenotypes, resulting from defects of pigmentary melanocytes. Recent years have seen remarkable progress in understanding these diseases, largely as a result of the systematic parallel study of human patients and inbred mice with similar phenotypes. These diseases may be most usefully considered as abnormalities of melanocyte development, melanocyte function, or melanocyte survival. This classification can provide keys to assist in correct diagnosis of these pigmentary disorders.

Disclosure(s): None

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Cardinal ocular signs of inherited systemic diseases. *EI Traboulsi*. Center for Genetic Eye Diseases, Cole Eye Institute, Cleveland Clinic Foundation.*

The eye is involved in at least one-third of all inherited systemic diseases, and ocular signs can be pathognomonic, providing definitive clinical evidence for the underlying illness and guiding further diagnostic and therapeutic interventions. Slit-lamp and indirect ophthalmoscopic examination by an experienced ophthalmologist who is familiar with the cardinal ocular signs of inherited systemic diseases should be performed early in the course of the work-up, and can save valuable time and resources.This presentation will review the major ocular manifestations of common and rare disorders that include some of the phakomatoses (NF1, NF2, von Hippel-Lindau, Tuberous sclerosis), Marfan syndrome, homocystinuria, familial adenomatous polyposis, the ceroid lipofuscinoses, Aicardi syndrome, incontinentia pigmenti, Alagille syndrome, Wilson disease, Fabry disease, albinism, Alport syndrome and the Bardet-Bield syndromes. High quality photographs will be used to illustrate the pathognomonic signs of the various disorders. The pathogenic mechanisms leading to the ocular manifestations will be briefly reviewed, and therapeutic interventions for the ocular complications will be highlighted.

Disclosure(s): None

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First trimester screening for chromosomal abnormalities: time to abandon age. *R Wapner*. Drexel University College of Medicine.*

Biochemical screening for fetal chromosome abnormalities in the second trimester has become standard of care for woman under the age of 35. This approach offers a trisomy 21 detection rate of approximately 60%-65% for a 5% false positive rate. The addition of inhibin-A increases detection to 70-75%. Recently first trimester screening has become possible using biochemical analytes (PAPP-A and free beta HCG) and ultrasound measurement of the fetal nuchal translucency. This results in a detection rate of approximately 80%. In woman 35 years old or older the detection rate is 92% with a 16% false positive rate. As screening performance has improved, the issue of whether age should remain an independent indication for invasive prenatal diagnosis must be addressed. Modeling shows that an approach in which first trimester screening is applied to woman of all ages will reduce the need for invasive testing from 12.1% of the pregnant population to 4.6%. This would increase the overall detection rate from 72% to 74%. Offering invasive testing to first trimester screen negative (<1:270) woman will result in 4.3 procedure induced miscarriages for every trisomy 21 pregnancy identified. If second trimester screening is used, 3 procedure induced losses will occur for every trisomy 21 identified.

Disclosure(s): None

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Research agenda for family history tools: analytic validity, clinical validity, clinical utility, and ethical, legal and social implications. *P Yoon*, M Scheuner, M Khoury. Office of Genomics and Disease Prevention, CDC, Atlanta, GA.*

Before family history can be embraced as a public health strategy for assessing risk of common diseases and influencing early detection and prevention strategies, the validity and utility of the tools and processes should be evaluated. The four components of a comprehensive evaluation would include analytic validity; clinical validity; clinical utility; and ethical, legal, and social issues that influence both validity and utility. Analytic validity addresses how accurately and reliably a family history tool identifies disease among a person's relatives. Clinical validity addresses how well family history of disease can be used to stratify disease risk and predict future disease in a person. The specific elements of clinical validity include sensitivity, specificity, and negative and positive predictive value. Clinical utility is an assessment of the impact and usefulness of the family history tool for individuals, families, and society. Perhaps the most important utility issue is whether family history information can be used as a motivator to change behavior. Assessing the ethical, legal, and social impact of using family history as a disease prevention strategy is also important. The use of a family history tool for public health purposes could only be successful if people perceived greater benefit than risk associated with revealing family medical information, if there were no stigma associated with being at above average risk, and if there were interventions and options for behavior change that could make a difference in reducing morbidity and mortality.

Disclosure(s): None