1. Genotype-phenotype associations in Juvenile Ceroid Neuronal Lipofuscinosis (JNCL; CLN3).

Heather R. Adams[1], Jennifer Kwon[1], Rachel Jordan, Frederick J. Marshall[1,2], Paul G. Rothberg[3], Amy Vierhile[1], Elisabeth A. deBlieck[2], David A. Pearce[4], Jonathan W. Mink[1]. [1]Department of Neurology; [2]Clinical Trials Coordination Center; [3]Pathology and Laboratory Medicine; [4]Center for Aging and Developmental Biology, University of Rochester Medical Center, Rochester, NY

Objective: The most frequent mutation causing Juvenile neuronal ceroid lipofuscinosis (JNCL) is a 1.02 kb deletion in the CLN3 gene (p12.1 region of chromosome 16) which removes exons 7 and 8, and accounts for approximately 80-85% of known mutations. Most of the remaining individuals are compound heterozygotes with one copy of the 1.02kb deletion. There are limited reports suggesting differential progression among patients who are heterozygous for the common deletion and a second mutation. The purpose of this investigation was to examine genotype – clinical phenotype differences in homozygous vs. compound heterozygous JNCL patients.

Methods: We evaluated subjects' clinical function using a neurobehavioral test battery and the Unified Batten Disease Rating Scale and (UBDRS). Genotyping was performed to ascertain if subjects were homozygous for the common deletion or were compound heterozygous (either a known or novel mutation).

Results: We evaluated 49 individuals with JNCL. Of these, 36 (73.5%) were homozygous for the common deletion, 12 were compound heterozygotes and one subject had 2 copies of the R334H deletion. Motor function did not differ by genotype, although homozygous subjects had more variable motor function in comparison to compound heterozygotes over the course of the disease. Anxiety symptoms were also significantly worse among patients homozygous for the 1.02kb deletion, on both the UBDRS and an independent, externally validated measure of child mood problems used in the neurobehavioral test battery.

Conclusions: Subjects homozygous for the 1.02kb deletion had greater mood dysfunction and more variable motor function compared to compound heterozygotes. While these data are preliminary, they are consistent with earlier reports of a milder clinical phenotype among compound heterozygotes. At least 40 disease-causing mutations in CLN3 have been identified, but the function of the protein encoded by this gene is as yet unknown. Identification of genotype-phenotypic patterns associated with CLN3 mutations may help further our understanding of genomic and biochemical correlates or modifiers of clinical disease, provide a focus for targeted interventions to anticipate and manage symptoms, and to improve models for future clinical trials.

2. Outcome Predictors for Pediatric Dilated Cardiomyopathy: A Systematic Review

Jorge A. Alvarez, AB; James D. Wilkinson, MD, MPH; Steven E. Lipshultz, MD
University of Miami Miller School of Medicine

Objective: Dilated cardiomyopathy comprises the largest group of pediatric cardiomyopathy functional types and is the most common indication for heart transplant in children over 5 years old. Prognostic factors for this condition have long been sought by many researchers.

Methods: We searched OVID MEDLINE from 1966 to February, 2007, with MeSH terms and key words including: dilated cardiomyopathy, congestive heart failure, prognosis, predictors, and risk factors. The search was limited to studies performed in children with idiopathic or primary DCM, including myocarditis. Studies were also limited to those systematically analyzing patients. Case reports and small case series were excluded. Reference lists of appropriate studies were reviewed for articles not identified in the MEDLINE search. Abstracted data included the dates of the accrual period, sample size, follow-up time, and univariate and multivariate predictors of mortality or "heart death," a composite endpoint of death or heart transplant.

Results: In a systematic review of these factors, we found 32 relevant articles published since 1976. Four studies report finding no predictive factors. In the remaining 28 studies, several factors indicating better prognosis stand out across multiple articles: younger age at diagnosis, higher left-ventricular fractional shortening and ejection fraction, and the presence of myocarditis. Results for other factors conflict across studies: severe mitral regurgitation, arrhythmias, and a family history of cardiomyopathy. Elevated left-ventricular end diastolic pressure was statistically significant in two studies, but it may be of limited utility as a result of its invasiveness. Although most children have congestive heart failure at presentation, only two studies found it to be a significant predictor of mortality. The largest study of this factor qualified the increased risk to 1 year after presentation. Other significant predictors that have not been analyzed or reported by more than one study group are right ventricular heart failure and impaired cardiac adrenergic innervation, as detected by radiolabeled meta-iodobenzylguanidine imaging. While 1- and 5-year overall survival rates have steadily improved, as more children with DCM receive cardiac transplants, event-free survival rates (the absence of "heart death" resulting in death or transplant) are similar to those from decades ago.

Conclusions: With few prognostic factors seen across studies, a unified risk algorithm may assist in clinical decision-making, but requires more studies. Other studies are needed to assess the post-transplant survival experience.

3. Development of Hepatocellular Carcinoma in BSEP Disease Due to Alterations in Oncogene Expression

Lee Bass Department of Pediatrics, Northwestern University, Chicago, Illinois

Objective: Progressive Familial Intrahepatic Cholestasis (PFIC) is characterized by hepatocellular cholestasis, low serum levels of gamma-glutamyl transferase (GGT) activity, autosomal recessive inheritance and development of cirrhosis unless treated.. Specific gene defects are identified in two subtypes of PFIC. Despite genetic distinctness, there are few clinical differences between PFIC-1(FIC1) and PFIC-2(BSEP). Both respond to partial external biliary diversion. A unique clinical feature of PFIC-2 is an increased risk of Hepatocellular Carcinoma (HCC) that persists despite bile diversion therapy with cholestasis reversal. Therefore, we hypothesize that expression of oncogenes in children with PFIC-2 leading to HCC result from specific loss of BSEP gene function and not the consequence of retention of toxic substances by the liver.

Methods: Livers from children with PFIC-2 will be compared to children with genetic cholestasis (PFIC-1 and Alagille syndrome), and children with non-genetic cholestasis (biliary atresia) not at risk for HCC to determine the relative expression of oncogenes using Affymetrix gene chip array mRNA expression analysis. The levels of polymerase III transcribed Alu RNA will be analyzed using Northern hybridization, production of Expressed Sequence Tags from Alu-cDNA libraries and tissue in situ hybridization. Finally, the relationship of oncogene expression will be compared to cholestatic toxicity: tissue bile salt concentration and hydrophobic index, expression of farnesoid X receptor response elements, and quantitative measures of parenchymal injury and fibrosis.

Results: The following BSEP livers have been genotyped and will be used in our investigation.

Sample #	Mutation 1 Nucleotide change	Mutation 1 Predicted effect	Mutation 2 Nucleotide change	Mutation 2 Predicted effect	Disease manifestation
1	890 A>G	Glu 297 Gly	unknown		BSEP def
2	890 A>G	Glu 297 Gly	unknown		BSEP def
3	890 A>G	Glu 297 Gly	890 A>G	Glu 297 Gly	BSEP def
4	890 A>G	Glu 297 Gly	IVS16-7 T>A	Splicing defect	BSEP def
5	890 A>G	Glu 297 Gly	890 A>G	Glu 297 Gly	BSEP def
6	890 A>G	Glu 297 Gly	IVS7+1 T>A	Splicing defect	BSEP def HCC
7	890 A>G	Glu 297 Gly	IVS7+1 T>A	Splicing defect	BSEP def
8	1416 T>A	Tyr 472 Ter	1416 T>A	Tyr 472 Ter	BSEP def HCC
9	1964 C>T	Thr 655 Ile	1935 delA	Lys 647 FS	BSEP def HCC
10	890 A>G	Glu 297 Gly	IVS19+1 G>T	Splicing defect	BSEP def
11	1723 C>T	Arg 575 Ter	890 A>G	Glu 297 Gly	BSEP def

Tissues are currently being processed for RNA, DNA, analysis of bile salts, and pathology. Data addressing specific aims are forthcoming.

4. Beyond VACTERL: Different Types of Anorectal Malformations Are Associated with Different Combinations of Other Congenital Anomalies

Michael D. Bates, Nicholas P. Matarazzo, Jareen K. Meinzen-Derr, George Rodriguez, Emily Louden, Richard A. Falcone, Marc A. Levitt, Alberto Peña; Cincinnati Children's Hospital Medical Center, Cincinnati, OH; Long Island Jewish Hospital, New Hyde Park, NY.

Objective: Anorectal malformations (ARMs) are congenital abnormalities of hindgut morphogenesis that occur in 1 in 2,000-5,000 live births. They represent a spectrum of disorders from simple (imperforate anus without fistula) to complex (cloaca) in which the distal gastrointestinal tract ends blindly or opens ectopically with a fistula to the skin or genitourinary tract. ARMs are often associated with other congenital anomalies, such as in the Vertebral-Anorectal-Cardiac-TracheoEsophageal-Renal-Limb (VACTERL) association. We tested the hypothesis that different types of ARMs would have different combinations of associated anomalies.

Methods: Clinical characteristics of patients with ARM seen by a single surgical group from 1980 through 2006 were collected prospectively and analyzed retrospectively. The most frequent ARM subtypes among the 1732 patients were cloaca (439), rectovestibular fistula (RV, 260), rectoprostatic fistula (RPro, 214), rectobulbar fistula (RBu, 205), rectoperineal fistula (RPer, 172), rectobladderneck fistula (RBN, 97), and no fistula (NF, 81); 264 patients had other types of ARM. Chi-squared analyses and hierarchical clustering were performed using SPSS.

Results: The different ARM subtypes in our series were associated with distinct sets of other congenital malformations: cloaca with various gastrointestinal, sacral, and genitourinary (GU)/renal anomalies; RPro with esophageal and duodenal atresia; RBN with GU/renal and vertebral and sacral anomalies; and RBu with developmental delay. Negative associations between specific ARM subtypes and other congenital anomalies were also observed. None of the ARM subtypes were associated with all of the defects making up the VACTERL association, and cloaca was the only ARM subtype that was associated with VACTERL association. For several of ARM subtypes (cloaca and RV in girls; RBN and RPer in boys), hierarchical clustering identified groups of patients with different sets of associated congenital anomalies.

Conclusions: Taken with our previous finding that certain subtypes of ARM (RV, RPer) are more likely to have a positive family history (*J Pediatr Surg* **42**:124, 2007), these results suggest that the various ARM subtypes result from different pathophysiological mechanisms. Patients with specific ARM subtypes may be further subclassified based on their associated congenital malformations. These results will assist in identifying homogeneous groups of patients for genetic analyses.

5. Identification of the X-Linked Infantile Spinal Muscular Atrophy (XL-SMA) Gene: Insights into Pathogenesis.

Lisa L Baumbach, Kemal Yariz, Mary Ellen Ahearn, Julianne Ramser, Alfons Meindl. *University of Miami, Miami Florida and Frauenklinik am Klinikum rechts der Isar, Munich, Germany.*

Objective: Autosomal recessive spinal muscular atrophy (AR-SMA) is associated with *SMN* mutations. SMN protein plays a critical role in mRNA metabolism. Significant reductions in SMN and associated proteins (gemins) have been reported in SMA type I patients. Our group has described a rare X-linked form of SMA, *lethal infantile spinal muscular atrophy [X-linked SMA (XL-SMA); MIM 301830*] with additional features of early onset/congenital contractures and/or fractures identified in fifteen unrelated families from North and Central America and Western Europe. We have recently reported mapping of the critical region to Xp11.3-centromere, with a cumulative LOD score is 8.71 at DXS1003. The purpose of this study was two-fold – 1) to identify *the XL-SMA* disease gene and 2) to address whether perturbations in the SMN/Gemin complex occur in X-linked SMA (XL-SMA) disease.

Methods: cDNA mutation screening was undertaken for all annotated genes in the *XL-SMA* critical region by *DHPLC - Wave* and DNA sequencing. SMN, Gemin-2 and Gemin-3 protein and RNA levels were quantitatively measured by Western blots and *Taqman* assays, respectively, in lymphoblastoid cell lines from XL-SMA patients, SMA Type I patients, and controls.

Results: We identified three rare mutant alleles (two novel missense mutations and a rare SNP) in a candidate disease gene within the highest LOD score region in five XL-SMA families. The missense mutations occur in two highly conserved amino acids within a highly conserved protein domain. Co-segregation of these gene variants with disease has been confirmed in 5/5families, and not detected in a large number of control chromosomes. These combined observations are highly suggestive of disease gene identification. We have also quantitatively measured SMN, Gemin-2 and Gemin-3 RNA and protein levels in XL-SMA and SMA cell lines. Our results suggest that XL-SMA patients have a selective loss (approx. 50%) of SMN and Gemin3 proteins as compared to SMA Type I patients and healthy controls. RNA expression studies suggest that these observed reductions occur post-transcriptionally.

Conclusions: These results illustrate how important collaborations can lead to rare disease gene discovery, and thus, provide not only important new information regarding understanding of *XL- SMA* disease, but possible interactions between the XL-SMA protein and the SMN/Gemin complex, and thus, long-term therapeutic approaches.

6. *Avp* is altered in mouse models of Rett syndrome and related *MECP2* disorders and is a transcriptional a target of MeCP2

Shay Ben-Shachar[1], Paolo Moretti[2], Sharyl Fyffe[1], James Carson[3], Christina Thaller[3] and Huda Y. Zoghbi[1,2] Departments of Molecular and Human Genetics[1], Neurology[2] and Biochemistry and Molecular Biology[3], Baylor College of Medicine, Houston, TX

Objective: Loss-of-function mutations as well as genomic duplication of the *MECP2* locus cause a variety of phenotypes. Typically, loss-of-function mutations cause Rett syndrome (RTT) in females, however, such mutations can cause a variety of neurological phenotypes including mental retardation and autism in males and females. Increased *MECP2* dosage in males causes mental retardation, seizures and hypotonia. MeCP2 contains methyl-CpG-binding domain and transcriptional repressor domain and is thus believed to function as a transcriptional repressor. To date, only a handful of genes were found to have increased expression in RTT mouse models. The relationship between some of those genes and the disease phenotype is not clear yet. Given the features in RTT and related disorders, we hypothesized that alterations of genes in the hypothalamus might underlie some of these phenotypes.

Methods: We analyzed hypothalamic RNA from *Mecp2*$^{308/Y}$ and wild-type littermates using Affymetrix 430.2 GeneChips. Alterations have been confirmed by using QPCR and *In Situ* hybridization. QPCR has been used to determine Arginine Vasopressin RNA (*Avp*) alterations in mouse models at different ages. Chromatin immunopreciptation analysis was performed from hypothalamic extracts DNA.

Results: *Avp* is increased in *Mecp2*$^{308/Y}$ and *Mecp2*$^{308/308}$ mice as well as in *Mecp2* null mice (fold changes 2.5, 1.8 and 1.4 respectively, P values<0.05). In addition, *Mecp2* ^{Tg} mice, which model the *MECP2* duplication syndrome, have an increased *Avp* expression as well (fold change 1.85, P value = 0.0002). The increased *Avp* expression was detected in symptomatic *Mecp2*$^{308/Y}$ mice (5 week-old and older, P values<0.01) but not in presymptomatic, 3 week-old animals. The increased expression was detected in both the paraventricular and the supraoptic nuclei, where *Avp* is normally expressed with no evidence of ectopic expression. Chromatin immunopreciptation revealed that *Mecp2* binds *Avp* promoter in *vivo*, thereby identifying *Avp* as a transcriptional target of MeCP2.

Conclusions: *Avp* expression is altered in different models of *MECP2* disorders. Given the important role of Avp for anxiety and social behavioral this alteration may contribute to the anxiety and possibly social behavioral phenotypes seen in mice and patients with *MECP2* disorders.

7. The Role of DNA Polymerase Gamma in Mitochondrial Disease

Sherine S. L. Chan and William C. Copeland
Mitochondrial DNA Replication Group, Laboratory of Molecular Genetics, NIEHS/NIH,
Research Triangle Park, NC 27709

Objective: Mitochondrial diseases affect 1/4000 people in the general population and consist of a wide spectrum of diseases, affecting both children and adults. *POLG*, the gene encoding the mitochondrial DNA (mtDNA) polymerase (pol γ) is a major locus for mitochondrial disease. More than 100 mutations in *POLG* are associated with disease, including the fatal early childhood Alpers syndrome, midlife-onset ataxia neuropathy syndromes to the late onset progressive external ophthalmoplegia (PEO).

Pol γ is a two subunit enzyme consisting of a catalytic subunit that has highly faithful DNA polymerase and proofreading activities, and a smaller accessory subunit for tight DNA binding and processive DNA synthesis. As pol γ is the only DNA polymerase within the mitochondrion, it is essential for replication and repair of mtDNA. Additionally, drugs used to treat HIV by causing chain termination also inhibit pol γ, thereby causing mitochondrial dysfunction. As such, we need to understand how and why pol γ defects cause such widely varying mitochondrial diseases.

Methods: Our multi-pronged approach encompasses the following methods:

1. Collaboration with clinicians to identify new mitochondrial disease mutations.
2. Transgenic mouse models of mitochondrial disease using pol γ disease mutations found in humans.
3. Structure-function and biochemical methods to characterize mutant pol γ proteins.

Results: We recently identified five new pol γ mutations in patients with Alpers syndrome. In a transgenic mouse model of the dominant Y955C PEO *POLG* mutation, cardiomyopathy, mitochondrial ultrastructural defects, mtDNA depletion and increased oxidative stress were observed, leading to premature death. We have found that some chain terminator drugs are less toxic to pol γ than others. We have biochemically characterized a number of the more common disease mutations in pol γ. Each mutant protein has it own unique set of defects, for example, the most common mutation, A467T, produces a protein that is defective in polymerizing DNA as well as binding its accessory subunit, whilst the defect of the Y955C protein lies in its inability to select the correct nucleotide for incorporation.

Conclusions: By using a wide array of methods, we are developing a clearer understanding of how mutations in pol γ contribute to mitochondrial disease as well as a greater understanding of the role of pol γ in mtDNA replication and repair.

8. Episodic Vertical Oscillopsia with Progressive Gait Ataxia: Clinical Description of a New Episodic Syndrome and Evidence of Linkage to Chromosome 13q

YH. Cha[†], MD; H. Lee, BS[‡]; JC. Jen, MD PhD[†]; JC. Kattah, MD[§]; SF. Nelson, MD[‡]; RW. Baloh, MD[†]
University of California Los Angeles, Department of Neurology[†] and Department of Human Genetics[‡], University of Illinois Peoria Department of Neurology[§]

Objective: To describe a new disorder characterized by late onset episodic vertical oscillopsia and gait ataxia and to determine ancestral sharing between the affected individuals.

Methods: We provide clinical descriptions of four families with late onset episodic vertical oscillopsia and progressive gait ataxia and performed Identity by Descent analysis (IBD) using a dense SNP array (Affymetrix 250K NspI array).

Results: Probands presented between the ages of 40 and 64 with initial symptoms of episodic vertical oscillopsia and interictal downbeat nystagmus. The downbeat nystagmus worsened during the spells. A mild gait ataxia developed over several years. Triggers included physical exertion, alcohol and caffeine. Patients did not respond to acetazolamide. Genetic screening for episodic ataxia types 1 and 2, SCA 1, 2, 3 and 6 were negative. Using ancestral IBD analysis and dense SNP genotyping throughout the genome, an interval of 28.6cM (~14.2Mb) on chromosome 13q12.11-q13.3 composed of 1259 SNPs, was shared between affected individuals in two of the four families and highlighted a region of suggestive linkage (LOD > 2.7).

Conclusions: Episodic vertical oscillopsia and progressive gait ataxia is a recognizable disorder in which the oscillopsia is likely due to intermittent worsening of downbeat nystagmus. The phenotype is remarkably similar between the families, in which two likely had a common ancestor. Genes in the region of IBD are currently being sequenced.

9. Two Japanese Women with Nontuberculous Mycobacterial Disease and the Q1352H CFTR Mutation

Rhonda E. Colombo, MD and Kenneth Olivier, MD
NIAID, NIH

Objective: Nontuberculous mycobacteria (NTM) are ubiquitous environmental organisms capable of causing pulmonary disease. Specific host factors predisposing to pulmonary NTM disease have not been fully elucidated. A 50% incidence of CFTR mutations has been described in adults with bronchiectasis and/or pulmonary NTM infection, suggesting a role for altered airways clearance (Ziedalski. *Chest* 2006). We are systematically evaluating patients with chronic airways disease to better define host susceptibility to infections from environmental organisms. We describe two Japanese women who have pulmonary NTM disease and a Q1352H CFTR mutation.

Methods: Cross-sectional investigation of airway host-defenses in individuals with recurring lung infections involving detailed investigation of clinical features, epithelial ion transport assessment, ciliary studies, and genetic evaluation.

Results: LF is a 47 yo woman with a history of chronic sinusitis, nasal congestion, and otitis media who presented to the NIH for pulmonary NTM disease. LF developed a chronic productive cough in adolescence and was diagnosed with bronchiectasis at the age of 42. She has had multiple pneumonias with pathogens including *Mycobacteria abscessus*, *Stenotrophomonas maltophilia*, and *Achromobacter xylosoxidans*. Genetic testing revealed a glutamine to histamine change at codon 1352 in exon 22 of the CFTR gene (Q1352H) as well as homozygosity for the M470V polymorphism. Sweat chloride and nasal potential difference testing were normal. However, mean nasal nitric oxide level was low at 60 nL/min. Coupled with her clinical presentation, this suggests an underlying diagnosis of primary ciliary dyskinesia.

KB is a 75 yo woman referred to the NIH for cavitary, biopsy-proven *Mycobacterium avium intracellulare* (MAI) pulmonary infection. She was hospitalized for worsening dyspnea, fever, and hemoptysis. *Stenotrophomonas maltophilia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Acinetobacter lwoffi* were subsequently isolated from sputum. KB improved on broad-spectrum antimicrobial therapy. Genetic testing revealed two CFTR mutations, Q1352H and 4006-4A→G, located on separate alleles, arousing suspicion for variant CF. However, sweat chloride testing and nasal potential difference studies were not consistent with CF. Mean nasal nitric oxide levels were also low at 85 nL/min.

Conclusions: These cases illustrate overlapping clinical, physiologic, and genetic features of disorders in mucociliary clearance that may predispose to adult-onset bronchiectasis and recurring infections. A combination of factors is likely involved and routine diagnostic tests may not detect more subtle features. Determining the significance of detected CFTR mutations may also be difficult.

10. Outcomes of Pregnancy in Hereditary Hemorrhagic Telangiectasia

E.M. de Gussem[1], A. Lausman[2], A. Beder[1], C. Williams[1], J.J. Mager[3] M.E. Faughnan[1].
[1]Department of Medicine, Division of Respirology, St Michael's Hospital, University of Toronto, Canada, [2]Department of Obstetrics & Gynecology, St Michael's Hospital, University of Toronto, Canada,
[3]Department of Respirology, St Antonius Hospital, Nieuwegein, The Netherlands.

Objective Hereditary Hemorrhagic Telangiectasia (HHT) is an autosomal dominant disorder affecting 1:5000 North Americans. It is characterized by telangiectasia, epistaxis and the presence of arteriovenous malformations (AVMs). The objective of this study is to demonstrate the outcomes of pregnancy in patients with HHT. Our hypothesis is that pregnancy and delivery (including epidural anesthesia) are low-risk for the mother and that outcomes will be similar to those in the general population.

Methods Retrospective review study of women in the database of the Toronto HHT Centre between the ages of 18-55 years, no exclusion criteria. The women received a letter to inform them about the study, two weeks later they were called for a telephone questionnaire regarding pregnancy, delivery, epidural anesthesia and fetal outcomes.

Results Two-hundred-eighty-eight women were eligible, 7 were deceased though none of them due to a pregnancy-related complication. To date we have recruited and interviewed 100 women. Of these, 62 had previously been pregnant and reported 174 pregnancies, of which 40 were miscarriages (23%) and 134 live births (77%). Twenty-four women reported pregnancy-related complications. Twenty-four women had HHT-related complications during pregnancy. Complications during delivery occurred in 3/134 (2%), postpartum complications in 12/134 (9%) and an ICU admission in 4/134 (3%) pregnancies, 2 because of postpartum complications. Three (2%) of the live births were a result of conception post IVF. Thirty-three (25%) of the deliveries were by ceasarian section. Twenty (15%) of the children were preterm and 16 (12%) had a birth weight of less than 2500 gram. Screening for a spinal AVM before pregnancy was performed in only 2 women. Women received epidural anesthesia in 66 (49%) deliveries, 3/66 (3%) suffered from minor complications.

Conclusions Pre-term delivery appears to occur more frequently in women with HHT, and the incidence of low birth weight appears to be greater, compared to Canadian population statistics. Complications form epidural anesthesia are rare in women with HHT, even though most were not pre-screened for spinal AVMs

11. Validation of an oligonucleotide-array CGH platform for the detection of deletions and duplications in the Dystrophin (DMD) gene. -Results of pilot study.

D. del Gaudio, Y. Yang, H. Pham, CM. Eng. Dept. of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Objective: The dystrophinopathies are X-linked recessive neuromuscular disorders affecting approximately 1 in 3500 males. Duchenne muscular dystrophy (DMD) presents in early childhood with delayed motor milestones, abnormal gait, and learning and speech problems. DMD is rapidly progressive leading to death in early adolescence, while Becker muscular dystrophy (BMD) generally has a later-onset muscular weakness. Mutations in the dystrophin gene leading to DMD/BMD include deletions (65%), duplications (6-10%) and point mutations (25-30%). The *DMD* gene consists of 79 exons across a 2.4 Mb genomic segment at Xp21. Current diagnostic testing for *DMD* deletions and duplications include multiplex polymerase chain reaction, Southern hybridization, Multiplex Ligation-dependent Probe Amplification (MLPA), and other quantitative-PCR techniques. The above methodologies are technically challenging, labor-intensive and often preclude an accurate identification of small rearrangements, particularly in female heterozygotes. The aim of this pilot study was to design and validate a high resolution array-CGH platform for identifying deletions and duplications involving the *DMD* gene.

Methods: A high-density oligonucleotide-based array which includes 8700 oligonucleotides spaced ~200 bp across the *DMD* gene was designed for the study. Phase I of the study included 39 retrospective cases with known deletions and duplications identified in our clinical laboratory. Phase II included prospective testing of 33 samples by array-CGH and conventional methods in parallel. Additional testing of 8 samples negative for rearrangements/mutations was undertaken to provide an estimate of the sensitivity of this new platform.

Results: In 38/39 cases tested in phase I, the rearrangements were accurately identified. These included 24 multi-exon rearrangements and 14 single-exon rearrangements. In one case, a single-exon deletion was not identifiable due to inadequate probe coverage for the specific genomic segment. The results were concordant for 32/33 cases in phase II, with 10 abnormalities identified by both methodologies. One case with single-exon duplication was identifiable by array-CGH only.

Conclusions: This study describes the application of an oligonucleotide-based array-CGH platform for the detection of chromosomal abnormalities in the dystrophin gene. This high-throughput approach enabled the detection of various deletions and duplications in the *DMD* gene and most importantly, allowed identification of a single-exon duplication that Southern analysis failed to identify. Additionally, this platform provides precise breakpoint identification, with considerable impact on deriving accurate genotype-phenotype correlations at a molecular level. In conclusion, this technology displays great potential towards supplanting current methodologies for the clinical diagnosis of DMD.

12. Clinical and Molecular Features of Mitochondrial DNA depletion due to Mutations in Deoxyguanosine Kinase

D. Dimmock1, C. Dionisi-Vici2, J. Shieh3, Q. Zhang1, C. Truong1, E. Schmitt1, M. Sifry-Platt4, R. Carrozzo2, S. Lucioli2, C. Ficicioglu5, K Wierenga6, G. Enns7, E. Arch8, N.Longo9, M. Lipson4, H. Vallance10, F. Scaglia1 & L-J. Wong1

1 Baylor College of Medicine, Houston, TX; 2 Children's Hospital "Bambino Gesu", Rome, Italy;

3 J. David Gladstone Institute at UCSF, San Francisco, CA; 4 Kaiser Permanente, Sacramento, CA;

5 Children's Hospital Philadelphia, Philadelphia, PA; 6 University of Miami Miller School of Medicine, Miami, FL; 6 Stanford University School of Medicine, Stanford, CA; 7 Massachusetts General Hospital, Boston, MA; 8 University of Utah, Salt Lake City, UT; 10 University of British Columbia, Vancouver, B.C., Canada.

Background: Deoxyguanosine kinase (DGK) is a nuclear gene that along with thymidine kinase-2 salvages deoxyribonucleotides (dNTPs) for mtDNA synthesis. Deficiency of either of these causes a mitochondrial depletion syndrome.

Methods: We have undertaken a retrospective analysis of two centers' 9 Kindreds representing 16 mutations, 13 of which are unpublished. These are compared with previously published cases to establish genotype/phenotype relationships.

Results: A total of 16 mutations were identified in 9 unrelated kindreds (One previously described (c763_c766dupGATT) and two (N46S and c591G>A) subsequently published. When performed, electron transport chain enzyme activities and mtDNA content were considerably reduced in both muscle and liver when compared with controls.

All patients have disturbances in hepatic function. The timing of presentation with abnormal newborn screening, cholestasis, hypoglycemia or fulminant liver failure was variable. Initial laboratory investigations show a cholestatic picture with significant for elevations in ALT, AST and GGT. Elevations in AFP and ferritin were variable.

Consistent with other published cases neonatal hepatic disease in some cases has an overlapping phenotype with neonatal haemochromatosis. Five of the children with a neurohepatic phenotype were assessed for renal tubular dysfunction. It was found in two of these children suggesting that this is a variable feature, unrelated to prognosis. In 7 of the 9 families failure to thrive has been a significant feature.

Liver biopsy showed lipid accumulation, fibrosis, giant cells and increased or normal numbers of mitochondria on electron microscopy.

Conclusions: Mitochondrial depletion caused by mutations in DGUOK should be considered in children with hepatic dysfunction or cholestasis even without neurological findings Full gene sequencing is warranted if DGUOK deficiency is suspected.

13. Electrogastrography in Children with Angelman Syndrome

Benjamin I. Enav, Pediatric Gastroenterology, Hepatology and Nutrition,
University of California Los Angeles, Los Angeles, California

Objective: Gastrointestinal manifestations have been well recognized in children with Angelman Syndrome (AS). Feeding difficulties, gastroesophageal reflux, retching, and vomiting have led to surgical interventions including gastrostomy tube placement and anti-reflux procedures (i.e. Nissen Fundoplication) for children with AS. There is no data to understand the origin of these problems and establish whether surgical versus medical therapy is the ideal treatment for these patients. AS is a neurogenetic syndrome with physical, clinical and behavioral aspects attributed to localized central nervous system dysfunction. In this pilot study, our *central hypothesis* is that children with AS have an underlying electrophysiological abnormality causing a functional gastrointestinal disturbance. Our second hypothesis is that understanding the underlying cause of gastrointestinal disorders in children with AS will lead to more appropriate decision making with regards to surgical versus medical therapy.

Methods: A randomized prospective pilot study will be conducted to assess the health related quality of life of patients with AS, correlate reported gastrointestinal symptoms with electrogastrography (EGG) findings and determine optimal therapies for individual patients. A questionnaire will be sent to AS patients specifically addressing feeding difficulties, swallowing disorders, gastroesophageal reflux, and abnormal gastric emptying symptoms. An IRB-approved letter of invitation with all standard elements of an informed consent will be included with the questionnaire. Once the patient questionnaire is reviewed and informed consent obtained patients will be scheduled at the earliest convenience to undergo EGG at the University of California at Los Angeles. We intend to evaluate up to 15 patients in this pilot study and findings will be presented in a descriptive manner. If we are able to consistently demonstrate EGG abnormalities in this population, we will further plan to expand the study to include all AS children with or without gastrointestinal manifestations.

Results: IRB approval for patient recruitment granted June 2007.

Conclusions: Feeding difficulties, gastroesophageal reflux, retching, and vomiting have led to surgical interventions including gastrostomy tube placement and anti-reflux procedures for children with AS. However, there has been no significant investigation into the etiology of gastrointestinal disorders in children with AS. A pilot study to further investigate the functional upper gastrointestinal difficulties seen in AS using electrogastrography will lead to a better understanding of the etiology and more appropriate decision making in regards to surgical versus medical therapy.

14. Developing Evidence-Based Guidelines for a Rare Disorder

M.E. Faughnan, V. Palda, S.E. Straus
St. Michael's Hospital, University of Toronto, Toronto, Canada.

Objectives: To develop evidence-based recommendations for the diagnosis and management for Hereditary Hemorrhagic Telangiectasia (HHT). International experts have identified significant care gaps in diagnosis and management of HHT, a rare genetic disorder,.

Methods: An internationally representative sample of HHT experts developed key questions reflecting the important aspects of HHT care using a modified Delphi process. Systematic searches of the medical literature were conducted to identify studies addressing these key questions. Results from studies meeting inclusion criteria were extracted into evidence tables. Experts, as well as other health professionals and patients with the disorder, convened at a conference to partake in a structured consensus process using the evidence tables generated from the systematic searches. With the assistance of methodologic facilitators, small groups generated recommendations for the key questions previously identified. The small groups assembled afterwards to vote (anonymously) agreement for all generated recommendations. Those recommendations achieving less than 80% agreement were further discussed, again with a facilitator, and re-voted.

Results: Fifty key questions were developed. Literature searches identified 2694 abstracts, of which 171 articles were found suitable for full review. Six groups representing expertise in the areas of HHT diagnosis, epistaxis, central nervous system vascular malformations, pulmonary arteriovenous malformations, gastrointestinal bleeding and liver vascular malformations generated 31 recommendations. Twenty-one/31 (67%) recommendations received >=80% agreement on first vote. Ten recommendations were further discussed and re-voted, resulting in a final count of 34 evidence-based recommendations, with >=80% agreement in 31/34 (91%). Post-conference feedback from participants suggested a high level of satisfaction with the process. In conclusion, this evidence-based consensus recommendation process for a rare disorder allowed development of 31 recommendations which met with >=80 expert approval.

Conclusions: In a controversial field, experts noted that the process allowed them to identify areas of agreement on key clinical questions and recommendations. The process allowed room for expert opinion while maintaining adherence to clarity and evidence. Patient participation provided valuable input.

15. Identification of 3-Methylglutaconyl-CoA Hydratase Deficiency by Newborn Screening

Jeffery D. Rivera[1], James L Beebe[1], Daniel G. Wright[1], Cory W. Porter[1], Erica L. Savino[2], Renata C. Gallagher[2]; 1. Colorado Department of Public Health and Environment, Denver CO
2. Clinical Genetics and Metabolism, The Children's Hospital, Denver CO

Objective: To report the identification of a rare inborn error of metabolism by expanded newborn screening, and the significance of this for rare disease research

Methods: On July 1, 2006 the Colorado Department of Public Health and Environment began expanded newborn screening via tandem mass spectrometry (MS/MS) for all births in Colorado, Wyoming, and contract affiliates.

Results: As of February 28, 2006 51,095 newborns had been screened by (MS/MS). Of those, 23 specimens had elevated C5-hydroxyacylcarnitine (C5-OH), a marker for multiple inborn errors of metabolism. Several of these disorders are in the leucine catabolism pathway, including 3-methylcrotonyl-CoA carboxylase deficiency, the most common organic aciduria identified on newborn screening.. This is a defect in the fourth step of leucine catabolism, and is of unclear clinical significance as many individuals are apparently asymptomatic. An abnormality of the fifth catabolic step of leucine, 3-methylglutaconyl-CoA hydratase deficiency, has been described in only a few individuals. The clinical significance of this is also unclear, because of the paucity of clinical reports. We report a child with likely 3-methylglutaconyl-CoA hydratase deficiency identified through newborn screening.. The newborn blood spot C5-OH concentration was 1.39 µmol/L (cut-off: 0.7 umol/L). Follow-up testing included urine organic acids that showed increased 3-methylglutaconic acid, and moderate 3-hydroxyisovaleric and lactic acids. Quantitative plasma 3-methylglutaconic acid was 12,432 nmol/L (144 +/- 98 nmol/L). These findings are strongly suggestive of 3-methylglutaconyl-CoA hydratase deficiency, an autosomal recessive disorder. One of two siblings had similar biochemical results. The children have had normal growth and development to date.

Conclusions: The identification of these two individuals through expanded newborn screening highlights the challenges of the care of individuals with rare diseases and the need for rare disease research. The challenges here include that clinical symptoms are not well defined, there is no accepted treatment regimen (e.g. limitation of the amino acid leucine), and the natural history is unknown. As children with such disorders, including mild variants of classical disorders, are identified through newborn screening, it is crucial that the features are systematically monitored and reported, so that the symptoms are delineated and evidence based treatment regimens are developed.

16. Activation of the Estrogen Receptor Contributes to the Progression of Pulmonary Lymphangioleiomyomatosis via MMP-induced Cell Invasiveness

Marilyn K. Glassberg[1], Sharon J. Elliot[1], Jason Fritz[1], Mylene Potier[1], Roger Donahue[1], William Stetler-Stevenson[2], and Michael Karl [1]Laboratory for the Study of Sex and Gender Differences, Miller School of Medicine, University of Miami, Fl, [2] Cell & Cancer Biology Branch, Center for Cancer Research, NCI, National Institutes of Health, Bethesda MD.

Objective: The role of estrogens (ER) in the pathogenesis of lymphangioleiomyomatosis (LAM), an aggressive and destructive, eventually fatal lung disease of women is poorly understood. To investigate whether the lung disease in LAM is estrogen-mediated, we isolated and propagated spindle-shaped "LAM cells" (LAMD-SM) from affected lungs and characterized estrogen interactions with matrix metalloproteinase (MMP)-2.

Methods: Cells were isolated and propagated from lung tissue removed from either LAM patients or age-matched women undergoing lung resection for lung carcinoma (control, n=7). In all experiments cells were exposed to either vehicle, 17β-estradiol (E_2, 1-10nM), or ICI 182,780 (10^{-6}M) or a combination. Reverse transcriptase PCR and western analysis were performed for ERα, ERβ**,** MMP-2), tissue inhibitor of metalloproteinases (TIMP)-2 and SRC-1. In some experiments, cells were treated with the proteasome inhibitor (MG132,1 µM) for 6h. Cell supernatants were collected and MMP-2 activity was measured by zymography. For transfection studies, cells were transfected with either a 4ERE-TATA-Luc or the human MMP-2-promoter-luciferase reporter gene construct and the β-galactosidase gene pRSV-βgal (0.4ug/well) to control for transfection efficacy. Cell invasion chambers were used to study E_2-mediated invasion. In some experiments an antisera to MMP-2 (5, 50, 500) or doxycycline 10mg/ml was added at the same time as E_2. A nonspecific rabbit IgG was used as control.

Results: LAMD-SM cells express functional ER which undergo rapid intracellular turnover in their unbound state. E_2 enhances the transcriptional ER activity and, likely by temporary stabilizing the activated ER complex, increases the responsiveness of LAMD-SM to estrogens. E_2-induced ER activation increases synthesis and activity of MMP-2 through post-transcriptional mechanisms in LAMD-SM. This process also involves upregulation of TIMP-2 expression. The E_2/ER-mediated increase of MMP-2 and TIMP-2 activity promotes LAMD-SM invasiveness, in assays *in vitro*, which were inhibited by specific antibodies against MMP-2 or doxycycline, an inhibitor of MMPs.

Conclusions: The invasion and destruction of lung parenchyma in LAM is, at least partially, an estrogen-MMP-driven process, which has major implications for therapeutic interventions. A two year pilot study of treatment with Doxycycline has been completed recently.

17. Preliminary experience with MRS at 3T in urea cycle disorders detects altered metabolism in partial ornithine transcarbamylase deficiency

Andrea Gropman 1,2, Ayichew Hailu2, Rebecca Seltzer2, Stanley Fricke2, John van Meter3 , Mark Batshaw4,5, Mendel Tuchman4,5, and the Urea Cycle Rare Disorders Consortium; Departments of Neurology 1 and Pediatrics 4 , Children's National Medical Center, Departments of 2 Neuroscience and 3 Neurology, Georgetown University, 5 Children's Research Institute, Washington, D.C.

Objective: To utilize ^{1}H MRS at 3T to assess the extent of biochemical variations in glutamine, myoinositol and NAA in symptomatic and asymptomatic female subjects heterozygous for ornithine transcarbamylase deficiency (OTCD) and hemizygous males with late onset disease.

Methods: Single voxel ^{1}H MRS was performed on 10 subjects (8 females, 2 males) and 10 controls (8 females, 2 males) using PRESS sequence at TE 30 msec. Data was corrected for partial volume and analyzed with LCModel, a user independent linear combination quantification software to yield absolute metabolite concentrations. All subjects also underwent IQ testing, dietary history analysis, and subjects had blood drawn for ammonia and plasma amino acids.

Results: All subjects were imaged in stable medical state. IQ scores were similar in subjects and controls. Two females with more severe disease manifest IQ in the low average range. Dietary protein intake ranged from 0.6 mg/kg to in subjects and 1.4 mg/kg in controls. Several biochemical changes identify subjects versus controls. Glutamine elevations were observed in thalamus of all subject compared to controls ($p<0.05$). Myoinositol depletion was evident in subjects versus controls in the parietal white matter ($p <0.05$)

Conclusions: Impaired metabolism as seen by ^{1}H MRS was common in subjects with partial OTCD, even those who are asymptomatic. The findings are important in that they confirm previous studies, but moreover, demonstrate abnormal metabolism in asymptomatic females which may provide an explanation of previous neurocognitive testing demonstrating impaired domains in females with OTC versus controls. This study also demonstrates the ability to use ^{1}H MRS to assess biomarkers that differentiate patients from controls which may be used to non invasively assess disease course, response to therapy and mechanisms of neural injury.

18. Liver Transplantation in Severe Hepatopulmonary Syndrome: Two-Center Experience

S Gupta[1], H. Castel[2], R. Rao[1], L. Lilly[3], G. Pomier-Layrargues[2], M.E. Faughnan[1]

[1]St. Michael's Hospital, University of Toronto, Toronto, Canada

[2]CHUM, Université de Montréal, Montreal, Canada

[3]University Health Network, University of Toronto, Toronto, Canada.

Objective: Hepatopulmonary syndrome (HPS) is defined by presence of portal hypertension, intrapulmonary vascular dilatation and hypoxemia (alveolar-arterial gradient ($AaDO_2$) > 20 mm Hg). HPS is a rare disease, with reported prevalence of 16% among end-stage cirrhotic subjects awaiting transplantation. The natural history is uniformly dismal, with progressive worsening in hypoxemia and mean survival of 2.5 years after diagnosis. Liver transplantation (LT) is the only effective treatment for HPS, but previous reports have indicated that severe HPS, with pre-transplant PaO_2<60mmHg carries a very high peri-operative mortality. We sought to review our experience in liver transplantation for severe HPS.

Methods: Nine severe HPS patients (ages 29-62 yrs, 5 male) received LT at the University Health Network (UHN) (University of Toronto) and Hôpital St-Luc (Université de Montréal) (10/2004 – 05/2007). A retrospective chart review was performed at both institutions.

Results: Two patients had living-related LT, seven patients had deceased-donor LT. All nine patients survived transplantation and are currently alive, with follow-up ranging from 91-1007 d (mean 444 d) post-transplant. All patients had post-transplant improvements in PaO_2, Seven/9 are now off oxygen, and two patients are currently being weaned off oxygen (post-op day 166, 91, respectively). Duration of mechanical ventilation ranged from 1-60 days, with two patients transiently requiring tracheostomy. Duration of ICU stay ranged from 1-62 days and duration of hospitalization from 22-90 days. Unique post-operative complications included anastamotic bile leak with *Candida albicans* peritonitis and sepsis, acute oliguric renal failure requiring dialysis, severe delirium, severe post-operative hypoxemia (requiring high-frequency oscillatory ventilation, Trendelenburg positioning, and inhaled NO), urosepsis, herpes simplex virus pneumonia, ARDS, and cholangitis.

Conclusions: LT in severe HPS is associated with a high risk of severe post-operative complications, but we have shown an excellent survival rate in this small series, contrary to current evidence in the literature. Also, living-related LT appears to be as effective as deceased-donor LT for severe HPS. Patients with HPS, even when severe, have an excellent prognosis with LT.

19. Association of BDNF Haploinsufficiency with Childhood Overweight in WAGR Syndrome

Joan C. Han[1], Carolyn M. Menzie[1], Rebecca L. Levinn[1], Diane C. Adler-Wailes[1], Ethan L. Sanford[1], MaryPat Jones[2], Felicitas L. Lacbawan[2], Owen M. Rennert[1], Jack A. Yanovski[1]

National Institute of Child Health and Human Development[1] and National Genome Research Institute[2]

Objective: Animal studies suggest that BDNF is important in energy homeostasis. Mice with heterozygous *BDNF* deletions exhibit hyperphagia and obesity, but little is known about the role of *BDNF* in human energy balance. WAGR Syndrome is caused by heterozygous contiguous gene deletions of variable size centered at 11p13, 4 Mb centromeric to the *BDNF* locus (11p14.1). Hyperphagia and severe childhood-onset overweight are reported in a subset of WAGR patients. The objective of this study was to investigate whether obesity in WAGR syndrome is associated with *BDNF* haploinsufficiency.

Methods: 28 subjects with WAGR Syndrome (age 11.8±7.4y) were recruited through the International WAGR Syndrome Association. Comparative genomic hybridization using a custom-designed oligonucleotide microarray, with an average spatial resolution of 400 bp spanning 11p and 35 kb genome-wide, was performed to determine location and extent of each subject's deletion. In subjects for whom both parents' DNA was available, genotyping was confirmed by analysis of 30 microsatellite markers spanning 11p12-14. Fasting serum BDNF concentration was measured by ELISA (sensitivity 15.6 pg/mL).

Results: 61% had heterozygous deletion of all or a portion of the *BDNF* gene (*BDNF*+/-). *BDNF*+/- had significantly higher BMI SD-scores at age 5y (1.96±1.10 vs. 0.11±1.82, p=0.008) and 10y (2.18±0.33 vs. 0.87±1.20, p=0.043) than those without *BDNF* deletion (*BDNF*+/+). These differences remained statistically significant after adjusting for parental BMI (5y: 1.98±1.11 vs. 0.08±1.11, p=0.002; 10y: 2.13±0.69 vs. 0.96±0.69, p=0.007). Only 1 *BDNF*+/+ subject was overweight by age 10y. Non-overweight subjects were found with deletions ending 74 kB centromeric of *BDNF*. All *BDNF*+/- subjects became overweight by age 10y (p<0.0001 vs. BDNF+/+). Consistent with their *BDNF* haploinsufficient genotype, *BDNF*+/- had serum BDNF concentrations that were reduced by 50% compared to *BDNF*+/+ (13.9±5.4 vs. 29.3±14.5 ng/mL, p=0.006). This difference remained statistically significant after adjusting for age, sex, and platelet count (15.3±8.3 vs. 27.1±8.2 ng/mL, p=0.002).

Conclusions: *BDNF* haploinsufficiency is associated with lower serum BDNF and higher BMI SD-score in subjects with WAGR Syndrome. These data provide strong evidence that BDNF plays an important role in human energy homeostasis.

20. Angelman syndrome mouse model with a large chromosomal deletion from *Ube3a* to *Gabrb3*

Yong-hui Jiang[1], Yan-Zhen Pan[1], Luis Landa[1], Corrine Spenncer[1], John Gardner[2], Murray Brillant[2] & Arthur L Beaudet[1].

[1]Department of Molecular and Human Genetics, Baylor College of Medicine. Houston. TX. [2]Department of Pediatrics, University of Arizona Health Science Center. Tucson, AZ 85724

Objective: Angelman syndrome (AS) is a neurobehavioral disorder associated with severe mental retardation, absence of language development, characteristic EEG abnormalities and epilepsy, happy disposition, and movement or balance disorders. The molecular defects underlying AS are heterogeneous including large chromosomal deletions of 15q11-q13 of exclusively maternal origin (70%), paternal uniparental disomy (UPD) of chromosome 15, imprinting mutations, and mutations in the E6-AP ubiquitin ligase gene (15%) (*UBE3A*). Previously, we have characterized a AS mouse model by inactivation of Ube3a gene in mice.

Methods: Using chromosomal engineering strategy by the cre-loxP and Hprt technique, we have generated mutant mice with a deletion from *Ube3a* to *Gabrb3* which inactivates both of these genes as well as *Atp10a* as is the case for the majority of human AS patients (70%) with a large chromosomal deletion of 15q11-q13.

Results: Homozygous mutant mice with this deletion died in the perinatal period largely due to the cleft palate resulting from null mutation of *Gabrb3* as previously reported. Mice with a maternal deletion are viable and have no apparent developmental defect. There is no significant difference for the expression *Atp10a* gene is sub-regions of brain between the maternal and paternal deletion which suggest that the *Atp10a* gene is biallelicly expressed in these regions. We have conducted a comprehensive behavioral analysis on mice with maternal deletion as well as paternal deletion. The behavioral analysis revealed significant impairments in motor function, spatial and fear conditioning learning and memory, prepulse inhibition, open-field, and light dark testing in maternal deletion mice. The hippocampal long-term potentiation is also impaired in maternal deletion mice. In addition, we have recorded ultrasonic calls emitted from pups with a maternal deletion. Interestingly, this analysis revealed a significant difference of ultrasonic calls between pups with a maternal deletion and wild type.

Conclusions: Mice with a deletion from *Ube3a* to *Gabrb3* provide another valuable mouse model in exploring the potentials of treatment strategy or making comparison to that of *Ube3a* maternal deficiency mice to dissect the molecular pathogenesis of Angelman syndrome.

21. Renal Graft Survival in Wegener's Granulomatosis (WG): Comparison to Systemic Lupus Erythematosus (SLE) from a National Database

Curry L. Koening, Carol A. Langford, Lester Kirchner, Gary S. Hoffman
Cleveland Clinic and Case Western Reserve University, Cleveland, OH

Purpose: Despite treatment with immunosuppressive drugs, up to 20% of patients with WG develop end stage renal disease (ESRD). For patients with ESRD, kidney transplantation is an important medical advance. Although case reports and small cohort studies have reported excellent graft survival rates in WG, it is not known how these results compare to other patient populations who undergo renal transplant. This study used a national renal transplant database to examine graft survival in patients transplanted for WG compared to patients transplanted for SLE.

Methods: Data for this study was extracted from the Organ Procurement and Transplantation Network's (OPTN) national transplant database which contains information regarding every organ donation and transplant event in the United States since 1987. Patients included in this study had renal transplantation for either WG or SLE between October, 1987 and February, 2006. Only 1st episode transplantations were analyzed. Log-rank tests, Kaplan-Meier survival curves and Cox regression models were used to compare graft survival times of WG to SLE.

Results: A total of 982 patients received renal transplantation for WG and 6,574 patients for SLE. 524 (53%) WG patients received a cadaveric donor allograft compared to 4,032 (61%) (P<0.0001) SLE patients. 142 (15%) grafts failed in patients transplanted for WG compared to 1,888 (29%) (P<0.0001) failed grafts in SLE. Recurrent disease was reported as the cause for graft failure in 6(4.3%) WG and 90(4.8%) SLE patients. The 6 month, 1 year, and 5 year graft survival rates for WG were 95.6%(95% CI 94.1-96.7), 95.2%(93.6-96.4), and 90.0%(87.9-91.7) respectively. Similarly, for SLE patients the graft survival rates were 92.7%(95% CI 92.1-93.3), 90.7%(90.0-91.4), and 79.7%(78.7-80.7) respectively. Patients transplanted for SLE had an unadjusted risk of graft failure of 2.16 (P<0.0001 95% CI 1.82- 2.56) compared to those transplanted for WG. After adjusting for gender, race, UNOS region, allograft type, and age as a time dependent variable, patients transplanted for SLE had a 71% (P<0.0001 95% CI 43%- 104%) increased risk of renal graft failure compared to patients with WG.

Conclusions: Patients who received renal transplantation for SLE had a higher risk of graft failure compared to patients transplanted for WG. The number of patients reported to have recurrent disease as a cause for their graft failure was small in both WG and SLE.

22. Wilson disease in Vietnamese children.

Hoang Le Phuc, Department of Gastroenterology, Children's Hospital # 1, Ho Chi Minh City, Vietnam

Objective: to identify the clinical, biochemical, ATP7B gene mutation features of children with Wilson disease (WD) in Vietnam.

Methods: Thirty-three children with confirmed WD admitted to Children's Hospital #1 from 2000 to 2006 were evaluated retrospectively for the clinical features at presentation and laboratory findings. Thirty-five blood samples from 8 families of the index cases were analyzed by direct sequencing of exons 4-21 of ATP7B gene at University Clinic for Internal Medicine IV, Vienna, Austria.

Results: All of the patients had the systemic WD score at least 4. The male: female ratio is 2:1 (22: 11). The mean age was 11.24+/-2.6 years; the youngest was 13 month old. Two cases were ethnic minors. The fulminant (H1), non-fulminant hepatic (H2), neurohepatic (N1) and neurological (NX) manifestations were 15.2%, 51.5%, 27.3% and 6.1%, respectively. The Kayer Fleischer ring was found in 100% patients with the neurological signs and symptoms but only 75.6% for the whole group. The most common biochemical abnormalities were decreased serum ceruloplasmin (100%), increased urinary copper (93.9%), increased prothrombin time (92%; 22 of 25 measured cases), increased liver enzymes (51.5%). The alkaline phosphatase (UI/L) : bilirubin (mg%) ratio is less than 2 in all five cases of fulminant WD (mean 1.04). Five identified disease-causing mutations are P767P-fs, R832K, T850I, E1173K and Q1372X. In addition the polymorphic variant A1140Vwas found. With identical mutations one sibling of the index case was diagnosed at 8 month old and being copper overloaded by 13 month old.

Conclusions: in this first report from Vietnam, pediatric WD manifestations were varied and challenging. The identified mutations are similar to China, Taiwan and European studies. Molecular diagnosis of WD is very useful in family screening of the index case. The 24h urinary copper should be tested at early age as 12 months in the genetic confirmed WD patient for early prevention of copper overload.

23. cyclic-di-GMP regulation of Pseudomonas aeruginosa virulence

Vincent Lee[1,2], Hemantha Kulasekara[1], Mauricia Matewish[1], Anja Brencic[1], and Steve Lory[1]
[1]Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115
[2]Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742

Objective: *Pseudomonas aeruginosa* is an opportunistic pathogen that can cause both acute and chronic infection utilizing distinct sets of virulence factors. Acute infection requires both activation of the host inflammatory response as well as neutralization of the host defenses by secreted toxins and type III secretion delivered effector molecules. In addition to transcriptional regulation of these factors, a novel secondary messanger molecule cylcic-di-GMP can modulate the activity of several of these virulence factors in a post-translational manner. We sought to determine the contribution of each enzyme that synthesis and degrade cyclic-di-GMP regulate the physiology of the bacteria. Furthermore, we sought to understand the mechanism of signal transduction pathway of this novel secondary signaling molecule.

Methods: The *P. aeruginosa* genome contains 40 genes that encode diguanylate cyclase and phosphodiesterase that regulate the production and degradation of c-di-GMP. Phenotypic assays were performed to understand the processes regulated by this small signaling molecule. The enzymatic activity of each protein in bacteria was measured by acid extraction followed by detection by high performance liquid chromatography. Genes containing putative cyclic-di-GMP binding motif was identified by bioinformatics and verified by direct binding to radiolabeled cyclic-di-GMP.

Results: We have systematically expressed each of these genes and foundt that only a subset of these enzymes has detectable cyclic-di-GMP synthesis or degradation activity. Phenotypically, overproduction of cyclic-di-GMP results in up-regulation of alginate and PEL polysaccharide synthesis which can lead to biofilm formation. To understand this regulation, we have now identified two cyclic-diGMP binding regulators, Alg44 and PelD, that specifically regulate production of alginate and PEL, respectively, in response to cyclic-di-GMP production.

Conclusions: *P. aeruginosa* appears to utilize a large number of gene products to detect the environment and regulate the level of cyclic-di-GMP. This secondary signaling molecule has a wide range of action on a number of cell-surface virulence factors. Our studies have begun to understand the regulatory process of the cyclic-di-GMP signaling pathways. Further studies will aim to understand the reciprocal regulation of acute and chronic virulence factors that appears to be programmed into all bacterial species

24. Working Towards Improved Outcome for Pediatric Localized Scleroderma (LS)

SC Li [1,2], MS Liebling[2], KA Haines[1,2], T Arkachaisri[3], AS Doria[6], F Ramji [4], I Foeldvari[7], M Punaro[5], A Mohanta[6], S Zhang[5], R Michalik[8], E Pope[6], KM O'Neil[4]
[1]Joseph M. Sanzari Children's Hospital, NJ; [2]Hackensack University Medical Center, NJ; [3]University Pittsburgh, PA; [4]University Oklahoma, OK; [5]Texas Scottish Rite Hospital, TX; [6]Hospital for Sick Children, University of Toronto, Canada; [7]Hamburger Zentrum Fur Kinder- und Jugendrheumatologie, am Klinikum Eibek, Germany; [8] Klinikum Eilbek, Hamburg, Germany

Objective: To work towards the development of a randomized clinical trial (RCT) for treating pediatric LS.

Background/Methods: Pediatric LS is associated with significant morbidity including limb growth disturbance, joint contractures, and brain involvement. There are no validated disease activity or outcome measures. This has made it difficult to develop RCT, and has hampered research into disease pathogenesis. We have found ultrasound (USG) detects several disease-related abnormalities (Rheumatology 2007 46: 1316-1319; doi:10.1093/rheumatology/kem120) and are working with other radiologists/sonographers and clinicians to develop outcome measures.

This is a CARRA (Childhood Arthritis and Rheumatology Research Alliance) approved project that involves several North American sites and one European site. Pediatric rheumatologists (PR), radiologists, and sonographers have come together at two meetings to review our USG data, learn our technique, and develop scoring measures. The radiologists/sonographers (using USG) and clinicians separately examined patient volunteers to aid in standardizing assessment. We surveyed PR to determine their current treatment practices for LS, including medications and doses used, and how LS subtype, lesion location, other organ involvement, and other features influence treatment. PR were asked to identify and rate features indicative of disease activity and severity to aid in developing clinical measures. At present, over 40% of CARRA members have responded.

Results: Two preliminary clinical measures (disease activity, disease damage) have been developed. The survey shows concordance for several features indicative of disease activity and damage, but much variation in treatment regimen and duration. An USG scoring measure has been developed that separately assesses dermis, hypodermis, and muscle for changes in thickness, echogenicity, and vascularity. The USG measure is being tested for intra- and inter-observer reliability on our existing patient scans (30+). Initial findings suggest that we may be able to generate separate USG activity and damage measures. A prospective study to validate the USG and clinical measures is being planned. Our collaborative group has over 300 patients.

Conclusion: We have developed preliminary clinical and USG outcome tools for pediatric LS. Once these are validated, they will be used to set up a RCT to evaluate the effectiveness of the most commonly used treatments, and possibly experimental therapies. Additional studies are planned to identify associated biological markers.

25. Alpha 1-Antitrypsin Deficiency and the Risk for Wegener's Granulomatosis

AD Mahr[1], JC Edberg[2], JH Stone[3], GS Hoffman[4], EW St. Clair[5], U Specks[6], PF Dellaripa[7], RF Spiera[8], FN Rouhani[9], ML Brantly[9], PA Merkel[1] for the WGGER Research Group.

[1]Boston University School of Medicine, Boston, MA; [2]University of Alabama at Birmingham, Birmingham, AL; [3]Johns Hopkins University, Baltimore, MD; [4]Cleveland Clinic, Cleveland, OH; [5]Duke University, Durham, NC; [6]Mayo Clinic, Rochester, MN; [7]Lahey Clinic, Burlington, MA; [8]Hospital for Special Surgery, New York, NY; [9]University of Florida, Gainesville, FL.

Background: Alpha 1-antitrypsin (AAT) deficiency, a genetic disease that predisposes to chronic obstructive pulmonary disease and liver cirrhosis, may also be a determinant of susceptibility to Wegener's granulomatosis (WG). Several small studies reported that 7–27% of patients with WG were carriers of the AAT-deficiency Z allele. It is unclear if the S allele, i.e. the other major AAT-deficiency variant, also contributes to risk for WG and whether carriage of AAT-deficiency alleles impacts on clinical phenotype in WG.

Methods: We studied the distribution of the AAT deficiency alleles Z and S in 395 unrelated Caucasians with WG and 423 ethnically matched controls without disease. Genotyping was performed by ABI TaqMan allelic discrimination and the results were compared between cases and controls using exact χ^2 tests. AAT-deficiency allele carriers and non-carriers were compared for clinical and laboratory features of WG.

Results: Among the patients with WG, the allele carriage frequencies of Z and S were 8.1% and 11.4%, respectively. The odds ratios (OR) of WG associated with allele carriage was 1.98 (95% CI, 1.06–3.82) for Z, 1.57 (95% CI, 0.95–2.62) for S, and 1.75 (95% CI, 1.17–2.63) for Z and/or S. Compared to the normal MM genotype, the OR for MZ or MS genotypes was 1.51 (95% CI, 1.00–2.31), and the OR for ZZ, SS or SZ genotypes was 16.16 (95% CI, 2.58–∞). Among the patients with WG, carriage of Z and/or S allele was associated with a lower frequency of lung nodules ($P = 0.03$); no other between-group differences were detected.

Conclusion: This large study provides strong evidence for the association between AAT deficiency and WG. The substantially higher risk in homozygous (ZZ, SS) or compound heterozygous (SZ) individuals supports a gene-dose effect and corroborates that the relationship between AAT deficiency and WG may be causal rather than due to linkage disequilibrium.

26. How Should Nasal Nitric Oxide Be Measured To Screen for PCD In Children?

Dimas Mateos-Corral, Robin Coombs, Hartmut Grasemann, Felix Ratjen, Sharon Dell

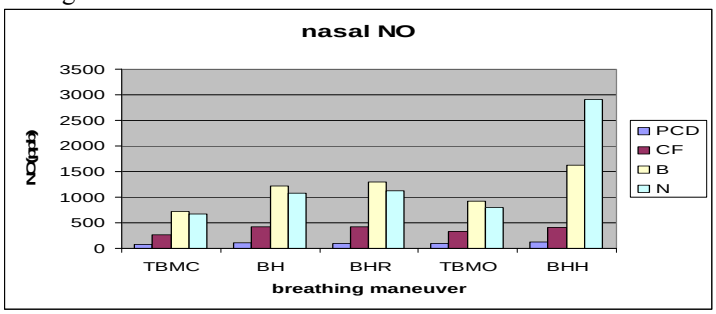
Division of Respiratory Medicine, The Hospital for Sick Children, Toronto, Canada

Objective: Nasal nitric oxide (NNO) is a reliable non-invasive screening test for Primary Ciliary Dyskinesia (PCD) in school age children and adults although the measurement technique is not standardized. The objectives of this project are: 1. to determine which breathing technique has the best ability to discriminate between PCD, normal controls and disease controls and 2. To determine if tidal breathing technique, which would be directly applicable to infants, has discriminative utility in children with PCD.

Methods: 120 children aged 5 to 18 years from four different groups (n=30 in each group) are being recruited: PCD, Cystic Fibrosis (CF), non-PCD bronchiectasis and normal controls. Using Echo physics chemiluminescence NO analyzer, 5 breathing techniques are being tested: Breath hold, exhaled resistance, tidal breathing mouth open & mouth closed and humming. The tests are repeated on a second visit within two months.

Results: To date, 79 subjects have been recruited. All 5 breathing techniques yielded statistically different NNO output in the PCD group versus other groups. No NNO peak was noted during humming technique in PCD and CF groups.

Conclusions: Sensitivity and specificity cut off values will be developed once the full sample size is recruited. Tidal breathing techniques are promising as a screening test for PCD in infants.



27. Gene mutations and clinical outcome after biliary diversion surgery for intractable pruritus in children with intrahepatic cholestasis

Alexander Miethke, Ursula Matte, Cong Liu, Frederick Ryckman, William Balistreri, Jorge Bezerra
Cincinnati Children's Hospital Medical Center

Objective: The post-operative outcome of biliary diversion (BD) surgery for refractory pruritus in children with cholestasis is variable and not reliably predicted by clinical measures. We hypothesized that mutations in the *ATP8B1, ABCB11, ABCB4,* and *JAG1* genes segregate with clinical response to BD.

Methods: All exons and intron boundaries for the 4 genes were sequenced using the JaundiceChip in 14 children after BD. The types of mutations were compared to post-operative changes in pruritus and serum bile acid (SBA) levels (follow-up period: 21–44 months).

Results: Disease-causing mutations were found in 12 of 14 children. Six of them had low serum GGT, of which 5 were found to have disease-causing mutations in *ATP8B1* or *ABCB11*. Pruritus improved in 3 of these 5 children; 2 of 3 also had reduced SBA levels following BD. These children had missense mutations or in-frame deletions in *ATP8B1*, or a splice-site mutation in *ABCB11*, none of them predicted to prematurely terminate transcription. In contrast, the 2 children without improvement had nonsense mutations in *ATP8B1* and *ABCB11*, both predicted to generate truncated proteins. Of the 8 subjects with high GGT, 7 had clinical and histological features of Alagille syndrome; 4 showed improvement in pruritus and 5 had reduced SBA levels after BD. Disease-causing heterozygous mutations in *JAG1* were detected in all 7 subjects, but the type of mutations did not segregate with different post-surgical outcome groups. All 3 subjects with poor outcome also had nonsynonymous mutations in *ATP8B1* or *ABCB11*.

Conclusions: 1) Nonsense mutations in *ATP8B1* or *ABCB11* were present in children with low GGT and poor response to BD, and 2) the *JAG1* mutations in children with Alagille syndrome did not segregate with types of clinical response, but children with poor outcome harbored coexisting nonsynonymous mutations in *ATP8B1* or *ABCB11*. Validation of these findings in a larger cohort will determine whether surgical treatment for intractable pruritus can be tailored to the patient's genetic makeup.

28. Brain size and cerebellar volume in individuals with PWS – what factors may influence brain development and IQ

Jennifer L Miller, Krista Schwenk, Michelle Long, Stephen Towler, Daniel J Driscoll, Christiana Leonard
University of Florida College of Medicine and College of Education

Objective: Both total brain volume and the cerebellum have been found to correlate with cognition. In a previous study we noted that individuals with Prader-Willi syndrome (PWS) and those with early-onset morbid obesity (EMO) of unknown etiology had lower cognitive function that their normal weight siblings. Therefore, we hypothesized that individuals with PWS and EMO would have smaller brain volume and cerebellar volume than normal weight controls.

Methods: We measured total brain volume and total cerebellar volume using three-dimensional MRI images in 18 individuals with PWS (8 females/10 males), 19 individuals with EMO (13 females/6 males), and 24 normal weight control siblings from both groups (14 females/10 males), all of whom were ages 4-30 years. These measurements were obtained by two independent raters who were blinded to age, gender, and diagnosis of the subjects.

Results: We found that the total brain volumes were similar in all groups ($p=0.4$), but the cerebellum ($p=0.004$) and the cerebellar/cerebral volume ratio was smaller in both individuals with EMO and PWS compared to controls ($p=0.03$). There was no difference in cerebellum/cerebral volume ratio between individuals with PWS and individuals with EMO, or between the two major sub-types of PWS (deletion vs. maternal uniparental disomy). General intellectual ability in these individuals was: PWS 65 ± 15; EMO 81 ± 12; and Controls 112 ± 7.

Conclusions: Decreased cerebellar/cerebral volume ratio in both individuals with EMO and PWS, along with the findings of decreased cognitive scores in both of these groups, compared to controls further suggests that the cerebellum plays a role in overall cognitive functioning. Additionally, since the only commonality between the individuals with PWS and those with EMO is the development of obesity early in life, our findings suggest that early-onset childhood obesity may result in decrease of cerebellar volume with concommitent decrease in cognitive function.

29. Impact of the Orphan Drug Act on Drug Development

Jun Mitsumoto, MPH; E. Ray Dorsey, MD, MBA; Joel Thompson, MPH; Robert C. Griggs, MD
Department of Neurology, University of Rochester Medical Center, Rochester, New York

Objective: To evaluate the impact of the Orphan Drug Act of 1983 on drug development from 1996 to 2006.

Methods: We used publicly available data from the U.S. Food and Drug Administration (FDA) to review all FDA designations and market approvals for orphan indicated products from 1996 to 2006. We evaluated the total number of approvals for orphan indicated products and compared them to approvals for all products. We searched the two major approval classifications: original new drug approvals, defined as new drug applications and biological license applications, and supplements to new drug applications and biological license applications, defined as changes requested to labeling, formulation, indication, patient population or other product modifications. New molecular and biological entity approvals, a subset of original new drug approvals, were defined as substances with no previously FDA approved chemical or biological equivalents.

Results: From 1996 to 2006, the average number of FDA orphan drug approvals per year was 16 (range 6 to 25). Over the last decade, drugs with orphan indications accounted for 0.7% of all original new drug approvals and supplemental applications combined, 11% of original new drug approvals alone, and 24% of all new molecular and biological entity approvals. Over the same period, the number of FDA orphan drug designations per year increased 143%.

Conclusions: Orphan drugs have played a substantial role in drug development over the past decade and account for nearly a quarter of all new molecular and biological entity approvals. Growth in the number of annual orphan drug designations highlights the influence the Orphan Drug Act continues to have on drug development.

30. Multicomponent Micro-Patterned Surfaces for Control of *ex vivo* human T-cell Activation: A Novel Tool for the Study of T-cell Abnormalities in Autoimmune Disease

Marc D. Natter[1,2], Darrell J. Irvine[1].
[1]Dept. of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA; [2]Dept. of Pediatric Rheumatology, Tufts-New England Medical Center, Boston, MA.

Objective: Proximal signaling pathways of human T-cells, aberrancies of which occur in autoimmune disease such as Systemic Lupus Erythematosus (SLE), are now known to function in the context of an Immunologic Synapse formed between T-cells and antigen presenting cells (APCs). Artificial Immunologic Synapse arrays for the study of activation behavior of murine monoclonal CD4+ T-cell blasts have been produced using a novel system developed in our laboratory, wherein T cells are seeded onto patterned surfaces that mimic the presentation of ligands by APCs. Human CD4+ Peripheral Blood Mononuclear Cells are smaller (~6μm vs. ~10μm) and less motile than murine T-cell blasts; additionally, quantification of activating stimulus strength is especially relevant to study of polyclonal populations. We therefore sought to develop techniques for producing Immunologic Synapse-like arrays with activating and adhesion ligand regions, specifically suited to the study of the activation behavior of human T-cells from normal controls and subjects with autoimmune disease such as SLE.

Methods: Strategies for photoresist processing and fluorescence labeling and attachment of signaling and adhesion ligands were optimized for human CD4+ PBMCs and baseline activation parameters obtained. Following isolation and incubation, cells were loaded with Fura-2 AM, a calcium-indicator dye. Morphologic changes and calcium fluxes were observed under time lapse utilizing 4-D epifluorescence microscopy and MetaMorph software for image acquisition/processing.

Results: Feature characteristics were repeatably obtained to diameters of 2 to 3μm. Fluorophore-labeled streptavidin was found to provide poorly reliable measurements of biotinylated ligand attachment at low ligand densities. Serial labeling of mouse anti-human anti-CD3 and ICAM-1-human-Fc fusion protein ligands with Alexa Fluor® 488 or 647 and then a long-linker biotin resulted in sensitive and specific measurements of ligand attachment. Control human CD4+ PBMCs showed morphologic changes of activation and calcium fluxing centered on anti-CD3 spots surrounded by an ICAM-1 background.

Conclusions: Artificial Immunologic Synapse-like arrays appear are a valid *ex-vivo* tool for qualitative and quantitative measurement of human CD4+ T-cell activation in normal controls. T-cell activation in subjects with SLE is ongoing. This model system holds promise as a platform for unique clinical assays in autoimmune disease states and in high-throughput methods for therapeutic drug screening.

31. Long Term Follow up in Apparent Mineralocorticoid Excess Patients

Saroj Nimkarn, Frances M Guevarra, Robert C.Wilson and Maria I.New
Mount Sinai School of Medicine, New York, New York 10029

Objective: Apparent mineralocorticoid excess (AME) is a rare inherited form of hypertension caused by 11ß-hydroxysteroid dehydrogenase type 2 deficiency. AME treatment primarily aims to correct hypokalemia and hypertension. End organ damage (EOD) is evident in AME patients, even at a young age. Our previous short term follow up (F/U) report (2-13 years period) in 6 patients showed a decrease in EOD for most patients who received treatment. This study aims to report a long term follow up of our cohort of AME patients.

Methods: We obtained data retrospectively from chart reviews. Consents were obtained in all patients.

Results: Nineteen AME patients (12M:7F) were identified by HSD11B2 mutations with age range of 0.1-14 years. Mutations located in exon 1, 3, 4 and 5 of HSD11B2 gene were found in homozygous fashion in 8 and compound heterozygotes in 2. F/U data is available in 10 patients (7M:3F), ranging from 3.6-27 years period. Two out of 19 patients died from unclear causes at 16 and 17 years old. Left ventricular hypertrophy (5/7) occurred more frequently than hypertensive retinopathy (2/7) at diagnosis. Despite persistent elevation of blood pressure in majority of the patients (8/10) at follow up, left ventricular hypertrophy and retinopathy improved in most patients. Although height of the patients improved from the time of diagnosis to the time of follow up, the patients were all below their target height (-1.1 ± 0.6 SDS). Nephrocalcinosis resolved in 8/10 patients at 4.1 to 14.5 years follow up. Two of the 10 patients developed renal insufficiency $\geq$10 years after diagnosis, despite normocalciuria and nephrocalcinosis resolution. One female had a stillbirth child and subsequent difficulty conceiving, and her affected younger brother was found to have azoospermia. Relatively low bone mineral density at L2-L4 was found in 2 out of 3 patients studied. Morbidities are associated with poor compliance, leading to uncontrolled hypertension and hypokalemia. Many with poor compliance had histories of frequent hospitalization of hypokalemia. AME patients who were compliant with the medication and diet fared better.

Conclusions: EOD evaluation is an essential part of the care for AME. Our data supports the notion that adequate control of blood pressure and hypokalemia is fundamental to the outcome of AME patients.

32. LGL Leukemia Serorecognition of HTLV-2: An Array-Based Serorecognition Approach Coupled with Array-Adapted Alanine Screening

Susan B. Nyland, Daniel J. Krissinger, Kendall Thomas, Thomas P. Loughran, Penn State Cancer Institute, Hershey, Pennsylvania

Objective: Of the known bone marrow failure disorders, LGL leukemia represents the best model for studying mechanisms of antigen-driven, pathogenic LGL proliferation. LGL leukemia patients demonstrate unique indeterminate seroreactivity patterns against human T cell leukemia virus (HTLV-1/2) antigens, especially against HTLV-1 Env BA21. Standard Western Blot alanine substitution screening for BA21 sequences revealed PPLEN as a reactive but non-specific epitope, and lacked the capacity to identify other leukemia-specific antigens within Env. Therefore, we analyzed serum recognition against an array of overlapping peptides derived from HTLV-1/2, with the overall goal of identifying antigens that are relevant to LGL leukemia.

Methods: We first screened patient sera against purified leukemia virus lysates and found significantly elevated HTLV-2 IgG levels. We then designed an array containing 240 overlapping peptides representing the major products of HTLV-2 reference genomes and tested our chip on sera from 12 patients, 10 normal donors and from groups of retrovirus-seropositive donors. Previously characterized epitopes were also probed by adapting an alanine substitution protocol to the array format.

Results: Five Env sequences were consistently recognized at elevated levels; one of these was noted as a candidate leukemia-specific site. Additionally, elevated recognition of some Pol and Gag sequences provided new information about other potential LGL leukemia-specific antigens. Selected peptides were tested for antibody recognition. After validating different peptide epitopes, we found that some but not all of the significant interactions were due to antibody binding. Env seroreactivity was associated with serum antibody, while Gag sequence recognition was mostly related to uncharacterized serum proteins.
Data from the array-adapted alanine screen confirmed that HTLV-1 PPLEN was most reactive for LGL leukemia patients, and was also predictive of its antigenicity with normal donor sera. Our array data indicated that HTLV-1 WGLN may provide a more LGL leukemia-specific target.

Conclusions: We developed a specimen-conserving approach to study serorecognition, and used it to screen hundreds of specific candidate epitopes. LGL leukemia sera contain antibodies and other serum proteins with significantly elevated recognition of sites that are most similar to HTLV-1 Env BA21. This supports the notion that this region, with other virus-like sequences, represents important LGL leukemia-associated antigens.

33. Genomic copy number variants and their role in bone marrow failure disorders.

Christine L. O'Keefe, Lukasz Gondek and Jaroslaw P. Maciejewski Experimental Hematology and Hematopoiesis Section, Cleveland Clinic, Cleveland Ohio

Objective: Predisposition to disease may arise from both single base differences (single nucleotide polymorphisms, SNPs) and copy number variants (CNVs) of large genomic regions. Regions of CNV account for approximately 12% of the human genome, including coding sequences. With the advent of high-throughput, high density array technology, global analysis of complex disease predisposition traits, including CNVs, can be performed. CNVs can range in size from kilobases to megabases and include duplications, deletions and insertions. For example, recent studies have investigated the correlation between CNVs and mental retardation or cardiovascular disease. We hypothesized that CNVs may also play a role in bone marrow failure disorders.

Methods: We performed whole genome scanning in a cohort of patients (N=252) and healthy controls (N=58) using the Affymetrix 250K SNP array.

Results: In controls, we identified 30 CNVs; all loci were listed in the Database of Genomic Variants (http://projects.tcag.ca/variation/). 16 regions were identified in 2 or more control samples, while 14 occurred in a single individual. CNVs ranged in size from 245.6 Kb to 2.32 Mb (average 805.9 Kb) and were identified on all chromosomes except 5, 13, 16, 18 and 21. A majority of CNVs had a frequency of <10% within the control cohort. Nonetheless, three regions (the pericentromeric regions of 14q and 15q and a locus on 17q21.31) were identified in over 20% of samples. We next compared the pattern and frequency of CNVs in patients with bone marrow failure syndromes, including myelodysplastic syndromes (MDS, N=164), aplastic anemia (AA, N=65) and large granular lymphocytic leukemia (LGL, N=23) to the distribution in healthy control individuals. The MDS, AA and LGL patients harbored 19, 15 and 6 of the CNVs, respectively. While for most CNVs, the frequencies found in patients were similar to those in controls, two regions, 3q29 and 8q24.23, were more frequent in patients (3q29 in MDS, $p=0.05$; 8q24.23 in MDS and LGL, $p<0.0001$; 8q24.23 in AA, $p=0.01$). The region at 3q29 contain genes and is a common breakpoint regions for hematologic malignancies such as MDS and AML.

Conclusions: Distinct patterns of CNVs can be identified in patients with bone marrow failure. In addition to SNPs, CNVs can act as disease predisposition markers that are amenable to high-density SNP array analysis.

34. 11C-Flumazenil PET imaging in Patients with SSADH Deficiency

Phillip L Pearl, Jacob Taylor, Stacey Trzcinski, Alex Sokohl, K. Michael Gibson, William H Theodore

Background: Succinic semialdehyde dehydrogenase (SSADH) deficiency is an autosomal recessive disorder of gamma-aminobutyric acid (GABA) metabolism characterized by elevated levels of GABA and gamma-hydroxybutyric acid (GHB). Clinical findings include mental retardation with disproportionate expressive language dysfunction, hypotonia, hyporeflexia, hallucinations, autistic behaviors and seizures. Autoradiographic labeling and slice electrophysiology studies in the murine model provide evidence for use-dependent down-regulation of the GABA(a) receptor. To investigate whether there is down-regulation of the GABA(a) receptor in human patients with SSADH deficiency, we investigated benzodiazepine receptor (BZPR) binding using [11C] flumazenil (FMZ) and positron emission tomography (PET).

Methods: FMZ binding was measured in 6 patients with SSADH deficiency, 10 unaffected parents (obligate heterozygotes) and 6 healthy controls. We performed PET on a GE Advance Scanner using a reference region compartmental model, with time-activity curve from pons as the input function. Relative parametric binding potential (BP) was derived, with MRI-based pixel by pixel partial volume correction, in regions of interest drawn on co-registered MRI.

Results: In hippocampus, amygdala, thalamus, caudate, frontal cortex, occipital cortex, and cerebellar vermis, patients with SSADH deficiency had significant reductions in FMZ BP compared to parents and controls. There was no effect of gender.

Conclusions: SSADH deficient patients show widespread reduction in BZPR binding on 11C-FMZ PET. Since previous studies of FMZ PET have shown that binding is higher in children, our results suggest that high endogenous brain GABA levels in SSADH deficiency down-regulate GABA(a)-BZPR binding site availability.

35. Revisiting Recombinant 8 Syndrome

Laura Pickler, MD, MPH; Rebecca Wilson, Psy.D.; Anne C-H Tsai, MD
Clinical Genetics and Metabolism, The Children's Hospital, Denver, Colorado

Objective: Recombinant 8 syndrome, also called San Luis Valley syndrome, is a rare but important cause for developmental delay and chronic illness among children that occurs with greater reported frequency in the mountain states region. The most recent review of the natural history of this syndrome undertaken in 1993 by Sujansky et al. revealed that families could expect medical complications and poor outcomes. Recently, there has been increasing anecdotal evidence that with modern acute and chronic illness management strategies these children have substantially better outcomes than would be predicted based on the literature. To test our hypothesis that modern medical management strategies may alter outcomes of patients with Recombinant 8 syndrome in regard to mortality, morbidity and neurodevelopmental perspectives, we sought to update the natural history of Recombinant 8 syndrome by completing a thorough medical and psychological assessment of affected individuals. The Medical Home Family Index was administered to examine the role of medical home in the management of these individuals.

Methods: Twelve affected individuals, ranging from 2 to 21 years of age, were recruited through word of mouth, an announcement on the Rec8 website, and by review of our current patient database (COMIRB Protocol Number 06-0226). A thorough medical history and physical examination was performed. Patients were assessed for cognitive performance and adaptive functioning skills by a licensed clinical psychologist using validated instruments appropriate for chronological age and developmental level.

Results: Our patients scored on in the mild to moderate cognitive functioning level (range 70-30, mean 50) with surprising preservation in the social/adaptive arenas. Physical phenotypic features were consistent with previous reports of the syndrome. The minority of patients had adverse surgical outcomes from correction of non-orthopedic anomalies. However, orthopedic surgery to ameliorate effects of spasticity was complicated by long recovery times and decreased ability to ambulate. Primary care that was provided with support from an invested specialist team within the context of a medical home was associated with improved medical outcomes and an overall more positive outlook from the family.

Conclusions: Our findings support a consistent phenotype that does not necessarily result in lethal outcomes. Efforts to encourage learning and developmental progress should not be withheld as quality of life for many of these individuals is considered good by their families and medical providers.

36. Early Lung Disease in Young Children with Primary Ciliary Dyskinesia

Jessica Pittman**, David Brown**, Margaret Leigh**, Lynn Fordham††, Stephanie Davis**. University of North Carolina, Departments of Pediatrics** and Radiology††

Objective: Primary Ciliary Dyskinesia (PCD) is a rare, autosomal recessive disease in which ciliary dysfunction leads to chronic lung, sinus, and middle ear disease. Little is known about onset and progression of lung disease in early childhood. We report 3 cases of biopsy-proven PCD in children less than 3 years of age with evidence of lung disease on infant lung function testing, bronchoscopy, and/or computed tomography (CT) of the chest.

Methods: Chart reviews were conducted in 3 children with ciliary ultrastructural defects (outer and/or inner dynein arm) consistent with PCD. Infant pulmonary function testing (PFTs), chest CTs, bronchoalveolar lavage (BAL) and deep pharyngeal cultures were reviewed. Infant PFTs included plethysmography and flow-volume curves obtained using the raised volume rapid thoracoabdominal compression technique described by Jones et al (AJRCCM, 2000). Chest CT scans were obtained using the controlled breathing technique described by Long et al (Radiology, 1999).

Results: Patients were diagnosed with PCD at 6, 25, and 29 months. Two of the three had situs inversus totalis; all had neonatal respiratory distress despite term gestation. All had bronchoscopy-diagnosed bronchitis and recurrent otitis medias. BAL and deep pharyngeal cultures grew *Moraxella cattarrhalis, Streptococcus pneumoniae,* and *Staphylococcus aureus.* Two had chest CTs which revealed evidence of multilobar bronchiectasis (at 31 and 24 months). Infant PFT results revealed: (1)forced expiratory volume at 0.5 seconds between 71 and 75% predicted, (2)forced expiratory flows between 25 and 75% of forced vital capacity between 66 and 78% predicted, (3)functional residual capacity between 92 and 134% predicted and (4)ratio of residual volume to total lung capacity between 134 and 184% predicted. These values were consistent with mild airway obstruction and hyperinflation.

Conclusions: PCD is often diagnosed in late childhood due to its rarity and technical difficulty of ciliary microscopy necessary for diagnosis. We report 3 young patients with evidence of early lung disease on bronchoscopy, infant PFTs, and/or chest CTs. This report suggests lung disease begins early and is identifiable by several diagnostic techniques. Identifying early lung disease could revolutionize clinical care for PCD infants and young children, with an emphasis on early detection and intervention. Further study is needed to determine if our findings translate to a larger population of children with PCD.

37. The Efficacy of Whole Lung Lavage in PAP Improves with Prone Positioning

Jonathan Puchalski, Michael Reed and Bruce Trapnell.
University of Cincinnati and Cincinnati Children's Hospital Medical Center.

Objective. Whole lung lavage (WLL) for pulmonary alveolar proteinosis (PAP) was described by Ramirez[1] in the 1960's. Since that time, refinements without major changes to the technique have been made. Still, it remains the most effective therapy for patients with PAP. The procedure is generally accomplished by selective ventilation of one lung and lavage of the other with 15-40 liters of saline. The addition of chest physiotherapy improves efficacy. The use of volumes up to 70 liters accompanied by manual percussion and late-phase prone positioning has been described.[2] Since deposition of the material within the alveoli can be likened to the "flakes in a snow globe," with rapid settling lessening the ability to remove the debris, we hypothesized that repeated prone positioning would improve the efficacy of the procedure in this case. The objective was to improve the efficacy of WLL in a patient failing to respond to traditional therapy. He had required bilateral lavages with 15-25 liters of saline at 1 month, 5 months, and 13 months after diagnosis with unilateral lavage at 15 months. Despite this and inhaled GM-CSF, the patient had worsened symptoms and quality of life.

Methods. The patient was intubated with a double-lumen endotracheal tube. He underwent serial instillation of warmed saline in 1 liter increments to a total of 50 and 63 liters with subsequent removal of effluent in volumes that ranged from 800 to 1300 ml. Each return was collected in a different bag for analysis. The patient was placed in the prone position twice during each lavage. The effluent was analyzed by visual inspection, turbidity at 450 nm, and dry weight.

Results. The visual and measured turbidity as well as the dry weight of the specimens increased consistently when the patient was rotated from a supine to prone position. The turbidity was 1.20-6.56 times higher after proning while the dry weight was 3.42 times higher immediately after the positional change. The patient was subjectively improved following the procedure.

Conclusion. The use of repeated prone positioning during WLL increased the return of lipoproteinaceous material and resulted in subjective improvement in this case. Consideration to prone positioning should be given to conventional lavage and to those who fail to respond to traditional treatment.

38. Prader-Willi syndrome is caused by paternal deficiency for the HBII-85 C/D box snoRNA cluster.

Trilochan Sahoo, Daniela del Gaudio, Jennifer R German, Marwan Shinawi, Sarika U Peters, Rick Person, Adolfo Garnica, Sau Wai Cheung, Arthur L Beaudet. Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Objective: Identification and characterization of a *de novo* microdeletion involving the HBII-85 C/D box snoRNA cluster at 15q11.2 in a patient with physical findings and clinical course meeting criteria for a diagnosis of Prader-Willi syndrome.

Methods: Array-based comparative genomic hybridization (array-CGH) and fluorescence in situ hybridization (FISH) studies revealed microdeletion involving segment at 15q11.2. DNA methylation analysis of the PWS-imprinting center had earlier ruled out deletion or imprinting abnormalities. A combination of quantitative Real-Time PCR and high-resolution oligonucleotide-based array-CGH defined the deletion size and approximate boundaries. Long-range PCR across this predicted breakpoint revealed a consistent junction fragment and the precise breakpoints. RT-PCR using RNA from lymphoblasts of the patient and appropriate normal and disease controls confirmed lack of expression of the transcripts within the deleted segment.

Results: Array-CGH revealed a loss in copy number detected by two clones estimated to encompass approximately 400 kb within the chromosome 15q11.2 PWS/AS critical interval. A combination of quantitative Real-Time PCR and high-resolution oligonucleotide-based array-CGH helped to define the deletion more precisely between positions 22.83 Mb (centromeric) and 23.01 Mb (telomeric). Long-range PCR across breakpoint revealed a consistent junction fragment of ~2.6 kb; sequencing revealed the breakpoints precisely at position 22835594 proximally and 23010179 distally with an insertion of 8 bp between the breaks; the size of the deleted segment was now accurately determined to include 174584 bp. The proximal breakpoint occurred between EST AB061178 and snoRNA box HBII-438A, and the distal breakpoint between snoRNA box HBII-52-23 and HBII-52-25 respectively.

Conclusions: Our findings reveal a unique microdeletion restricted to a genomic segment within the PWS-AS critical interval and encompassing the entire HB-II-85 and a portion of the HB-II-52 cluster of snoRNAs. Together, these data provide relatively conclusive evidence that the PWS phenotype is caused by paternal deficiency for the HBII-85 snoRNA cluster of 29 copies. Previous evidence from balanced translocation cases was less conclusive because the HBII-85 cluster was still present with no access to data for expression in the brain. This interpretation would represent the first well documented example of a human phenotype caused by deficiency of a snoRNA, a subclass of regulatory noncoding RNAs.

39. Clinical and Laboratory Features of Lower Extremity Ulcers in Connective Tissue Diseases

Victoria Shanmugam, Virginia Steen, Christopher Attinger, Blanche Mavromatis, Craig Kessler, Thomas Cupps. Georgetown University Hospital, Washington, DC

Purpose: Lower extremity ulcers affect 9% of patients with rheumatoid arthritis (RA) and are seen in other connective tissue diseases (CTD). Although they are often attributed to vasculitis, their pathogenesis is usually multifactorial. The aim of this study was to determine the clinical, immunologic, and hypercoagulable characteristics of lower extremity ulcers in CTD.

Methods: A retrospective chart review of 95 patients with lower extremity ulcers evaluated in our clinic between 1998 and 2006 identified 34 patients with concomitant CTD and leg ulcers. Data was collected on demographics, number and distribution of lesions; underlying medical conditions; autoimmune, inflammatory, and hypercoagulable evaluations; and response to therapy.

Results: The 34 patients with CTD associated ulcers included 13 patients with rheumatoid arthritis (RA), seven with systemic sclerosis (SSc), four with mixed connective tissue disease (MCTD) and ten with systemic lupus erythematosus (SLE). Ulcers were a late complication of CTD, occurring on average 19 years after the initial CTD diagnosis. In RA lesions were perimalleolar in location, but in the other CTD they were more widely distributed. Biopsies were available in 16 patients. A non-specific mixed inflammatory infiltrate was present in 56%, four patients had evidence of small vessel fibro-occlusion, and three had vascular proliferation. Only one had evidence of vasculitis. At least one hypercoagulable abnormality was identified in 65% of patients tested. Lupus anticoagulant was present in 46%, anti-β_2 glycoprotein I antibodies in 41%, and anti-cardiolipin antibodies in 50%. Of the patients undergoing genetic evaluation, plasminogen activator inhibitor gene mutation was seen in 66%, and methyl-tetrahydrofolate reductase gene mutation in 60%. Hyperhomocysteinemia was identified in 41%. After a mean follow-up of 2.41 years, only 32% of lesions had healed. An initial response to oral prednisone was seen in 33%, but lesions often recurred. Immunosuppressive therapies were generally ineffective. Patients treated with coumadin (n=8) and aspirin (n=8) did not experience wound healing. However, of six patients receiving low molecular weight heparin (LMWH), five had complete healing and one demonstrated improvement but was lost to follow-up. These patients all had at least one positive antiphospholipid antibody, and five had multiple coagulation defects.

Conclusions: Lower extremity ulcers are a late complication of CTD. We found little evidence to support a vasculitic etiology, but identified hypercoagulable abnormalities in 65%.

40. Mitochondrial Transcription in Patient-Derived Samples – Pilot Study

Neal Sondheimer* and Narayan G. Avadhani**. *Section of Biochemical Genetics, The Children's Hospital of Philadelphia and **Department of Animal Biology, The University of Pennsylvania School of Veterinary Medicine

Objective: "Pediatric mitochondrial disease" is a catchall phrase for the disorders of energy production that present in childhood. As clinicians, we fail to identify a genetic basis of disease in more than 70% of the patients who present with an illness meeting standard criteria for mitochondrial disease. It is possible that some children who lack diagnosis have a defect in transcription of their mitochondrial DNA. In this study we adapt methods for the study of mitochondrial transcription to skin biopsy samples, which can be readily obtained for study.

Methods: By varying culture conditions, we increased mitochondrial activity to improve protein and RNA yield. RNA harvested from fibroblast cell culture was used as the basis for analysis of mitochondrial RNA production.

Results: Growth of cells with 400μM clofibrate increased the yield of mitochondrial RNA as determined by RTPCR and the levels of expressed subunits of the electron transport chain. S1 analysis of mitochondrial transcription was used to map and identify the 3' ends of the L-strand and long H-strand transcripts. Since these two transcripts produce all of the mitochondrial mRNAs, quantitation will provide an overall picture of mitochondrial gene expression. Mapping of the 5' end using primer extension allowed the discrimination of mRNA productive transcription from transcription producing rRNA. A high throughput assay system was also developed for the rapid analysis of mitochondrial transcription from multiple patient samples.

Conclusions: Although fibroblast samples have low levels of mitochondrial function, they are adequate for the demonstration of mitochondrial transcription. Use of altered culture conditions can increase mitochondrial function, improving the speed and reliability of analysis. These preparations will allow the planned screening of patients with undiagnosed mitochondrial disorders.

41. Role and Regulation of Barx1 in the zebrafish pharyngeal arches

Steven Sperber and Igor Dawid

Laboratory of Molecular Genetics, National Institute of Child Health and Human Development

Objective: BARX1 (9q12), most prominently expressed in the pharyngeal arches, is implicated in human craniofacial anomalies and syndromic malformations that include dolichocephaly, beaked nose, and receding chin; however, little is understood of its function or regulation in patterning the osteochondroprogenitor elements of the developing viscerocranium. We characterized the role and regulation of the zebrafish barx1gene during pharyngogenesis (24-72 hours post fertilization, hfp).

Method: Gene expression was examined during craniofacial development. Functional analysis was determined by attenuating *barx1* translation by microinjection of antisense morpholino oligonucleotides. The influence of signaling pathways on *barx1* and chondrocyte condensation were assessed using FGF3 and FGF8 deficient fish, and by FGF8, BMP4 and ET1 imbued beads implanted at the initiation of pharyngogenesis.

Results: Expression of the zebrafish *barx1* is restricted within the head to the pharyngeal arches. Embryos microinjected with *barx1* morpholinos exhibit mildly delayed cranial neural crest (CNC) migration as visualized in *fli1:GFP* transgenic fish. Expression analysis of *inca* and *dlx2a* indicate normal initial patterning of the CNC. By 48-72 hpf, a period of significant pharyngeal outgrowth, *barx1* morphants stained with phosphohistone H3 exhibit reduced cellular proliferation resulting in hypoplastic arch tissues, and micrognathia. Histology (72 hpf) revealed reductions in chondrocyte differentiation and poor cellular condensation and perturbation of *col2a1* and *chondromodulin* chondrogenic markers. Alcian blue stained morphants (120 hpf) lack or exhibit dysmorphic cartilage elements. FGF signaling, necessary for pharyngeal arch patterning, maintains *barx1* expression in a coordinate manner. Exogenous FGF8 reduces cellular condensation; BMP4 maintains *barx1* interzone expression between elements while ET1 causes a loss of expression suggesting all have roles in modulation of *barx1* and chondrocyte condensation.

Conclusions: *Barx1* is essential for cellular proliferation, aggregation and condensation of prechondrocytes within the zebrafish pharyngeal arches. Lack of expression in the ethmoid plate and trabeculae exemplifies an alternate genetic program necessary for endochondral bone formation within the chondrocranium. FGF8, BMP4, and ET1 influence *barx1* expression necessary for proper shaping of the anlagen that presages the viscerocranium. Together, these results suggest a critical role for *barx1* in chondrogenesis and the patterning of cartilage that presages osteogenesis within the zebrafish viscerocranium. Investigating the zebrafish *barx1* provides new insights into the human BARX1 ortholog that may be implicated in agnathia, micrognathia or other craniofacial malformation syndromes.

42. The Nondystrophic Myotonias: genotype-phenotype correlation and longitudinal study. Clinical phenotype characterization

J Statland, Y Wang, RJ Walch, B Bundy, RJ Barohn, and the CINCH study group

Objectives: To collect initial standardized clinical data from participants with nondystrophic myotonias(NDM) and better define the relationship between investigator-suspected subtype and clinical features.

Background: NDM are a heterogeneous group of neuromuscular disorders caused by mutations in skeletal muscle sodium and chloride channels. The relationship of genotype to phenotype and the natural history are not well understood.

Methods: 45/75 planned subjects were enrolled from 6 academic centers across the United States, England, and Canada. Patients were categorized by investigator's impression into myotonia congenital (MC), paramyotonia congenital (PMC), and other myotonic disorders (OMD). We examined the demographic features, clinical symptoms and physical findings in this initial cohort.

Results: 15 female and 30 male subjects were enrolled. Initial clinical diagnosis: 20 MC (1 with molecular confirmation at time of enrollment); 16 PMC(12 with molecular conformation at time of enrollment); and 9 OMD (3 with Myotonic Dystrophy type II, and 2 with Hyperkalemic Periodic Paralysis). The mean age at enrollment for the population was 43 (range 12-73). The most prominent symptom for the total population was stiffness in 31/45, and by subgroup: 17/20 MC, 11/16 PMC, 3/9 OMD. On physical exam hand grip myotonia was present in 34/45 participants, and by subgroup: 19/20 MC, 12/16 PMC, 3/8 ODM. Objective warm-up phenomenon during repetitive hand grip was seen in 19/45 participants, and by subgroup: 18/20 MC, and 1/16 PMC. Paradoxical myotonia on exam was only seen in participants with a clinical diagnosis of PMC (14/16). For participants who had no genetic confirmation prior to entering the study 11/12 with a clinical diagnosis of MC had a CLCN1 mutation, and 1/5 with a diagnosis of PMC had a SCN4A mutation.

Conclusion: Clinical features are useful to distinguish NDM subtypes. Interestingly, paradoxical myotonia was only seen in patients with a clinical diagnosis of PMC. And for patients with a clinical diagnosis of MC in the absence of genetic testing, 11/12 had a CLCN1 mutation. We plan to correlate these findings with genotype in all subjects when the data is available.

43. A Preliminary Summary of an Angelman syndrome Natural History Study

Wen-Hann Tan[1,2], Carlos A. Bacino[1,3], Steven A. Skinner[1,4], Arthur L. Beaudet[1,3], Terry Jo Bichell[1,5], Lynne M. Bird[1,6]
[1]NIH Rare Diseases Clinical Research Network – Angelman, Rett, Prader-Willi Syndromes Consortium; [2]Children's Hospital Boston, MA; [3]Texas Children's Hospital, Houston, TX; [4]Greenwood Genetic Center, SC; [5]Vanderbilt University, Kennedy Center, Nashville, TN; [6]Rady Children's Hospital San Diego, CA

Objective: Angelman syndrome (AS) may be caused by either a loss of function or a mutation in the maternal copy of *UBE3A*. This loss may be due to a deletion in the AS critical region on chromosome 15, paternal uniparental disomy, or imprinting defects. The natural history of AS due to the different molecular defects remains unclear. Therefore, we are conducting a study to obtain a better understanding of the natural history of AS, including the behavioral phenotype, neurodevelopment and morbidity in children and adults with AS.

Methods: A 5-year multi-center longitudinal study on the natural history of Angelman syndrome (AS) is being conducted. Patients with either a molecular or clinical diagnosis of AS, are being recruited, from newborns to adults aged 60 years. At each annual visit, a set of detailed evaluations is performed, including physical and neurological examinations, and developmental assessment by clinical psychologists; electroencephalogram (EEG) and evaluation for autism is performed every other year.

Results: To date, we have enrolled 82 AS patients –71% have a deletion in the AS critical region, 15% have UBE3A mutations, 3.7% have paternal uniparental disomy, 2.4% have imprinting defects, and 8.5% have methylation abnormalities due to a molecular defect that remains to be determined. Among the more common characteristic behavioral features are: "mouthing" behavior in 76% of our patients; short attention span in 72%, easy excitability in 71%, fascination with water and sleep difficulties, both of which were observed in 63% of our patients. In contrast, inappropriate laughter and hand-flapping, which has traditionally been associated with AS, were seen in only 51% of our patients. We also observed that 54% of our patients had either no vocalizations or had only grunts, while 49% did not have any means of non-verbal communication.

Conclusions: Our findings suggest that the published literature on the natural history of AS is out of date, and we hope that our findings will help health care providers recognize AS, leading to earlier diagnosis and intervention for these children.

44. The Natural History of Rett Syndrome: A comparison of genotype and phenotype.

Daniel C. Tarquinio, RS consortium, University of Alabama Birmingham

Objective: Rett syndrome is an X-linked dominant neurodevelopmental disorder associated with over 200 mutations in *MECP2*. Currently, no reports exist with large enough sample size to develop robust genotype/phenotype correlations between these mutations. We analyzed clinical data from 263 RDCRN participants affected with one of 8 of the most common mutations (R106W, R133C, T158M, R168X, R255X, R270X, R294X, and R306C), and examined their genotype/phenotype correlations using eleven clinical criteria.

Methods: All information was collected by RDCRN Rett syndrome investigators to maintain consistency and stored in the DTCC database. The database was queried for eleven clinical criteria: Age at diagnosis, Clinical Severity Score (CSS), Motor-Behavioral Analysis (MBA), Seizure Frequency, Body Mass Index (BMI), and scores for Hand Use, Ambulation, Scoliosis, Language, and two quality of life measures (CHQ and SF-36). Univariate analysis was performed on age at diagnosis and compared to that of individual mutations. Two published severity scales, the CSS and MBA, were used to assess clinical severity. Individual scores were extracted from these severity scales and examined separately for each mutation. ANOVA analysis was then performed using the Tukey-Kramer method. The two previously validated quality of life scores were evaluated within individual mutations and then compared using ANOVA. Correlation coefficients for the two disease severity scales were calculated.

Results: The frequency of the 3 most common mutations in our study was consistent with previous studies. The CSS and MBA showed mutation-specific trends indicating that R133C, R294X, and R306C result in a less severe phenotype compared to the other 5 mutations, a trend noted elsewhere. Although differences on CSS and MBA did not achieve statistical significance on ANOVA, when language, hand use, and ambulation were analyzed separately, these three were significantly milder than the other 5 mutations. The CSS and MBA were internally consistent with a 0.70 correlation score. R168X yielded a Scoliosis Score higher than any other mutation but a Seizure Score that was second lowest (R306C had the fewest seizures). BMI in R270X and R306C participants trended lower than the others although these were not statistically significant.

Conclusions: A statistically significant difference exists in clinical phenotype regarding language, ambulation, and hand use among the 8 most common mutations associated with Rett syndrome. Trends in seizure frequency, scoliosis, and BMI exist, although these were not statistically significant.

45. Statistical methodology using Random Forests for whole genome association studies in rare diseases

Aaron D. Viny,[1,2] Hemant Ishwaran,[3] Jaroslaw P. Maciejewski.[1,2]

[1]Cleveland Clinic Lerner College of Medicine of Case Western Reserve University; Departments of [2]Experimental Hematology & Hematopoeisis and of [3]Quantitative Health Sciences, Cleveland Clinic, Cleveland, OH, USA.

Objective: Along with the technological advances for investigating genome-wide genetic variation using high-density single nucleotide polymorphism (SNP) arrays, has come a tremendous burden in data analysis. SNP array platforms now include up to 10^6 SNPs per array, and carry an equally high positive inference mistake rate (α-error). This is particularly cumbersome for investigating rare diseases, such as large granular lymphocyte (LGL) leukemia, where sample size is extremely small.

Methods: A cohort of LGL patients (n=37) were analyzed for SNPs that co-segregate with disease versus controls (n=56) using the Illumina 12K non-synonymous SNP genotyping platform. In the presence of a complex genetic disease like LGL we anticipate a complex inter-relationship between genes, more closely reflecting the biology of a non-Mendelian inherited disease. Therefore, our analysis used Random Forests, a nonparametric tree method, whereby all SNP information was used multivariately to predict disease. Averaging over trees, in combination with the randomization used in growing the base tree learner, enables Random Forests to approximate large classes of decision functions. In order to preserve low generalization error, and mitigate high false positive rates, regularization was imposed on the number of candidate SNPs used to split a node within a tree. SNPs identified as predictive were subjected to technical validation using allele-specific PCR and biological validation in an external validation cohort and were compared to results from traditional analysis using Exemplar statistical software.

Results: Our analysis identified two highly predictive SNPs—rs11136300 and rs1063635 within *C8orf31* and MHC class-I related-chain A gene (*MICA*) respectively, but only the latter was identified using Exemplar. Additionally, 4 other MICA SNPs were identified of moderate predictability. These 6 SNPs have been subjected to technical validation and rs1063635 has been validated in an external cohort (n=40) with PPV=73% and NPV=90%.

Conclusions: Our data suggests that MICA SNP rs1063635 is a major predictor of disease. Further studies will be needed to determine the functional implications of this SNP and other polymorphisms in linkage disequilibrium. Flow cytometric results have shown an increased expression of MICA on patient neutrophils compared to controls, further confirming the molecular significance of this SNP, or SNP in linkage disequilibrium.

46. Adaptive and Maladaptive Behavior in Deletion 22q13 Syndrome Compared to 5p- Syndrome

Jeannie Visootsak, MD[1]; Elisabeth Dykens, PhD[2]; Mary C. Phelan, PhD[3]; John M. Graham, Jr, MD, ScD[4]

[1]Department of Human Genetics, Emory University School of Medicine, Atlanta, GA; [2]Department of Psychology and Human Development, Vanderbilt University, Nashville, TN; [3]Molecular Pathology Laboratory Network, Maryville, TN; [4]Medical Genetics Institute, Cedars-Sinai Medical Center, UCLA School of Medicine, Los Angeles, CA

Objective: Deletion 22q13 syndrome is a microdeletion syndrome associated with global developmental delay, hypotonia, and a specific physical and behavioral profile. The incidence of deletion 22q13 syndrome has not been determined with only 360 cases identified worldwide. Cri du Chat syndrome results from a deletion of the short arm of chromosome 5 (5p-). Clinical features include a high-pitched cat-like cry, distinct facial dysmorphism, microcephaly, and mental retardation. It is a rare disease with an incidence ranging from 1:15,000 to 1:50,000 livebirths. Herein, we compare the adaptive functioning and define behavioral problems in individuals with deletion 22q13 syndrome and 5p- (cri du chat) syndrome and assess the extent to which these behaviors are unique or shared.

Methods: The study includes 17 individuals with deletion 22q13 syndrome, ranging from 5 to19 years, with a mean age of 10.4 years and 64 individuals with 5p- syndrome, ranging in age from 6-20 years, with a mean age of 12.2 years. Standardized questionnaires are used: 1. Vineland Adaptive Behavior Scales and 2. Aberrant Behavior Checklist.

Results: Based on the Vineland Adaptive Behavior Scales, both groups manifest strengths in socialization, and weaknesses in communication and daily living skills. For maladaptive functioning, the two comparison groups demonstrate significant hyperactivity and impulsivity. They are also prone to temper tantrums, aggressiveness, and irritability. However, deletion 22q13 syndrome subjects frequently has stereotypic behavior and repetitive movements with increased vulnerability to social isolation and difficulties with transitions.

Conclusion: The behavioral features of deletion 22q13 syndrome and 5p- syndrome are influenced by their global developmental delay and lower adaptive functioning. Both groups share similarities in activity level and frequent externalizing behavior. However, deletion 22q13 syndrome has a higher probability of social deficits and stereotypic behavior, which may increase their risk for manifesting autism spectrum disorder. These findings may provide insights for early intervention and anticipatory guidance.

47. Interactive Voice Response Diary and objective myotonia measurementas endpoints for clinical trials in nondystrophic myotonia

Yunxia Wang, Jeffery Statland, Ronan J Walsh, Richard Barohn, and the CINCH study group

Objectives: To evaluate interactive voice response dairy (IVR) system and quantitive myotonia measurment as potential outcome measures for nondystrophic myotonias(NDM) clinical trials

Background: NDM is a heterogeneous group of neuromuscular disorders caused by mutations in skeletal muscle sodium and chloride channels. There are no established treatments for myotonia despite the availability of agents that deserve careful study. An outcome measure of myotonia is important for clinical trials.

Methods: 45 subjects were enrolled from 6 academic centers across the United States, England, and Canada. Patients were categorized as myotonia congenita (MC), paramyotonia congenita (PMC), and other myotonic disorders(OMD) Three possible myotonia(syptoms/signs) measures were assessed: relaxation time following maximum voluntary isometric contraction of the finger flexors (QMA); myotonic discharges on needle EMG, an Interactive Voice Response Diary(IVR) where participants called in once a week up to 8 weeks to rank symptom severity on a scale of 1-9(stiffness, pain, weakness, fatigue) and frequency(1-7 days).

Results: Initial clinical diagnosis: 20MC; 16PMC; 9OMD. Myotonic discharge potentials were seen in all subjects with no difference in their degree and location among the subtypes. Of 33 participants for which QMA test results were available, 15 showed a delay in relaxation; however for subjects with ≥ 2 s hand grip opening on clinical exam 14/16 showed a delay in relaxation on QMA. IVR data: frequency of symptoms for the total population was: stiffness 91%, pain 60%, weakness56%, fatigue 65%. Average severity for each symptom: stiffness 4.1, pain 4.0, weakness 4.2, fatigue 4.2. Standard deviation of reported symptom severity within subject: stiffness 1.2; pain, 1.1, weakness 1.3, tiredness, 0.9. For participants reporting symptoms for a given week, the average number of days they experienced symptoms were stiffness 5.2, pain 5.2, weakness 4.4, and fatigue 4.5.

Conclusion: The IVR data showed consistency in patient self rated clinical symptoms related to myotonia, which makes this a potentially useful endpoint for future clinical trials. Quantitive myotonia measure is a less sensitive outcome measure since only minority patients showed abnormal results.

48. Correction of chloride channelopathy and myotonia in a transgenic mouse model of DM1 by oligonucleotide-mediated skipping of ClC-1 exon 7a

Thurman M. Wheeler[1], John D. Lueck[2], Robert T. Dirksen[2], and Charles A. Thornton[1]

[1]Departments of Neurology and [2]Pharmacology and Physiology, University of Rochester, Rochester, NY

Objective: elucidate the physiological basis of myotonia in myotonic dystrophy type 1 (DM1).

Background: The mechanism of myotonia in DM1 is controversial. Expression of chloride channel 1 (ClC-1) is reduced in DM1 muscle. It is unknown, however, whether chloride channelopathy is a unitary explanation for the myotonia. Reduced expression of ClC-1 may result either from misregulated alternative splicing (Mankodi, 2002) or reduced transcription (Ebralidze, 2004). DM1 is associated with inappropriate inclusion of ClC-1 exon 7a, which leads to truncated ClC-1 protein. Like DM1, the HSA^{LR} transgenic mouse model displays myotonia, reduced ClC-1 expression, and spliceopathy.

Methods: The goal was to induce skipping of ClC-1 exon 7a using antisense oligonucleotides. We designed a morpholino oligonucleotide targeting this exon. Antisense or control morpholino was injected into tibialis anterior and flexor digitorum brevis muscles of HSA^{LR} mice. Entry of morpholino into muscle fibers was enhanced by electroporation in vivo. Splicing of exon 7a was determined by RT-PCR. Expression of ClC-1 protein was assessed by immunofluorescence. ClC-1 function was examined by patch clamp analysis of single muscle fibers. Electromyographic myotonia was assessed by a blinded examiner.

Results: Antisense morpholino had the intended effect of inducing skipping of ClC-1 exon 7a. Correction of ClC-1 spliceopathy was associated with (1) restoration of ClC-1 protein to the surface membrane; (2) normalization of chloride current density; and (3) reversal of myotonia. Effects persisted up to 8 weeks after a single injection. Injection of control morpholino had no effect.

Conclusions: These results show that myotonia in a transgenic mouse model of DM1 results from chloride channelopathy, that loss of ClC-1 function results from spliceopathy rather than reduced ClC-1 transcription, and that antisense-mediated exon skipping is a viable therapeutic strategy in DM1.

49. The Heparin-PF4 ELISA Confirmatory Test Is Valuable For Diagnosis Of HIT

Nicole L Whitlatch[1], Stephanie L Perry [1], David F Kong[1], Thomas L Ortel[1]

1. Duke University Medical Center, Durham, NC

Background: Diagnosis of heparin-induced thrombocytopenia (HIT) requires certain clinical features along with the development of platelet activating antibodies induced by heparin interaction with platelet factor 4 (PF4). Clinical features include thrombocytopenia in the absence of other causes of platelet count reduction, occurring 4-15 days after heparin exposure, with or without thrombosis. Laboratory testing includes the PF4 ELISA, which although a highly sensitive test, is not specific. It is therefore recommended that a confirmatory test be performed on all positive PF4 ELISA results by adding excess heparin to the sample. A positive reaction decreases antibody binding by 50% or more. We sought to determine the clinical value of this procedure.

Methods: We retrospectively identified 116 patients with positive anti-PF4/heparin antibodies at Duke Medical Center during 2005. Anti-PF4/heparin antibody titers were determined by ELISA (GTI) using a confirmatory step with excess heparin. The "4-T" rule (Warkentin, Br. J. Haematol, 2003) classified patients as HIT-positive or HIT-negative. Chi-square tests, t-tests, and logistic regression models estimated the relationship between patient characteristics, laboratory findings, and clinical HIT status. The peak PF4 titer was log-transformed to satisfy model assumptions.

Results: There were 99 confirmatory positive and 17 confirmatory negative patients. There was no detectable collinearity between peak PF4 titer and confirmatory assay results. We found no relationship between age, race, or gender and HIT status. In univariate logistic models, peak PF4 titer, confirmatory positive status, and surgical service correlated significantly with HIT+ status (Table). In multivariate analysis, surgical service did not independently contribute to the model.

Variable	Degrees of Freedom	Likelihood Ratio Chi Square	P Value	c-index
Log PF4	1	15.72	<0.0001	0.70
Confirmatory +	1	18.83	<0.0001	0.65
Surgical Patient	1	4.04	<0.0445	0.60
Confirmatory + and Log PF4	2	35.12	<0.0001	0.78

Conclusion: The confirmatory result and the peak PF4 titer both contribute significant information to the estimated probability of HIT. The confirmatory assay is a valuable adjunct to the laboratory diagnosis of HIT.

50. Outcomes in Children with Cardiomyopathy: The NHLBI PCMR Study

James D. Wilkinson, MD, MPH; Jorge A. Alvarez, AB; Natalya Bublik, MPH; Steven E. Lipshultz, MD

Objective: Cardiomyopathy (CM) is a serious disorder of the heart muscle. Although rare, it is potentially devastating in children. Accounting for only 1% of pediatric cardiac disease, CM leads to significant morbidity and death. 40% of children presenting symptomatically will receive a heart transplant or die within the first two years. The Pediatric Cardiomyopathy Registry (PCMR) was designed to describe the epidemiology and clinical course of selected CMs in patients 18 years old or younger and to promote the development of etiology-specific prevention and treatment strategies.

Methods: Two registry cohorts were established: a prospective population-based cohort of patients diagnosed from 1996 and on, residing in New England and the Central Southwest; and a cohort of patients diagnosed from 1990 to 1995 and enrolled upon retrospective review of medical records from selected large tertiary care centers in the United States and Canada.

Results: Data collected by the PCMR has resulted in many important findings. A more accurate estimate of the incidence of pediatric CM in two large regions of the United States was found to be 1.13 cases per 100,000 children. Only 1/3 of children had a known etiology at the time of CM diagnosis. Diagnosis was associated with certain patient characteristics, family history, echocardiographic findings, laboratory testing, and biopsy. Greater incidence was found in boys and infants (<1 yr) for both dilated and hypertrophic cardiomyopathy (DCM, HCM) and black race for only DCM. In DCM, prognosis is worse in older children (>1yr), heart failure (HF) at diagnosis or idiopathic etiology. For HCM, worse prognosis is associated with inborn errors of metabolism or combination of HCM and another CM type. The best outcomes were observed in children presenting at age >1 yr with idiopathic HCM. Standard of care and real-world practice for the treatment of pediatric CM has changed little over the past several decades. Many state-of-the-art interventions undergoing evaluation in adults have not been fully evaluated or developed for pediatric use. PCMR data have enabled analysis of patients with Noonan Syndrome (NS) and CM: diagnosis in early infancy (age <6 months) and HF present at diagnosis were associated with decreased survival in these patients.

Conclusions: Findings from the PCMR have shed light on incidence, etiology, and outcomes of pediatric cardiomyopathy, as well as contributed to the improvement of current therapy practices.

51. Diffuse Lung Disease in Young Children: Application of a Novel Classification Scheme

Lisa R. Young*, Gail H. Deutsch*, Robin R. Deterding, Leland L. Fan, Sharon D. Dell, Judy A. Bean, Alan S. Brody, Lawrence M. Nogee, Bruce C. Trapnell, Claire Langston, and the Pathology Cooperative Group for the Children's Interstitial Lung Disease Network, Rare Lung Diseases Consortium, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, United States.

Objectives: Considerable confusion exists regarding nomenclature, classification and management of pediatric diffuse lung diseases due to the relative rarity and differences in the spectrum of disease between adults and young children. A multi-disciplinary working group was formed to: 1) Develop and apply consensus terminology and diagnostic criteria for disorders presenting with diffuse lung disease in infancy; and 2) Describe the distribution of disease entities, clinical features, and outcome in young children who currently undergo lung biopsy in North America.

Methods: Eleven pediatric centers provided pathologic material, clinical data and imaging from all children less than 2 years of age who underwent lung biopsy for diffuse lung disease from 1999 to 2004.

Results: Multidisciplinary review categorized 88% of 187 cases. Disorders more prevalent in infancy, including primary developmental and lung growth abnormalities, neuroendocrine cell hyperplasia of infancy, and surfactant-dysfunction disorders, comprised the majority of cases (53%). Lung growth disorders were often unsuspected clinically and under-recognized histologically. Cases with known surfactant mutations had characteristic pathologic features. Age at biopsy and clinical presentation varied among categories. Pulmonary hypertension, presence of a primary developmental abnormality or *ABCA3* mutation was associated with high mortality, while no deaths occurred in cases of pulmonary interstitial glycogenosis or neuroendocrine cell hyperplasia of infancy.

Conclusions: This cross-sectional study identifies a diverse spectrum of lung disorders, largely unique to young children. Application of a classification scheme grouped clinically distinct patients with variable age of biopsy and mortality. Standardized terminology and classification will enhance accurate description and diagnosis of these disorders.

52. Microbial Communities in Children with Cystic Fibrosis and Clinically Stable, Mild Lung Disease

Edith T. Zemanick, J. Kirk Harris, Scott D. Sagel, and Frank J. Accurso

Institutions: Department of Pediatrics, University of Colorado at Denver and Health Sciences Center, Denver, CO, USA.

Objective: Chronic lung infections are the leading cause of morbidity and mortality in cystic fibrosis (CF) patients. Microbial culture of airway samples currently guides antibiotic treatment. However, culture techniques are designed to target known CF pathogens and thus may miss unsuspected microbes. Also, airway cultures are negative in some CF patients with pulmonary symptoms and airway inflammation. Molecular techniques allow identification and quantification of bacteria without the need for culture. We hypothesized that microbial identification and quantification using 16S rRNA gene sequencing and quantitative real-time polymerase chain reaction (qPCR) detects uncultured bacteria from CF airway samples.

Methods: We studied throat swab, expectorated and induced sputum samples from 5 well-characterized, clinically stable CF patients age 10-18 years at a baseline and one month visit. All airway samples underwent standard microbial culture for CF pathogens and 16S rRNA gene sequencing. For 3 subjects, we quantified total bacterial DNA and three bacteria of interest (*H. influenzae, Prevotella melaninogenica,* and *P. oris)* using qPCR.

Results: 16S rRNA gene sequencing identified a mean of 7.3 ± 2.3 bacterial genera from each airway sample with 3.1 ± 0.7 genera representing ≥ 10% of the clone library. There was no significant difference in the number of genera identified in throat swab, expectorated or induced sputum samples. The most common bacterial genera identified were *Prevotella*, *Streptococcus*, *Staphylococcus*, *Haemophilus*, and *Veillonella*. *H. influenzae* and *S. aureus* were identified by gene libraries when present in quantities $>10^5$ cfu/ml by microbial culture. Total bacterial DNA in airway samples from 3 subjects was quantified with a mean 6.45 x 10^7 bacterial rDNA copies/ mL detected from each sample. Quantification of specific bacteria yielded the following (expressed as mean rDNA copies/mL): *H. influenzae* 7 x 10^6, *P. melaninogenica* 6.8 x 10^5 and *P. oris* 2.5 x 10^4. In 9/18 samples, *H. influenzae* was detected by qPCR but not by culture.

Conclusions: 16S rRNA gene sequencing identifies uncultured bacteria, including anaerobes, from CF airway samples. Gene sequencing appears less sensitive than culture, missing CF pathogens present in $<10^6$ cfu/mL. Conversely, qPCR may be more sensitive than culture and allows quantitation of bacteria without the need for culture.

53. Identification Of Specific Epitopes Recognized By PR3-ANCA.

Francisco Silva, Amber Hummel, Ulrich Specks.
Thoracic Disease Research Unit, Mayo Clinic, Rochester, Minnesota.

Objective: Anti–neutrophil cytoplasmic antibodies against proteinase 3 (PR3-ANCA) are useful for the diagnosis of WG and have been implicated in the pathogenesis. Even though a correlation between PR3-ANCA and disease activity or extent has been shown in groups of patients, the role of serial ANCA testing in the clinical management of individual patients remains unclear. Inhibition studies with monoclonal antibodies (moAbs) against PR3 have shown that PR3-ANCA react with a small group of epitopes on the target antigen. However, these epitopes have not been identified. The recognition of subsets of PR3-ANCA reacting with specific epitopes could clarify our understanding of their pathogenic role and possibly provide better biomarkers of disease activity or prognosis than standard ANCA testing. The aims of this study were to characterize specific epitopes for PR3-ANCA on PR3 and evaluate the presence of subsets of PR3-ANCA in patients with WG recognizing these epitopes.

Methods: TALON-beads were coated with recombinant human PR3 (hPR3) and 10 chimeric human/mouse PR3 variants (mH1-5 and Hm1-5) identifying 5 specific epitopes not conserved between human and mouse PR3. The recognition of these antigens by moAbs and patient PR3-ANCA was analyzed by flow cytometry using FITC-conjugated secondary antibodies. For competition studies, unlabeled moAbs and patient sera were used as competitors for FITC-conjugated moAbs. The recognition pattern of the chimeric PR3 by sera of 48 PR3-ANCA(+) patients was evaluated.

Results: First, our moAb competition studies confirmed a previous report indicating that anti-PR3 moAbs can be grouped based on epitope recognition: 6A6, 12.8, PRG-2 (group 1), PR3G-4 (group 2), 4A5, WGM2 (group 3), 4A3 and MCPR3-2 (group 4). Second, the analysis of previously uncharacterized moAbs allowed their assignment to group 3 (MCPR3-3), group 4 (MCPR3-1), and an additional group 5 (MCPR3-7, MCPR3-11). The differential recognition of epitope-specific chimeric PR3 molecules by the different moAbs allowed us to identify the epitopes recognized by group 1, group 2 and group 5 moAbs. The analysis of 48 patient sera showed the presence of different patterns of epitope recognition in individual patients.

Conclusions: The previous epitope map is confirmed and a topographic assignment of the epitopes recognized by anti-PR3 moAbs was achieved. Subsets of PR3-ANCA were recognized in patients defined by different epitopes on PR3. Their clinical significance as potential biomarkers deserves further study in well-characterized patient populations.

54. Lessons from the Amish

Hilda Morillas MD[1], Maimoona Zariwala PhD[1], Margaret Leigh MD[1], Tom Ferkol MD[2], Susan Minnix RN[1], Mariana Schmajuk BS[1], Johnny Carson PhD[1], Milan Hazucha PhD[1], Eric Puffenberger MD[3], Kevin Strauss MD[3], Holmes Morton MD[3], Michael Knowles MD[1]
[1]University of North Carolina, Chapel Hill, NC [2]Washington University, St Louis, MO, [3]Clinic for Special Children, Lancaster, PA

Objective: Primary ciliary dyskinesia (PCD) is a rare, autosomal recessive disorder associated with abnormalities in the structure/function of respiratory cilia and sperm flagella. It is a genetically heterogeneous disease caused by loss of function mutations in the inner and outer dynein arms of respiratory cilia. Thus far, disease-causing mutations have been identified in many PCD patients in *DNAH5* and *DNAI1*, which encode for proteins in the heavy and intermediate chains of the outer dynein arms (ODA), respectively. In rare instances, PCD has been found to be caused by mutations in *DNAH11*, *RPGR* and *OFD1*. Prior experience studying a consanguineous Amish community in Missouri led us to understand that the disorder is genetically heterogeneous in this community

Methods: A child from an Amish community in Pennsylvania with congenital heart disease and situs inversus totalis was referred to our Genetic Disorders of Mucociliary Clearance research center for diagnostic evaluation for PCD; plus one of his siblings had a history suggestive of PCD. Both had low nasal nitric oxide (nNO) and EM's with absent ODA. Subsequently, we visited the Clinic for Special Children to educate and evaluate multiple patients from several families with the typical clinical phenotype. Twenty-two subjects were evaluated for PCD with a history and physical exam, echocardiogram, spirometry, nNO measurements, nasal scrape for ciliary ultrastructure EMs, and blood samples for DNA analysis.

Results: Seven patients within this Amish community were found to be homozygous for the same disease-causing mutation in *DNAH5*, but others did not have this mutation in *DNAH5*, or *DNAI1*.

Conclusion: PCD is a heterogeneous genetic disorder that is seen in high prevalence in some consanguineous communities. However, not all affected individuals are homozygous for mutations in the same gene; thus using homozygous gene mapping to identify disease causing mutations in such a community can occasionally miss the causative genetic mutation. Utilizing whole genome SNP testing in this population may assist in identifying PCD loci and therefore allow novel mutations to be discovered. However, the analysis must consider genetic heterogeneity.

55. Limited Utility of Rapamycin in Severe, Refractory Wegener's Granulomatosis

Curry L. Koening M.D., Jose Hernandez-Rodriguez M.D., Eamonn Molloy M.D. Tiffany Clark N.P., Carol A. Langford M.D. M.H.S, Gary S. Hoffman M.D. M.S.

Introduction: New therapeutics that can induce and maintain remission without incurring substantial toxicity are needed in the treatment of WG. WG is a disease in which enhanced macrophage, neutrophil, T cell, and B cell activation plays an important role in pathogenesis. Rapamycin, a macrocyclic lactone, inhibits T and B cell proliferation and activation by inhibiting the function of mammalian target of rapamycin (mTOR). We report our experience with the use of rapamycin in 8 patients who failed to achieve sustained remission or developed substantial toxicities from conventional therapy.

Methods: A retrospective chart review was conducted on 8 patients who fulfilled the 1990 ACR criteria for WG and received oral rapamycin between February 1, 2004 and April 1, 2007. To have received rapamycin, patients had to have developed toxicities precluding the use of conventional therapeutics or failed to achieve remission after receiving a cytotoxic agent plus prednisone at doses of ≤10mg/day.

Results: Eight patients with WG were treated with oral rapamycin with doses ranging between 1 and 4 mg / d. The median time from disease onset to the start of rapamycin was 6.5 years (range 1-14) with a median trial of 4.5 (range 3-8) different therapeutics prescribed prior to the initiation of rapamycin. As of April 1, 2007, 3 of 8 patients continued to take rapamycin. Only one had a sustained remission defined as no features of active disease while receiving ≤10mg/day of prednisone. Another patient had a stable but persistent lung nodule with no other features of active disease while a third patient had a disease relapse after 45 weeks on rapamycin. Even though no patient was able to discontinue prednisone, all three were able to taper to ≤10mg/day of prednisone. For 5 of 8 patients in which rapamycin was discontinued, two patients stopped the drug due to continued disease activity. One continued to have progressive lung nodules and later developed invasive lung aspergillosis while the other had progressive orbital WG. Two patients had their rapamycin stopped after developing cancer while a fifth patient developed pseudomonas pneumonia and had to discontinue the drug. Of the three patients who had a relapse while on rapamycin, each required additional immunosuppressant agents to induce remission. Other adverse events included one patient with supra-therapeutic drug levels and elevated liver function tests that improved with lowering the rapamycin dose. One patient developed oral ulcers and another, leukopenia. Both improved once the dose of rapamycin was lowered.

Conclusion: For theoretical reasons, rapamycin would appear to be a promising agent in the treatment of WG. Although our treatment group was small and our patients treatment resistant, the absence of clear cut efficacy and the presence of adverse events has discouraged us from further investigation of this agent in WG.

56. Atypical Findings of Fixed Weakness in a Family with Paramyotonia Congenita

Mohammad Kian Salajegheh[a], Kristen Whiteside[a], CINCH Study Group[b]; Anthony A. Amato[a]

[a] Brigham and Women's Hospital, Harvard Medical School, Boston, MA

[b] Consortium for Clinical Investigation of Neurologic Channelopathies, University of Rochester, Rochester, NY

Objective: To present a family with typical features of paramyotonia congenita (PMC) and fixed weakness of the deep finger flexors.

Methods: We describe two patients from a family with PMC. We present their clinical findings, the results of their ulnar-abductor digiti minimi (ADM) short and long exercise studies, needle electromyography (EMG) and response to cooling. We compare our findings with previous reports in the literature.

Results: Our patients included a father and a son, from a family with members demonstrating an autosomal dominant pattern of muscle stiffness and weakness since childhood. Their clinical presentation included episodes of stiffness followed by muscle weakness induced by cold and exertion, involving the face, tongue, pharynx and limb muscles; eye closure paramyotonia; handgrip myotonia; mild fixed proximal limb weakness and forearm atrophy in the father; and deep finger flexor weakness, severe in the father and mild in the son. On electrophysiologic studies in both patients, the long exercise test demonstrated reduction in the ulnar-ADM compound muscle action potential (CMAP) post-exercise, with delayed recovery to baseline; the short exercise test demonstrated post-exercise myotonic potentials (PEMP), and reduction in the ulnar-ADM CMAP post-exercise that worsened with repetition; the needle EMG demonstrated widespread myotonic potentials; in response to cooling to -20 degrees Celsius, there was severe post-exercise reduction in the ulnar-ADM CMAP and disappearance of myotonic potentials, accompanied by clinical weakness of the ADM muscle. The electrophysiological findings were similar to what has been described as pattern 1 of Fournier and consistent with that of patient with PMC. Basic laboratory workup was normal for both patients, except for mild elevation in serum creatine kinase levels in the father. We are in the process of determining the genetic mutation responsible for the channelopathy present in the two patients.

Conclusions: Patients with typical clinical and electrodiagnostic features of paramyotonia congenita may present with fixed deep finger flexor weakness and forearm atrophy, similar to what has been described for patients with inclusion body myositis.