

N-Carbamylglutamate Accelerates Ureagenesis in Patients with Hyperammonemia

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The urea cycle is responsible for nitrogen regulation and balance in humans. Disruption of normal urea cycle function leads to hyperammonemia, neurological sequelae and can result in death. Current treatment for hyperammonemia is based on dietary protein restriction and ammonia-scavengers, but these interventions commonly fail to prevent hyperammonemia and avert brain damage. The carbamyl phosphate synthase I (CPSI) reaction, the first and rate-limiting step of the urea cycle, requires an allosteric activator, N-acetylglutamate (NAG). A shortage of NAG causes decreased flux through CPSI and is responsible for hyperammonemia in many conditions. Though orally administered NAG is hydrolyzed *in vivo*, N-carbamylglutamate (NCG) is a stable analog of NAG which is resistant to hydrolysis.

Objectives: In this study, we investigate the use of N-carbamylglutamate (NCG), a new therapeutic agent for a subset of hyperammonemic conditions which are caused by a deficiency of NAG, including NAG synthase deficiency, propionic acidemia (PA) and methylmalonic acidemia (MMA) as well as in partial CPSI deficiency.

Methods: Urea cycle function was assessed through stable isotope ¹³C studies before and after a 3-day trial of oral NCG in a total of 14 subjects with NAGS deficiency, partial CPSI deficiency, PA or MMA. Plasma urea, ammonia, and amino acids were also measured.

Results: Oral NCG improves urea cycle function and decreases plasma ammonia levels in patients with NAGS deficiency, partial CPSI deficiency, PA and MMA. In 2 patients with NAGS deficiency, peak ¹³C-urea increased from 0.25 and 0.07 $\mu\text{mol/l}$ to 2.3 and 2.0 $\mu\text{mol/l}$, respectively. In our largest cohort, 7 patients with PA, ¹³C-urea peak increased from 2.2 (95% CI: 1.5-3.1) to 3.8 $\mu\text{mol/l}$ (95% CI: 2.9-5.0).

Conclusions: NCG may be a useful adjunct in the treatment of acute hyperammonemia of various metabolic etiologies that are due to a lack of NAG. Also, NCG can restore ureagenesis in NAG synthase deficiency and is therefore curative for patients with this condition.

Cellular and Genetic studies in the Neonatal Progeroid Syndrome (Wiedemann-Rautenstrauch)

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Objective: Wiedemann–Rautenstrauch syndrome (WRS) is a rare autosomal recessive disease with features of premature aging recognizable at birth, characteristic that makes it different from Hutchinson-Gilford progeria syndrome (HGPS). The etiology of WRS remains unknown. The present study aims to elucidate the cellular and genetic basis WRS.

Methods: We clinically delineate four WRS patients. The entire Lamin A/C coding regions were amplified in four WRS patients using PCR, and the fragments were purified and sequenced. Fibroblasts from two patients were analyzed for Lamin A/C expression profile, growth kinetics, telomere length (real-time PCR) and nuclear morphology (confocal microscopy), and compared them with control and HGPS fibroblasts. To begin the genetic mapping of the causative gene associated to WRS, we performed homocigosity mapping using Affymetrix 6.0 platform.

Results: Mutations of lamin A were excluded as the cause of WRS. HGPS cells have irregular shape of its nuclear envelope, associated to expression of progerin. In contrast, WRS cells have small and rounded nuclei, even when compared to control cells, and did not express progerin. Growth kinetics is slower than normal cells and the expression of senescent markers occurs earlier. WRS telomere-shortening rate is similar to control cells, but much slower than in HGPS. However the critical telomere shortening in WRS occurs in earlier population doublings compared to controls and is associated to increase expression of P16. Homozygosity mapping highlight two candidate regions (chromosomes 6 and 8) in which the gen responsible for WRS may reside.

Conclusions: These results suggest that alteration of lamin A/C is not the cause of WRS. However, we could not rule out the possibility that a gene controlling nuclear volume may be the cause of this neonatal progeroid syndrome. Telomere-shortening rate in SWR fibroblasts do not drive in a determinant way the senescence process, contrary to what happen in HGPS. Studies of candidate genes present in chromosomes 6 and 8 are under way.

Funded by COLCIENCIAS and Universidad Nacional de Colombia.

Early Identification of Rare Syndromic Forms of Diabetes Mellitus

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Objective: Syndromic type 1 diabetes (T1DM) represents a rare subset of heterogeneous disorders. Many (including Wolfram syndrome and mitochondrial DNA disorders) share common syndromic phenotypes despite diverse molecular etiologies.

Methods: We have initiated screening for rare forms of diabetes at the Barbara Davis Center for Childhood Diabetes (seeing 80% of Colorado children with T1DM). Patients with new onset T1DM were tested for GAD, IA2, IAA, and ZnT8 autoantibodies, which detect >90% of autoimmune diabetes. We examined individuals with type 1 diabetes diagnosed under age 25 who had known clinical and antibody status. When suspected, further commercial molecular testing was performed in search of monogenic etiologies.

Results: Of 2,603 subjects, 320 (12%) were negative for all 4 autoantibodies. One autoantibody negative child, with onset of diabetes at age 4 was autoantibody negative and had HLA class II haplotype considered protective for autoimmune diabetes (DQA1*0102-DQB1*0602). Thus, it was suspected that the child did not have common (immune mediated) T1DM. Visual acuity changes developed with macular granularity at age 6 and macular disc pallor at age 8. *WFS1* gene analysis was pursued and showed two mutations (P540X and R147fsX163) indicating Wolfram syndrome (Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy, Deafness or DIDMOAD). Initial analysis of 2,603 subjects revealed 23 known genetic diagnoses including Down syndrome (9), Marfan (2), Turner, Shprintzen, Beckwith-Wiedemann, Kabuki, Potocki-Lupski, Duchenne muscular dystrophy, Friedrich Ataxia, osteogenesis imperfecta, cystic fibrosis, alpha-1-antitrypsin deficiency, chromosome 16 aberration, and fetal alcohol syndrome. In the remaining subjects comorbidities possibly associated with syndromic T1DM included seizure disorder (18), cognitive deficits (32), ophthalmologic abnormalities (7), hearing loss (21), and psychosis/bipolar disorder (18). Correlating state-of-the-art autoantibody testing with clinical signs of syndromic T1DM is currently underway.

Conclusion: As a sub-category of T1DM, syndromic (often monogenic) diabetes mellitus represents a unique and heterogeneous group of disorders. With recent advancements in serologic and molecular testing identifying the etiology of these disorders has improved. Further characterization could offer insight into links between endocrinologic, cardiologic, ophthalmologic, psychiatric and neurologic diseases, as well as improve clinical outcome by early detection. Testing all four known diabetes-associated autoantibodies as well as closer investigation of atypical clinical signs is likely a good first step to distinguish monogenic/syndromic T1DM from typical T1DM.

18F-DO PA PET/CT Scan for Pre-Operative Localization of Focal Lesions in 105 Infants with Hyperinsulinism

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Objectives: Half of infants with medically-uncontrollable congenital hyperinsulinism have focal adenomatosis lesions in the pancreas due to isodisomy for a paternally derived recessive mutation of the ATP-dependent potassium channel genes, *ABCC8* or *KCNJ11*. Pre-operative diagnosis and localization is important to allow potential cure of the lesion by local resection. The purpose of this study was to prospectively determine the sensitivity and specificity of F-DOPA PET/CT in infants who required pancreatic surgery and had a histologic diagnosis of focal or diffuse hyperinsulinism.

Methods: Results were made available to the surgeon to use in searching for focal lesions after the first 5 cases. Patients with diffuse hyperinsulinism on intra-operative frozen section examination underwent near-total pancreatectomy. Patients with no evidence of diffuse hyperinsulinism underwent exploration and additional biopsies to detect and resect the focal lesion.

Results: From 12/04 to 12/08, 105 infants with medically-uncontrollable congenital hyperinsulinism underwent pre-operative 18F-DOPA PET/CT imaging of the pancreas. Of these, 93 cases were evaluable for this study. 52 cases had focal adenomatosis. F-DOPA PET/CT identified a focal area of uptake in the pancreas in 46 of the focal cases. In 6 cases, focal lesions were not detected by F-DOPA PET/CT, but were found at surgery. Five of these were small lesions < 0.5 cm in diameter and all were identified and resected during surgery; one case had an extensive adenomatosis lesion occupying 80% of the pancreas. 14 of 52 focal cases required resection of the head of the pancreas and a Roux-en-Y anastomosis of the tail to the jejunum. In all of the diffuse cases, F-DOPA PET/CT scans were read as diffuse uptake.

Conclusions: In this series, F-DOPA PET/CT imaging had a high rate of success in detecting focal lesions and was 100% accurate in localization. These results indicate that FDOPA PET/CT imaging should be considered in those infants with congenital hyperinsulinism who require surgery.

CpG Island Methylation Profiling in Human Salivary Gland Adenoid Cystic Carcinoma

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Objective: A better understanding of key molecular changes during the pathogenesis of salivary gland adenoid cystic carcinoma (ACC) could impact strategies to reduce recurrence and mortality from this cancer. Two epigenetic events involved in the pathogenesis of cancer are hypermethylation of tumor-suppressor gene promoters associated with transcriptional repression and hypomethylation associated with gene reexpression and genomic instability. The aim of this study was to identify genes (1) strongly deregulated by epigenetic CpG island methylation, (2) involved in development and survival of ACC.

Methods: We analyzed 16 patients (tumor ACC and matching normal tissue) for aberrant hypermethylation using the methylated CpG island amplification and microarray (MCAM) method. For validation, CpG island regions of four genes, showing highest changes in methylation with the MCAM method, were analyzed by Pyrosequencing.

Results: The hyper- and hypomethylation tables showed variable ratio between ACC and normal tissue: 9 - 37 fold increase in hypermethylation for 13 associated genes, and 9 -11 fold changes in hypomethylation for only two associated genes. Strongest hypomethylations occur in regions near the TSS of PARVG, and has-mir-339. Strongest hypermethylations occur in regions around TSS of genes encoding predominantly transcription factors: EN1, PITX1, FOXE1, LXB2, IRX1, FOXI2, FOXA1, TBX4, and GBX2, except for 4 other genes: epoxide hydrolase 3 (EPHX3, removal of mutagenic epoxides), MT1G (or MT1H, cellular processes on binding, storage, and transport of Zn /metal ions), prestin (SLC26A5, transmembrane transport,) and FNDC1 (desmoplastic response). 4 CpG island regions, showing highest hypermethylation in MCAM, and associated with EN1, PITX1, FOXE1, and TBX4 were validated by Pyrosequencing. This method supported the MCAM results, with an average difference of 50% in CpG hypermethylation over all 4 gene regions investigated. The resulting 13 genes associated with the highest CpG island hypermethylations in neoplastic tissue imply, that these genes are actively expressed in normal tissue and silenced in salivary gland ACC. These results are supported by literature, where therefore mentioned genes with their expressed proteins are known to be involved in various types of cancers, e. g. PITX1 as activator of the tumor suppressor p53.

Conclusion: The 13 strongest hypermethylated genes in ACC of salivary gland, with 9 of them expressing transcription factors, and 4 expressing other genes are known to be involved in developmental, apoptotic and other cancer inducing or supporting processes. These silenced genes most probably constitute main molecular markers for quality and severity in outcome of ACC in salivary gland. These genes furthermore provide clues, for which cancer-related pathways need to be intercepted for therapeutical approaches to suppress ACC.

Multiplicity of Brain AVMs in Hereditary Hemorrhagic Telangiectasia

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Background: Hereditary hemorrhagic telangiectasia (HHT) is a rare autosomal dominant vascular disorder characterized by mucocutaneous teleangiectasias and visceral AVMs, most commonly of the lungs, brain (BAVM), gastrointestinal tract, and liver. The diagnosis is primarily clinical, however genetic testing can be helpful in some cases. The prevalence of BAVMs has been known to be elevated in patients with HHT, estimated at 5-15%. In addition, it has been suggested that the prevalence of multiple BAVMs is greater in patients with HHT than in those with sporadic BAVMs, however the magnitude of this difference remains unclear.

Purpose: To compare the frequency of BAVM multiplicity in patients with HHT compared to those with sporadic BAVMs.

Methods: Retrospective review of clinical and imaging records in all patients with BAVMS referred to two large North American tertiary referral centers over the past 25 years. We present here our preliminary results.

Results: A total of 1998 patients were included. Out of all BAVM patients, 2.8% had diagnosed HHT and 1.9% had multiple BAVMs. Out of patients with multiple BAVMs, 59% had HHT. Of all BAVM patients with HHT, 39% had multiple BAVMs, while in BAVM patients without HHT, 0.7% had multiple BAVMs. Multiple BAVMS were significantly more common in patients with HHT. The odds ratio for HHT in patients with multiple BAVMs was 83 (95% CI: 40-174).

Conclusion: These results indicate that multiplicity of BAVMs is much more common in patients with HHT than in patients with sporadic BAVMs, indicating that this may be a useful feature for identification of these patients.

The GLP-1 Receptor Antagonist Exendin-(9-39) Elevates Fasting Blood Glucose Levels in KATP Hyperinsulinism

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Background: Congenital hyperinsulinism due to mutations in the KATP channel (KATPHI) is a rare disease characterized by severe hypoglycemia unresponsive to current medical therapies. In preclinical studies we demonstrated that the glucagon-like peptide-1 receptor (GLP-1r) is constitutively active in islets lacking KATP channels and that antagonism of the GLP-1r by exendin-(9-39) (Ex-9) suppresses insulin secretion and corrects fasting hypoglycemia in *SUR-1^{-/-}* mice. We hypothesized that antagonism of the GLP-1r by exendin-(9-39) will increase fasting blood glucose levels in subjects with KATPHI.

Objective: To evaluate the effect of exendin-(9-39) on fasting blood glucose, plasma insulin, glucagon and GLP-1 levels in subjects with KATPHI.

Design/Methods: Subjects (n=9, 6F) with KATPHI ages 15-47 yrs were admitted to the CTRC unit. After an overnight fast, subjects received an intravenous infusion of vehicle (0.9%NaCl) for 1 hr followed by exendin-(9-39) at three different doses (100, 300 and 500 pmol/kg/min) for 2 hrs each or vehicle for 6 hrs. Subjects underwent vehicle and exendin-(9-39) infusions in randomized order and on different days. Samples for blood glucose, insulin, glucagon, and GLP-1 levels were obtained at multiple time points.

Results: In all subjects fasting blood glucose levels were higher during exendin-(9-39) infusion compared to vehicle (105±23.2 vs. 84.2±12.2 mg/dL, p<0.01). Six of the subjects had hypoglycemia during vehicle infusion while none of them had hypoglycemia during exendin-(9-39) infusion. Fasting insulin to glucose ratio was significantly lower during exendin-(9-39) infusion compared to vehicle (0.08±0.03 vs. 0.12±0.04, p=0.01). Fasting glucagon and GLP-1 levels were not different during exendin-(9-39) infusion compared to vehicle.

Conclusions: Our findings suggest that GLP-1 and its receptor may play a role in the pathogenesis of hyperinsulinism. The promising results with exendin-(9-39) in subjects with KATPHI suggest that GLP-1r antagonism may have a therapeutic role in this disorder.

Pilot Study of Primary Prevention and Screening on Environmental Factors in Pathogenesis of Sjogren's Syndrome Associated Salivary and Lacrimal Gland Lesions

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Introduction: Sjogren's syndrome is chronic autoimmune inflammatory rheumatic disease, characterized by lymphocytic infiltration of salivary and lacrimal glands, causing dryness of mouth and eye. Sjogren's syndrome affects mainly women 90% of cases in their 40-50s, though younger age onset is also characteristic. Later disease is progressed and other organs are involved as well. There is substantial risk of development of malignant lymphomas and characterized by 44-fold increased risk compared to general population. Environmental factors are thought to trigger inflammation in individuals with a genetic predisposition for the disease.

Objectives: The goal of primary prevention is to avoid the development of cancer by reducing or eliminating exposure to cancer-causing factors. Primary prevention of Sjogren's syndrome is directed to increase awareness of harmful environmental conditions, which may be avoidable, also to educate recognition of early warning symptoms of this condition. Timely diagnosed lesion is better treatable and late onset complications are also preventable. The aim of "Georgian Sjogren's Syndrome Association" is to perform screening and provide prevention of Sjogren's syndrome in Georgia. In cooperation with the "Alliance for Rare Diseases of Georgia" we apply our joint efforts in the prevention of this disease among women population in Georgia.

Methods: With the support and assistance from the specialized ophthalmologic clinics we performed primary prevention and screening (pilot study) of susceptible individuals on 12 June 2010 during Rustavi Women's Health Fair - Celebrating Healthy Women (organized by Peace Corps Georgia Volunteers and Young Initiators. Primary prevention at the population, as well as individual i.e. patient-doctor communication level was performed to avoid initiation of pSS by means of declining impact of harmful environmental factors, such as smoking, air conditioning, etc. Activities for primary prevention were undertaken, particularly: I. Short presentation on the following topics: Age related hormonal changes, environmental factors and genetic predisposition in triggering primary Sjogren's syndrome associated salivary and lacrimal gland lesions. Recommendations and measures on primary prevention. Role of stress in the development of autoimmune diseases. Stress management techniques. What is the benefit of prevention of autoimmune diseases for the women in their peri/post menopausal periods of life. II. Training - Primary prevention for Sjogren's syndrome - recognition of the causes and outcomes of dry eye and mouth symptoms. III. Health consulting - Examination of dry eye and dry mouth complaints in susceptible population. Individual and population level primary prevention and screening (pilot study).

Results: Out of 85 examined patients 8 revealed combined dryness of eye and mouth, 7- isolated eye, and 3 - mouth dryness.

Conclusion: Based on the results of pilot study for the further study was chosen individual-level i.e. secondary screening and prevention at the patient-physician level versus population-level approach.

Microarray Analysis of Multiple Intestinal Atresia with Immunodeficiency and Identification of Candidate Genes

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Objective: Multiple intestinal atresia with immunodeficiency (MIA-I) is characterized by multiple atretic regions throughout the gut, variable combined immunodeficiency, and extremely poor survival. Although the pathogenesis is unknown, familial case reports suggest an autosomal recessive pattern of inheritance. We used single nucleotide polymorphism (SNP) and expression microarray platforms to identify candidate genes and cell signaling pathways in a cohort with MIA-I.

Methods: DNA was isolated from whole blood or saliva of living affected individuals and their families, and from paraffin-embedded tissues of deceased affected individuals. RNA was isolated from whole blood and primary T cell cultures from an affected female infant and her unaffected parents. Gene expression profiles were examined using an mRNA expression microarray platform. Genotyping and copy number variation (CNV) analysis was completed using a genome-wide SNP microarray platform. Transmission/disequilibrium test analysis was performed to identify associations with the MIA-I phenotype. Genes and pathways of significance based on log-fold change (affected versus unaffected) and p-value were evaluated using dCHIP software and a curated web-based analysis software program.

Results: Initial homozygosity mapping from two affected siblings identified regions of homozygosity, including loci at 1p35.2, 2p23.1, 4q21.1, and 11q22.1. Candidate genes and regions of interest within these loci include: *XDH*, the *CXCL* locus (chromosome 4), *SERINC2*, and *FABP3*. CNV analysis did not identify significant differences. Top canonical pathways of highest significance in expression microarray analysis include: 1) The antigen presentation pathway, 2) NFAT in regulation of the immune response, 3) IL-4 signaling, 4) CD28 signaling in T helper cells, and 5) T cell receptor signaling. Within these pathways, mRNAs of interest with significant underexpression (log ratio ≤ -2) include *c-fos*, and Fc γ RI alpha (CD64a). *TCF7*, involved in Wnt signaling and T cell development, demonstrated a 4.2-fold decrease in expression.

Conclusions: MIA-I results in total loss of gut function and has an extremely poor prognosis. Microarray analysis of MIA-I affected against unaffected samples in one family demonstrates altered signaling in multiple T cell-associated pathways. SNP and expression microarray analysis can be used to identify gene candidates in this rare disorder that would not otherwise have been considered in a hypothesis-driven approach.

Use of Informatics and Social Networking Recruitment Strategies Conducting Research on Children with Klinefelter Syndrome

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Objective: Children with genetic conditions pose unique challenges in study design, recruitment, and logistics of participation. The purpose of this poster is to describe traditional and novel strategies that were successful in recruitment of children with Klinefelter Syndrome (KS) and lessons learned.

Background: The prevalence of KS is estimated to be between 1 in 500-700 male births, but many individuals with KS remain undiagnosed or are diagnosed later in life. The pool of potential research subjects is therefore limited. Personal networking with families using an education-based approach has provided an extra measure of success in recruiting for this study.

Method: We are conducting a cross-sectional study of 40 KS boys between the ages of 8 and 18 years at an academic medical center in New York City. We began subject recruitment using traditional approaches such as direct mail, community-based posters and clinical referrals. Our recruitment strategy expanded with the use of information technologies such as email, patient advocacy organization list serves, a recruitment website, teleconference and computer-based social networking. Figure 1 illustrates the study website response after placing an advertisement on a social network site.

Results: Currently the enrollment is 30 (26% by traditional recruitment and 64% using novel approaches). The addition of information technologies, education-based networking and offering off-site participation opportunities has created a net recruitment improvement of 38%.

Conclusions: Traditional approaches to recruitment failed to achieve subject recruitment goals. Ascertainment of potential recruits requires innovative recruitment strategies. While small sample size continues to be a challenge, integration of novel recruitment strategies has drawn an international sample to our study.

The Manton Center: A Home for Orphan Diseases

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Objectives: To create the first comprehensive center focused on the investigation of any and all rare diseases. To provide the funding and infrastructure to support, emphasize and stimulate the efforts of the institution's faculty with the goal of developing better diagnostic, therapeutic, and ultimately, curative approaches for rare diseases. To foster collaborations within the rare disease community.

Methods: First, we identified the weaknesses of rare disease research:

- small population size
- difficulty in finding start-up funding for research
- lack of interest for career focus on rare disease
- lack of collaboration
- lack of infrastructure for continued support and collaboration

We combated these problems and created funding opportunities for research, sponsorship for patient and health care provider events, funding for research efforts of investigators who are the experts in their respective fields, and state-of-the-art human research protocol, the "Gene Discovery Core" (GDC) that equally benefits participants and physicians. The GDC is a repository established for studying and storing samples and medical/family histories. By collecting DNA/tissue samples and associating them with clinical information the GDC serves as a resource that investigators can utilize to further the study of orphan diseases. Participants in our research receive the dual benefit of being part of a de-identified rare disease repository (GDC) and have their care providers perform targeted analysis without a specific IRB-approved protocol. This expedites the discovery of new mechanisms of human disease. The Center provides the enrollment, data collection, sample collection, and DNA extraction for investigators as well fund the efforts of smaller investigators.

Results: Several individuals with rare and unclassified disorders have been enrolled and genetic analysis has begun. Ongoing work is proving the above efforts to be improving research initiatives on orphan disease research and is strengthening the correct work of staff and harboring interest in rare diseases for young investigators.

Conclusions: The Manton Center has created the infrastructure needed to efficiently carry out research on rare diseases. Several grant projects have been initiated which are making strides in identifying new genetic etiologies in various rare disorders. Because of the new opportunities the Center is creating, junior faculty members are expressing interest, stimulating a new generation of researchers invested in careers centered around orphan diseases. The Center's programs reduce the obstacles between rare disease research and scientific discovery.

The Diagnostic Dilemma of Primary Ciliary Dyskinesia: Experience of the GDMCC Consortium

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Objective: Primary ciliary dyskinesia (PCD) is a heterogeneous recessive genetic disorder characterized by abnormal ciliary function manifest as neonatal respiratory distress, chronic oto-sino-pulmonary disease, bronchiectasis, and infertility; 50% have situs inversus or heterotaxy. Identification of hallmark defects in ciliary ultrastructure by electron microscopy (EM) is the gold standard for PCD diagnosis, but PCD occurs in patients with normal EMs, as validated by genetic mutations in *DNAH11*. Nasal NO (nNO) is a useful screening test for PCD, but EM is confirmatory. We sought to assess the accuracy of "presumptive" diagnoses of PCD in the US.

Methods: We reviewed diagnostic evaluations from 551 patients referred for "genetic disorders of mucociliary clearance", which included a detailed history, physical exam, chest radiographic studies, nNO measurements, and EM evaluation of ciliary ultrastructure using a standardized protocol and blinded review by three experienced investigators. Using all available data, we assessed the accuracy of the diagnosis of PCD.

Results: There were 464 patients referred with a "presumptive" diagnosis of PCD, and we were able to confirm (n = 205; 44%) or refute (n = 142; 31%) a diagnosis of PCD in 347 (75%). the remaining 117 (25%) patients had "probable PCD", defined as a compatible clinical phenotype and low nNO, with either normal EMs or adequate EMs not yet completed. A subset of 153 subjects referred with the diagnosis of PCD, reportedly "confirmed" by EM abnormalities at outside institutions, underwent repeat nasal ciliary EM imaging. Confirmation of a hallmark PCD EM defect was made in only 115 (75%), whereas 38 (25%) had normal EMs. Of the 87 patients without a presumptive diagnosis of PCD on referral, 1 (1%) was confirmed to have PCD by ciliary EM defects, and an additional 9 (10%) were thought likely to have PCD based on nNO and phenotypic presentation, but no EM evidence of a ciliary defect.

Conclusions: Making the diagnosis of PCD relies on interpreting and integrating evidence of key features of clinical phenotype, nNO measurements, EM, and genetic studies, when possible. The current use of ciliary EMs in the general medical community to support a diagnosis of PCD is constrained by technical limitations of EM quality and reader experience, limiting sufficient standardization.

Supported by NIH Research Grant 9 U54 HL096458-06, funded by the Office of the Director, and supported by ORDR and NHLBI, NIH.

Development of a Test to Screen Newborns for the Neurodegenerative Disease Cerebrotendinous Xanthomatosis

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Objective: The prevalence of cerebrotendinous xanthomatosis (CTX) in the US population is estimated to be as high as 3 to 5 per 100,000, predicting a minimum number of 8,400 affected individuals. CTX is therefore almost certainly severely under diagnosed with known patients in the US numbering less than 100 (<http://ctxinfo.org/>). As treatment of CTX from the preclinical stage appears to prevent the onset of disease complications, the value of an early diagnosis for this disease cannot be overstated. We set out to develop an LC-MS/MS (MS²) based test capable of identifying bloodspots from newborns affected with CTX.

Methods: Blood bile alcohols (BAs) possessing a 3-oxo-4-ene and 3-oxo structure were detected in a targeted manner utilizing derivatization with Girard's P reagent (1-(carboxymethyl) pyridinium chloride hydrazide) and LC-ESI-MSⁿ analysis performed with an LTQ-Orbitrap instrument. Girard's P reagent reacts with the BA oxo moiety to form charged hydrazone derivatives that are sensitively detected with ESI and that provide a characteristic neutral loss of 79 Da (pyridine) with MS² analysis. Girard's P-tagged BAs were resolved using HPLC and identified by high resolution exact mass analysis (± 5 ppm), with MS² and MS³ spectra obtained to confirm BA identity. In addition to a dominant [M-79]⁺ fragment ion observed in the MS² ([M]⁺→) spectra, structurally informative fragment ions are observed in the MS³ ([M]⁺→[M-79]⁺→) spectra.

Results: Although the atypical BAs produced in adult CTX patients have been well-characterized, little is known about BAs that may be present in patients at birth. Utilizing Girard's P derivatization and LC-ESI-MSⁿ analysis we were able to profile BAs present in retrieved newborn bloodspots of patients diagnosed with CTX as teenagers. Differences in free BAs were observed that could be used to discriminate between retrieved CTX bloodspots (n=2) and residual bloodspots from unaffected newborns (n=10).

Conclusions: The ability to identify bloodspots from newborns affected with CTX enables screening for this disease at birth. Early identification and treatment of patients from birth can prevent development of the severe neurological complications associated with this rare disease.

Differences in Attention and Executive Functioning Between MPS Types I and II: Analysis of Test Performance and Quantitative MRI

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Objective: Specific profiles of neurobehavioral functioning and brain structure among MPS Types I and II are not well understood. Although patients with MPS Type IH are more cognitively impaired than some other MPS types, little is known about differences in attentional skills and executive functions, which are critical to academic progress and development of independent living skills. We seek to identify and describe differences in brain functioning, specifically attention and executive skills, as well as to correlate brain structures between MPS Types I and II.

Methods: Data were gathered from the first year of a prospective longitudinal study of MPS, which is funded by the Lysosomal Disease Network. We conducted neuropsychological testing and quantitative MRI studies of children with MPS Types I and II. Comparisons were made between children with MPS Type IH (MPSIH, N=10) who have undergone hematopoietic cell transplantation (HCT) and children with MPS Type II (MPSII, N=8).

Results: MPSIH had lower overall IQs than MPSII, $t(16)=-2.99$, $p<.01$. MPSIH also obtained lower scores than MPSII on a measure of attention and impulse control, $t(14)=-2.53$, $p<.03$ and spatial working memory (the ability to hold information in mind and manipulate it), $t(15)=-4.28$, $p<.002$. There was no difference in performance on a measure of reaction time, where both groups were low average, nor on a test of planning and problem solving, where both groups were average as compared to typically developing peers. Volume of the corpus callosum was positively correlated with spatial working memory ($p<.01$) for both groups combined.

Conclusions: Children with MPSIH had lower scores on attention, impulse control, and spatial working memory than those with MPSII. These findings are consistent with the broader HCT literature, which indicates that HCT has adverse effects on attention and executive functioning. Given that children with MPSIH do not have abnormal behaviors, their struggles with attention and executive functioning may go unnoticed due to quiet, compliant behavior in the classroom and at home. Evaluating for and treating these difficulties proactively will have important educational benefits. Additional correlations of brain volumetrics with these findings will be presented.

Plasma Bile Acids in Children with Smith-Lemli-Opitz Syndrome

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Objective: SLOS is a multiple congenital anomalies/mental retardation syndrome, due to deficiency of 7-dehydrocholesterol reductase in the final step of cholesterol biosynthesis. Most patients have cholesterol deficiency. Since cholesterol is the immediate precursor of bile acids, we hypothesized that bile acid synthesis would be reduced, and potentially qualitatively different. This study aims to analyze the plasma bile acid profile in SLOS patients.

Material and Method: Plasma bile acids, GCA (glycocholic acid), GUDCA (glycoursodeoxycholic acid), GCDCA (glycochenodeoxycholic acid), GDCA (glycodeoxycholic acid), TCA (taurocholic acid), TCDC (taurochenodeoxycholic acid), CA (cholic acid), and CDCA (chenodeoxycholic acid) were analyzed by LC-MS.

Results: 5 SLOS plasma samples (4F, 1M) were analyzed. The mean age at sample collection was 3 yrs (range 2-11 yrs).

Table 1: Plasma bile acid profiles in SLOS patients

Bile acids (mmol/L)	Patient #1	Patient #2	Patient #3	Patient #4	Patient #5	Mean (SD)	SEM
GCA	0.19	0.62	0.03	0.06	0.00	0.18 (0.25)	0.13
GUDCA	ND	ND	ND	ND	ND	ND	ND
GCDCA	1.34	3.39	0.61	0.60	0.56	1.30 (1.21)	0.61
GDCA	0.12	0.22	0.11	0.12	0.00	0.11 (0.08)	0.04
TCA	0.01	0.16	0.12	0.00	0.00	0.06 (0.08)	0.04
TCDC	0.55	0.48	0.00	0.07	0.05	0.23 (0.26)	0.13
CA	0.06	0.13	0.11	0.09	0.06	0.09 (0.03)	0.02
CDCA	0.08	0.18	0.32	0.09	0.15	0.16 (0.10)	0.05
Total bile acids	2.34	5.18	1.30	1.02	0.82	2.13 (1.80)	0.90

Table 2: Comparison of bile acid profiles from SLOS patients, healthy newborns and healthy adults

Bile acids (mmol/L)	Newborns ¹ Median (99 th percentile)	SLOS patients Mean (SD)	Adult ² Mean (SD) (Serum)
GCA	1.43 (7.18)	0.18 (0.25)	0.41 (0.36)
GUDCA	0.01 (0.08)	ND	0.28 (0.15)
GCDCA	0.01 (7.00)	1.30 (1.21)	1.71 (0.80)
GDCA	0.03 (0.13)	0.11 (0.08)	0.38 (0.26)
TCA	1.53 (5.20)	0.06 (0.08)	0.048 (0.083)
TCDC	2.01 (6.70)	0.23 (0.26)	0.21 (0.23)
CA	0.08 (0.52)	0.09 (0.03)	0.2 (0.18)
CDCA	0.01 (0.22)	0.16 (0.10)	0.34 (0.28)

Conclusion: In a limited number of SLOS patients, major bile acid species appear to be reduced compared to normals. No unusual pattern or atypical bile acids were identified in SLOS patients. Additional work is needed to quantify bile acids in age matched controls and to identify novel bile acids in SLOS.

Acid Alpha-Glucosidase Delivery Using Adeno-Associated Virus-2/9 in Adult Animals Improves Respiratory and Cardiac Function in Pompe Disease

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Objective: Pompe disease is an autosomal recessive genetic disorder characterized by a deficiency of the enzyme, acid α -glucosidase (GAA), responsible for degradation of lysosomal glycogen. Respiratory muscle and cardiac dysfunction are primary features of this disorder and we have incorporated an animal model of Pompe disease (*Gaa*^{-/-} mouse) to characterize this aspect. We will compare the efficacy of rAAV2/9-DES-hGAA (AAV9) vs enzyme replacement therapy (ERT) for treatment of Pompe disease.

Methods: Adult *Gaa*^{-/-} mice received one of the following treatments; A) single systemic injection of lactated ringers (*Gaa*^{-/-}), B) single systemic injection of AAV9, or C) bi-monthly injection (duration 3 months) of ERT. Determination of diaphragmatic contractile function was measured *ex vivo* between 0 and 200Hz. *In vivo* cardiac measurements were obtained using a 4.7T Bruker magnetic imaging scanner and analysis was completed using CAAS MRV FARM 2.0. Electrocardiographic measurements were analyzed using Powerlab software.

Results: Assessment of contractile function 3 months after initiation of treatment revealed a significant increase in specific force measured ≥ 60 Hz in AAV9 and ERT groups when compared to untreated *Gaa*^{-/-} animals. Compared to saline treated *Gaa*^{-/-} animals, AAV9 or ERT groups demonstrated improvement in ejection function at one month (*Gaa*^{-/-} 63.21 \pm 1.27%; AAV9 66.35 \pm 2.25%; ERT 66.63 \pm 1.66%) and significantly at three months (*Gaa*^{-/-} 62.65 \pm 1.22%; AAV9 73.51 \pm 3.13%; ERT 73.29 \pm 3.59%). AAV9 resulted in a reduction in end diastolic cardiac mass at one month (*Gaa*^{-/-} 4.46 \pm 0.18 mg/g; AAV9 4.06 \pm 0.27mg/g; ERT 4.68 \pm 0.11mg/g) and at three months AAV9 and ERT groups show significant reduction in left ventricular mass (*Gaa*^{-/-} 4.96 \pm 0.16 mg/g; AAV9 3.75 \pm 0.41mg/g; ERT 4.11 \pm 0.11mg/g). ECG analysis revealed a significant elongation in the PR interval at three months in AAV9 animals (*Gaa*^{-/-} 39.45 \pm 0.32ms; AAV9 42.46 \pm 0.76ms; ERT 40.89 \pm 0.73ms) compared to ERT and saline-treated *Gaa*^{-/-} animals.

Conclusions: These results indicate that a single systemic administration of rAAV2/9-DES-hGAA vector is as effective as the current prescribed ERT regimen and can significantly augment respiratory muscle and cardiac function while improving cardiac morphology at three months post-treatment in a murine model of Pompe disease.

Using Induced Pluripotent Stem Cells as a New Approach to Correct Human Lipoprotein Lipase Deficiency

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Objective: Lipoprotein lipase (LPL) plays a central role in lipoprotein metabolism and energy homeostasis. Human LPL deficiency (type I hyperlipoproteinemia) is a rare autosomal recessive disease (1 in 1,000,000) characterized by severe hypertriglyceridemia and variably associated with abdominal pain and at times life-threatening pancreatitis. LPL deficiency is refractory to lipid lowering drugs, and enzyme replacement therapy has been ineffective due to the short half-life of exogenous LPL in plasma. To remain free of severe hypertriglyceridemia, type 1 patients must remain on a strict diet over a lifetime with dietary fat intake lowered to less than 15% calories, and even then fasting and postprandial plasma TG levels remain elevated. Gene therapy vectors for long-term human LPL transgene expression have been developed and used correct LPL deficiency in mice with LPL deficiency and to some extent in humans. However, the application of such techniques in human patients has been limited by the efficiency of the vectors. The recent breakthrough in generating induced pluripotent stem cells (iPS) from fibroblasts provides an opportunity to correct rare diseases such as LPL deficiency with a new approach.

Method: We propose to conduct a series of proof-of-principle experiments using a murine model developed in our laboratory that has severe hypertriglyceridemia due to a deletion of LPL in both cardiac and skeletal muscle (MLPL^{-/-} mice). iPS will be generated from mouse fibroblasts. A naturally occurring active mutant of LPL-(S447X) will be introduced into iPS to correct the LPL deficiency. LPL-(S447X) transgene stably expressed iPS will be differentiated into several different self-renewing tissue types and transplanted back into MLPL^{-/-} mice to test the efficiency of this method to correct the severe hypertriglyceridemia. In addition, this model will be used to assess the unlikely possibility of adverse effects of overexpressing LPL.

Result: We are currently in the initial stages of establishing the iPS technique.

Conclusion: Once proof of concept is accomplished in our murine model, the approach outlined above will be tested in human subjects with homozygous LPL deficiency.

Prevalence of Self-Reported Photosensitivity and Quality of Life in Lupus

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Objectives: Little is known about self-reported photosensitivity among patients with cutaneous lupus erythematosus (CLE). The purpose of this study was to assess the prevalence of self-reported photosensitivity and evaluate its impact on quality of life in a U.S. lupus population.

Methods: Patients completed the Skindex 29+3, which includes two photosensitivity items (I worry about going outside because the sun might flare my disease; my skin disease prevents me from doing outdoor activities) that were averaged to generate a subscale. The CLASI, a validated outcome measure for assessing CLE skin-specific disease severity was completed. Patients were asked about photosensitivity symptoms. Patients were categorized into a PS group (those with both a history of and current photosensitivity), a NOT PS group (those that denied a history of and current symptoms) and a PS Equivocal group (those reporting a history of or new onset photosensitivity).

Results: Of the 169 patients, 46% had discoid lupus (DLE), 25% had subacute cutaneous lupus (SCLE), 9% had tumid lupus (LET), 7% had acute cutaneous lupus (ACLE), 7% had systemic lupus with nonspecific skin lesions, and 6% had other forms of chronic CLE. 68% reported symptoms of photosensitivity (PS and PS Equivocal groups), with the PS group accounting for 54% of the sample. 82% of ACLE, 76% of SCLE, 63% of LET, and 46% of DLE were classified into the PS group. The PS group engaged in sun protective behaviors ($X^2 = 16.63$, $p = 0.002$) and developed lesions in sun exposed areas ($X^2 = 5.88$, $p = 0.015$) more frequently than the NOT PS group. The PS group had higher CLASI activity scores (9.20 ± 9.4) compared to the NOT PS group (4.6 ± 5.56), $p = 0.005$. Mean Skindex 29+3 subscale scores for symptom, emotion, function, and photosensitivity were significantly higher for the PS group compared to the NOT PS group ($p = 0.007$; $p = 0.003$; $p = 0.0001$; $p = 0.000$, respectively).

Conclusions: Self-reported photosensitivity is very common among patients with CLE. Photosensitive patients have increased cutaneous lupus disease activity and poor quality of life related to disease symptoms. Photosensitive patients have poorer functional and emotional quality of life. Future studies should attempt to characterize photosensitivity phenotypes and to identify factors in blood and skin that might contribute to the pathology of photosensitivity.

Ciliary and Cytokine Expression Responses in Primary Respiratory Epithelial Cell Cultures from Patients with Pulmonary Nontuberculous Mycobacterial Infection and Healthy Controls

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Introduction: Nontuberculous mycobacteria (NTM) are ubiquitous environmental organisms associated with pulmonary disease in persons with known genetic disorders of mucociliary clearance or idiopathic bronchiectasis. The basis for susceptibility to pulmonary NTM (PNTM), outside of certain groups, is currently unknown but family studies support an autosomal dominant genetic predisposition.

Objective: To identify and characterize differences in ciliary beat characteristics and cytokine expression profiles of respiratory epithelium from PNTM patients and healthy controls in response to endotoxin or mycobacteria.

Methods: Respiratory epithelial cells were harvested via nasal scrapes from controls and patients with idiopathic bronchiectasis with PNTM or known genetic disorders of mucociliary clearance (cystic fibrosis, primary ciliary dyskinesia) on IRB approved protocols. Respiratory epithelium monolayers were grown to differentiation at an air-liquid interface and exposed to mycobacteria or endotoxin for varying periods of time. Samples from the apical and basal surfaces were collected at predetermined time points and analyzed for cytokine levels. High speed video microscopy, confocal microscopy, and gene expression were used to further characterize the effects on the respiratory epithelium.

Results: There is a wide variance in ciliary beat frequency in PNTM compared to healthy controls. In some PNTM patients, ciliary beat frequency was 60% of normal at rest with little to no change when challenged with NTM. In contrast, we found a 27 to 35% decrease in ciliary beat frequency in healthy individuals when exposed to NTM. Cytokine levels from the basal media suggest that PNTM patient cells produce high levels of IL-8 and IFN-g in response to NTM; mRNA expression levels correlate with this cytokine response.

Conclusion: This technique allows examination of local cellular responses of human cells obtained by a minimally invasive technique that allows for repeated collection. We can characterize respiratory epithelial cell function within and between populations and assess responses to epithelial microbial exposure. These preliminary data suggest that differences exist in the responses of the respiratory epithelium of patients with PNTM and normals.

Supported in part by intramural funds of the NIAID and by NIH 9 U54 HL096458-06

The Association of Brain-Derived Neurotrophic Factor Haploinsufficiency & Autism Symptoms in WAGR Syndrome

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Objective: WAGR syndrome (Wilms tumor, aniridia, genitourinary anomalies, mental retardation) is a rare genetic disorder caused by heterozygous chromosome 11p13 deletions of variable size. Due to its role in brain development and function, we hypothesized that haploinsufficiency of brain-derived neurotrophic factor (*BDNF*), a gene that is often deleted in patients with WAGR syndrome, may play a role for autism spectrum disorder (ASD) development in this population.

Methods: Twenty patients with WAGR syndrome (10F/10M, age 6-37y) were recruited to the NIH through the International WAGR Syndrome Association. ASD symptoms were assessed using Autism Diagnostic Interview-Revised (ADI-R, parent interview, N=20), Autism Diagnostic Observation Schedule (ADOS, direct behavioral observation, N=15), and clinical judgment of doctoral level psychologists. Deletion boundaries were determined by array comparative genomic hybridization. Prevalence of ASD was compared by Fisher's exact test.

Results: The deletion sizes ranged from 2.9 to 15.1 Mb. Thirteen subjects had heterozygous *BDNF* deletion (+/-) while 7 had intact *BDNF* (+/+). All but one subject met criteria for mental retardation, ranging from mild to severe. Visual impairment ranged from legal blindness to complete lack of vision. Using ADI-R, 7 out of 13 *BDNF*+/- subjects met "ever" ASD criteria compared to none out of 7 *BDNF*+/+ subjects [54% (95% CI: 25-80%) vs. 0% (95% CI: 0-41%), p=0.04]. However, using ADI current codes, ADOS, and clinical judgment, only 1 out of 9 *BDNF*+/- subjects met ASD criteria (with 1 other also meeting criteria only on the ADI-R) compared to none out of 6 *BDNF*+/+ subjects [11% (95% CI: 0-48%) vs. 0% (95% CI: 0-45%), p=1.00].

Conclusions: Because WAGR syndrome includes visual impairment, mental retardation, and serious medical illness in early childhood, current functioning may be more reliable than ADI-R "ever" codes in assessing autism symptoms in this population. Further exploration of cognitive and/or visual impairments is needed to validate the observation of an association between *BDNF* haploinsufficiency and a history of autism symptoms.

This research was supported by the Intramural Research Program of the NICHD and NIMH, NIH.

Clinical and Laboratory Features of Dopamine Beta-Hydroxylase Deficiency

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Objective: To describe the clinical characteristics of Dopamine beta-hydroxylase (DBH) deficiency, a rare autonomic disorder caused by the absence of DBH, the enzyme required to synthesize norepinephrine from dopamine.

Methods: We reviewed the research and medical records for all patients with DBH deficiency evaluated at the Vanderbilt University Autonomic Dysfunction Center (n=8). Data on another 12 patients were obtained from a review of the literature.

Results: DBH deficiency is characterized by normal parasympathetic and sympathetic cholinergic function with absent sympathetic noradrenergic function. Affected individuals exhibit profound deficits in autonomic regulation of cardiovascular function, but only subtle signs of central nervous system dysfunction. In the perinatal period, DBH deficiency has been complicated by vomiting, dehydration, hypotension, hypothermia, and profound hypoglycemia requiring repeated hospitalization. Delay in opening of the eyes has occurred and ptosis is seen in most affected infants. Children with DBH deficiency have markedly reduced exercise capacity, perhaps because of exaggerated hypotension engendered by physical exertion. The syncope associated with postural hypotension often mimics seizures, and many patients receive trials of anticonvulsive medication despite normal electroencephalograms. Mental and physical development are normal. Symptoms generally worsen in late adolescence. Based on the limited number of reported cases, clinical features reported in affected individuals include profound orthostatic hypotension (in 100% of reported patients), ptosis (92%), nasal stuffiness (100%), and high arched palate (100%). Males experience retrograde ejaculation. Elevated blood urea nitrogen noted in five individuals in the United States may be evidence of reduced renal function. Biochemical features characteristic of DBH deficiency include very low/absent plasma norepinephrine and epinephrine and elevated (5-10 fold) plasma dopamine. The treatment of choice is L-threo-3,4-dihydroxyphenylserine (L-DOPS or droxidopa). Droxidopa is converted directly to norepinephrine by L-aromatic amino acid decarboxylase, thereby bypassing DBH. Administration of droxidopa restores plasma norepinephrine to the normal range, and concomitantly alleviates orthostatic hypotension and other symptoms.

Conclusions: Although it seems unlikely that an individual without norepinephrine could survive, there are rare patients with DBH deficiency, in which norepinephrine and epinephrine are absent. Droxidopa, which can be converted into norepinephrine via decarboxylation, is an effective treatment for DBH deficiency.

Autonomic Tone and Cardio-Respiratory Control in RETT Syndrome.

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Objective: Rett syndrome (RTT) is a developmental disorder resulting from mutation in the gene encoding MECP2, a transcription regulatory protein. Autonomic dysfunction is one of the main clinical manifestations of the disease. In RTT patients, autonomic dysfunction has been attributed to an apparent imbalance between sympathetic and parasympathetic tone that results in a relative "hyperactivity" of the sympathetic nervous system. This imbalance has been suggested to result from brainstem immaturity leading to decreased parasympathetic tone.

Methods: Fifteen Rett syndrome females, ages 12-18 years, with documented classic MECP2 deficiency and 15 age matched controls will be recruited for this study. All subjects will undergo full night polysomnography testing during which continuous EEG, EMG, respiratory rate, PETCO₂, and ECG will be recorded. Data will be separated by sleep stage and analyzed in five minute arousal free segments for autonomic tone (frequency analysis of ECG R-R intervals, respiratory rate and PETCO₂). Specifically, power in the high-frequency range (HF; >0.15 to 0.40 Hz), considered a function of cardiac parasympathetic nervous system activity to the heart, and the LF-to-HF power ratio of R-R variability (LF_{R-R}/HF_{R-R}), considered an index of the balance of sympathovagal input to sinoatrial node activity will be calculated. Arousals and respiratory events will also be extracted and analyzed for respiratory rate and PETCO₂ responses both before and after the events. Five breath averages of respiratory rate and PETCO₂ prior to respiratory events will be used for comparison. In addition, breath-hold duration, PETCO₂ and respiratory rate will be analyzed in the same fashion post respiratory events. Averages will then be compared between groups for NREM sleep and wakefulness periods via independent t-tests.

Results: Data pending.

Conclusions: Our first hypothesis is that individuals with Rett Syndrome will have decreased parasympathetic and increased sympathetic measures during NREM sleep as compared to controls. Our second hypothesis is that the ventilatory response to breath hold (apnea) following periods of hyperventilation that remove the central and peripheral drive to breath via decreased end-tidal carbon dioxide (PETCO₂) is attenuated in Rett Syndrome as compared to controls.

North American Cystinosis Research Platform

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Objective: Cystinosis is a rare disease, and clinical research presents a challenge. Patients are scattered, laboratory testing is not uniformly available and no single medical center encounters enough patients for clinical trials. Yet the impact of cysteamine therapy on long-term outcome is still unknown, valuable historical data and stored tissues are difficult to access and the infrastructure for testing new therapies is absent. However, this may soon be changed by the advent of cystinosis patient registries which afford a mechanism to generate a central database available to the next generations of investigators. We propose a North American cystinosis research platform that can tap emerging registries and: A) chart the current natural history of cystinosis; B) link individual CTNS genotypes to clinical data; C) link existing tissue repository samples to individual patients in a registry.

Methods: 1) Develop a case report form. To characterize the important clinical milestones for North American cystinosis patients, we will develop a case report form that engages the patient's physician and captures essential clinical data in a format which can be easily uploaded into a secure patient registry. Milestones will include: a) initial clinical features and diagnostic data; b) effect of cysteamine treatment on leukocyte cystine; c) snapshot of organ function at age 10 years; d) renal replacement therapy; e) late complications. 2) Develop a CTNS mutation form. To generate a mutational database that could be linked to individuals in a registry, we will develop a coded report form to specify the mutation on each CTNS allele for each individual. We will develop a method for displaying and categorizing the various mutations in a database and a method for linking genotype data to clinical parameters. 3) Develop a virtual tissue repository. To link existing cystinosis DNA or tissue samples to a patient registry, we will develop lists of anonymized samples from known sites across North America. These coded lists could then be integrated into a patient registry to provide potential access to samples for approved projects, anonymity and patient consent.

Association of Vascular Physical Examination Findings and Angiographically-Detected Arterial Lesions in Subjects with Large Vessel Vasculitis

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Objective: The vascular physical examination (PE) to assess for pulses, bruits, and systolic blood pressure (SBP) readings is considered essential in the clinical evaluation of patients with large vessel vasculitis (LVV), including Takayasu's arteritis (TAK) and giant cell arteritis (GCA). We examined the relationship between findings on PE and angiographically-detected arterial lesions in subjects with established LVV.

Methods: 100 subjects (TAK=68, GCA=32) enrolled in a longitudinal, observational cohort underwent standardized PE and magnetic resonance angiography of the aorta and its primary branches. Sensitivity and specificity was calculated for the association between PE findings (absent pulse, bruit, and inter-arm SBP difference) and angiographic lesions defined as stenosis, occlusion, or aneurysm. Analysis was restricted to bilateral carotid, subclavian, and axillary arteries.

Results: Participants were predominantly female (92%), Caucasian (87%), and had mean ages of 40 years (TAK) and 69 years (GCA). 67% had at least one PE abnormality: absent pulse (41%-TAK, 38%-GCA), bruit (54%-TAK, 28%-GCA), or ≥ 15 mmHg SBP difference (52%-TAK, 29%-GCA). Lesions in any of the carotid, subclavian, or axillary arteries were detected in 75% of subjects (82%-TAK, 59%-GCA). Individual PE findings had poor sensitivity (range 14-51%) and good-excellent specificity (range 72-98%) to detect arterial lesions. Sensitivity improved (range 52-71%) and specificity worsened (range 59-86%) if *any* of the 3 different PE maneuvers considered in combination were abnormal. Sensitivity worsened (range 6-30%) and specificity improved (range 88-100%) if 2 *or more* of the PE findings were abnormal or if individual PE findings were compared to regional vessels without imposing single vessel anatomic correlation.

Conclusions: In subjects with established LVV, vascular PE findings have low sensitivity but high specificity to detect arterial disease. Abnormal PE findings are highly associated with the presence of arterial lesions, but normal PE findings do not rule out the possibility of arterial disease. Even when considering PE findings in combination, sensitivity is still inadequate to rule out arterial lesions. Specificity improves when PE findings are evaluated in association with a broader region of arterial lesions rather than a specific anatomically correlated vessel, suggesting that PE findings do not always accurately localize disease. While valuable in the assessment of arterial disease in LVV, the PE should not form the sole basis of assessment and should be supplemented by angiography.

Patients with Rare *KCNJ11* Mutations Exhibiting Variable Developmental Delay and Response to Sulfonylurea Treatment Identified Through the US Neonatal Diabetes Registry

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Objective: Neonatal diabetes (NDM) has an identifiable monogenic cause in nearly 2/3 of cases diagnosed under 6 months of age. NDM caused by heterozygous activating mutations in the genes (*KCNJ11/ABCC8*) encoding the ATP-sensitive potassium channel usually responds to treatment with oral sulfonylureas in place of insulin and may also exhibit neurodevelopmental disabilities. Although genetic diagnosis can lead to dramatic improvement in diabetes control and possibly neurodevelopment, many cases likely remain unidentified.

Methods: Subjects with diabetes diagnosed under a year of age were recruited through our IRB-approved website, <http://NeonatalDiabetes.org> to participate in comprehensive longitudinal survey data collection and gene sequencing. Mutations were confirmed in a CLIA-certified laboratory.

Results: Of 139 subjects currently enrolled in the Registry, we report those with extremely rare mutations in *KCNJ11* about which previous clinical information including response to sulfonylureas is limited. A girl with the previously unreported V59A mutation exhibited significant developmental delay with slow progress after transition to an unusually high dose of glyburide (2.2 mg/kg/day). A boy with R50P was treated with glyburide since diagnosis at birth but continues to exhibit significant delay. A boy with Y330C transitioned off insulin at the age of 12.5 months with subsequent improvement in developmental delay not described in the two other known cases. A boy with E322K previously described to respond to glyburide at 4 yo subsequently exhibited significant behavioral concerns with an extreme ADHD-like phenotype. A mother and 2 daughters with E179A exhibit a mild degree of cognitive impairment despite successful transition. A girl with G53D was treated empirically with glyburide at birth, prior to a genetic diagnosis that was uncovered only through inclusion in the Registry. Importantly, her lack of significant developmental delay by 24 months is in contrast to three published G53D cases with significant neurological impairment.

Conclusions: A web-based registry is an effective means for identifying and surveying patients with NDM and provides a foundation for prospective clinical studies, including careful longitudinal neurodevelopmental assessment. Involvement in such a national registry is critical for uncovering variability among patients with rare mutations that may be related to differences in treatment or other clinical characteristics.

Leptin Resistance in Patients with Bardet-Biedl Syndrome

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Objective: Bardet-Biedl syndrome (BBS) is a genetically heterogeneous disorder of the primary cilium associated with obesity. In some BBS mouse models, ciliary dysfunction leads to impaired leptin signaling and hyperleptinemia before obesity onset. To study the pathophysiology of obesity in BBS, we compared patients with BBS and BMI-Z matched controls.

Methods: 50 patients with BBS were matched 2:1 with 100 healthy controls by age (BBS vs. control: 14.8±11.1 vs. 15.3±11.0y, p=0.68), sex (50%F each group), race (98% Caucasian each group), BMI (31.7±9.7 vs. 31.5±8.4kg/m², p=0.96), and BMI-Z (2.17±0.89 vs. 2.10±0.84, p=0.60). Patients with BBS and control subjects were compared for differences in body composition (DEXA, abdominal MRI), blood pressure (BP-Z, standardized for age, sex, height), and fasting concentrations of leptin, lipids, insulin, and glucose. Patients with BBS were also compared by genotype.

Results: Patients with BBS had body fat% similar to control (42.1±8.5 vs. 39.3±10.5%, p=0.19), but higher visceral adiposity (%total fat at L2/3+L4/5: 26.9±10.4 vs. 19.7±6.1, p<0.001), higher DBP-Z (0.72±0.80 vs. 0.14±0.73, p<0.001), higher leptin (42.1±28.5 vs. 23.7±16.3ng/mL, p<0.001), and higher triglycerides (182±133 vs. 112±88 mg/dL, p<0.001). BBS1 (27%) and BBS10 (30%) mutations were the most prevalent. Patients with BBS10 mutations had significantly higher BMI-Z (2.63±0.13 vs. 1.90±0.15, p=0.002), greater visceral adiposity (35.6±3.0 vs. 20.3±2.8%, p=0.006), and more severe insulin resistance (6.45±0.62 vs. 2.78±0.62, p=0.002) than those with BBS1 mutations

Conclusions: Patients with BBS had higher leptin than expected for their degree of adiposity, consistent with the notion that ciliopathy-induced leptin signaling dysfunction is associated with leptin resistance. The preferential deposition of fat intra-abdominally in patients with BBS may indicate a predisposition for metabolic complications, including hypertension and hypertriglyceridemia. The observation of disparate results in the BBS10 versus BBS1 mutation groups is the first demonstration of physiologic differences between BBS genotypes. These results suggest the obesity of BBS is distinct from nonsyndromic obesity.

This study was supported by the NIH Intramural Research Programs of NICHD and NHGRI. JCH, DN, and JAY are commissioned officers of the US Public Health Service.

Pundi Rasayana Improves Cognition in Mouse Models Relevant to Alzheimer's Disease of Dementia Type

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Objective: Cure of cognitive disorders such as amnesia, attention deficit and Alzheimer's disease is still a nightmare in the field of medicine. Nootropic agents such as piracetam, aniracetam and choline esterase inhibitors like Donepezil are being used to improve memory, mood and behavior, but the resulting side effects associated with these agents have made their use limited. The present study was undertaken to assess the potential of Pundi Rasayana (PR) as a memory enhancer. The roots of the herb included in the formulation is used by the gondas tribal of Karnataka for various mental ailments.

Methods: PR (2, 4 and 6 mg/kg p.o.) was administered for eight successive days to both young and aged mice. β -amyloid, scopolamine, ibotenic acid, CO₂ and aging induced amnesia were the experimental models. Basal forebrain lesion induced decrease in cerebral Ach and ChAT activity were assessed, Concentrations of Norepinephrine, Epinephrine, Dopamine, 5-HT in cerebral cortex, cerebellum, hypothalamus, hippocampus, and corpus striatum of rats and mice were measured by HPLC analysis.

Results: PR significantly improved learning and memory in young mice and reversed the amnesia induced by scopolamine, β -amyloid, ibotenic acid, CO₂ and natural aging. PR inhibited KCN and Carotid artery ligation induced hypoxia, reversed amnesia, neurodegeneration and produced normalizing action on discrete regions of brain and controlled alterations in neurotransmitter levels due to neurodegeneration.

Conclusions: PR might prove to be a useful memory restorative agent in the treatment of dementia seen in elderly.

SC4MOL Deficiency, A New Genetic Disorder In Cholesterologensis

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Objective: SC4MOL deficiency is a new genetic disorder in cholesterol biosynthesis pathway, characterized by significant accumulation of dimethyl and monomethyl sterols in the human body which can be detected in blood, skin flakes or primary skin fibroblast. We previously reported mutations in SC4MOL, a gene in the PSORS9 region on 4q32-34 as the cause of an autosomal recessive syndrome that includes psoriasis, arthralgias, microcephaly, congenital cataracts and developmental delay. We previously reported the first patient with SC4MOL deficiency.

Methods: We performed sterol analysis by GC-MS method and direct exon sequencing of SC4MOL were performed on the patients with elevated methylsterols.

Results: A second affected child has now been identified at age 3 ½ years who has milder dermal symptoms than the original patient, emphasizing that the disorder is likely to be heterogeneous. This gene encodes a sterol C4 methyl oxidase catalyzing demethylation of methylsterols in the cholesterol synthesis pathway. The methylsterols are meiosis activating sterols (MAS), and they are the first family of cholesterologensis intermediates found to have functions other than *de novo* cholesterol biosynthesis. Our *in vitro* studies provided evidence that methylsterols also play a role in cell proliferation and immune regulation. With treatment with a statin and supplementation of cholesterol and bile acids, we have been able to normalize the methylsterol level in this patient. In the two years following institution of therapy, the patient's weight and growth improved dramatically with a growth velocity of 0.8 cm/month. Her height has increased from below the 3% at 9 month old and 50% for a 10½ year old at age 13 years To the 10% for her current age of 18 years.

Conclusions: These findings underscore the important extra-hepatic functions of C4 methyl oxidase and provide insights into the pathogenesis of cholesterologensis disorders.

Comparison of Methylation-Specific Multiplex Ligation-Dependent Probe Amplification (MLPA) Kits for the Identification of Prader-Willi and Angelman Syndrome Genetic Subtypes

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Objective: Both Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are complex neurodevelopmental disorders caused by loss of expression of imprinted genes from the 15q11-q13 region generally due to similar defects in the region but differ in parent of origin. A methylation-specific multiplex ligation-dependent probe amplification (MLPA) kit from MHC-Holland consisting of 43 probes was recently validated to detect copy number changes and methylation status in the 15a11-q13 region from 62 PWS and 10 AS individuals (Bittel et al, *Genet. Test.* 11:467-475, 2007). We now report our experience in the use of two versions (original A1 and newer B1) of the MLPA-PWS/AS kit with a new cohort of PWS subjects to identify methylation abnormalities and to determine copy number (i.e. deletion) status including microdeletions of the imprinting center.

Methods: Blood or buccal cell DNA was isolated from PWS subjects or control subjects including parents. The MLPA procedure and subsequent computational analysis were performed according to manufacturer's protocol using the MLPA-PWS/AS-A1 and/or the MLPA-PWS/AS-B1 probe set for each subject.

Results: Both MLPA kits effectively distinguished between type I (32%), type II (26%) and atypical deletions (6%). When copy number variations were not observed, subjects were determined to have uniparental disomy (UPD, 30%) or imprinting center defects (9%). The MLPA-A1 kit has a higher density of probes to identify atypical deletions at the telomeric end of the deleted region, while the MLPA-B1 kit contains more probes in the imprinting center including snoRNA.

Conclusions: The MLPA-PWS/AS-A1 kit includes two probes (*GCP5* and *CYFIP1*) between breakpoints 1 (BP1) and 2 (BP2) and 2 probes for the *P(OCA2)* gene close to the distal BP3 within the 15q11-q13 region while version B1 contains more probes in the vicinity of the imprinting center, but fewer probes in the proximal and distal areas of the 15q11-q13 region. The A1 kit is sufficient for identifying the typical (type I and type II) or atypical deletions while the B1 kit is more informative for analyzing imprinting defects. MLPA is a relatively simple, cost-effective technique useful for methylation and copy number status and analysis of genetic subtypes in both PWS and AS, as well as other chromosome 15 abnormalities.

Defining Cross-Species Conserved Glomerular Transcriptional Networks of Early Diabetic Glomerulopathy in Man and Mouse.

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Objective: Mouse models of diabetes are invaluable tools to study the pathogenesis of diabetic glomerulopathy. Several mouse models have been employed, however none reliably mimic human disease, delaying the identification of specific factors that cause or predict diabetic nephropathy (DN). This study aims to define where mouse models faithfully recapitulate the human disease on a functional level using a cross-species comparison to identify shared transcriptional networks in DN.

Methods: Using Affymetrix microarrays and transcriptional pathway mapping and promoter modeling tools, we defined networks activated in humans and in three AMDCC models of murine diabetic complications. The human transcriptional network was derived from microdissected glomeruli from albuminuric (>30 mg/g ACR) versus nonalbuminuric (<30 mg/g) Pima Indians, a cohort with early DN. Similarly, mouse glomerular transcriptional networks were derived from streptozotocin treated DBA/2 mice, db/db mice, and eNOS-deficient db/db mice, each versus control. Networks were generated by integrating the gene expression alterations with available biological knowledge through co-citation in current literature, resulting in complex networks of 1000s of genes linked by multiple co-citations and promoter binding sites. TALE (Tool for Approximate Large Graph Matching, University of Michigan) was used to align the human transcriptional network with the mouse transcriptional networks to derive three conserved network structures for each human-mouse comparison, each comprised of less than 100 nodes representing key hubs of conserved regulatory events.

Results: Shared gene nodes were found in all three networks, many of them reflecting established pathogenic mechanism of DN, including JAK-STAT and PPAR signaling pathways. Analysis of the top biological processes in each conserved network reveals enrichment for angiogenesis pathways in streptozotocin treated DBA/2 mice, but enrichment for inflammatory responses in the eNOS-deficient db/db BKS mouse model.

Conclusion: Using graph-matching tools on transcriptional networks allowed us to define key regulatory hubs conserved between human and murine DN. This approach can guide the selection of the most relevant mouse models to study specific human DN disease process. In addition, key pathways only detected in humans can be altered in mice with the goal of creating more human-like models of DN.

Neurofibromatosis Networking – Building the Tools to Advance Research Discoveries to the Clinic

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Objective: Neurofibromatosis (NF) encompasses a group of rare disorders affecting 100,000 Americans. The clinical course of NF is unpredictable: it can lead to deafness, blindness, amputation or disfigurement. The genes for the two major forms, NF1 and NF2, were identified in the early 1990's, and the molecular signaling pathways are well understood. The primary funders of US NF research are the Congressionally Directed Medical Research Program for Neurofibromatosis Research (\$10M-\$25M/year) and NIH (\$11-\$15M/year). Major tools developed in the last 10 years include transgenic mouse models representing individual NF manifestations, and a Phase II Clinical Trials Consortium. In 2006, the Children's Tumor Foundation (CTF) examined the NF research landscape looking for 'funding gaps' that if filled could rapidly advance NF discoveries to the clinic. The goal was to implement recommendations within 5 years.

Methods: We first mapped 'bench to bedside' the dollar amounts of NF grants issued by NIH and CDMRP 1996-2005, then convened an expert strategic planning workshop. We concluded that the NF community had a reasonable understanding of NF candidate drug targets; good preclinical drug screening tools; and a Phase II clinical trials consortium; but that areas in need of funding were: i) Coordinated preclinical drug screening; ii) pilot clinical trials for initial advancement of promising compounds; iii) NF clinic network; iv) patient registry; and v) tissue bank to utilize for identifying new biomarkers.

Results: CTF has now launched initiatives in all five 2005 strategic recommendation areas. These include: a NF Preclinical Consortium and Drug Discovery Initiative to test candidate drugs in multiple NF tumor models; a Clinical Trial Awards program for proof of concept studies; an NF Clinic Network of 45 US clinics; and with Genetic Alliance, a BioBank and Patient Registry.

Conclusions: In 2010 CTF is reviewing these initiatives for the next five years' strategic planning. Lessons learned have included vagaries of managing multisite legal agreements - particularly when collaborating with a commercial entity; setting realistic goals; and managing expectations. On the positive side, we have a significant increase in industry's interest in NF as a marketable condition. Preclinical testing, clinical trials and clinical care are becoming progressively linked and the NF community can now offer industry the opportunity to engage in NF research at any point from pilot preclinical studies to large scale clinical trials.

Deuterated Vitamin A Clinical Stargardt's Disease

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Objective: Stargardt's disease has an incidence of 1:10,000 and is the most prevalent cause of inherited blindness. The disease is thought to originate from genetic defects in the *ABCA4* gene resulting in deficient processing of vitamin A through the visual (also called vitamin A) cycle. Premature accumulation of ocular lipofuscin, retinal "spotting" or "flecks" and central vision loss, are all classical hallmarks of the disease. Incorporating deuterium atoms at carbon twenty on vitamin A (C20-D3 vitamin A) seems to slow signs of disease progression in mouse models of Stargardt's. This poster summarizes preclinical work with C20-D3-vitamin A and discusses plans and obstacles for clinical testing.

Methods: C20-D3-vitamin A was administered for up to 12 months to wild type rodents (mice and rats) and to *ABCA4* -/- knock-out mice, the mouse model of Stargardt's disease. We compared ocular changes in the above treated groups with control animals given normal vitamin A. In order to assess safety and determine suitability of C20-D3-vitamin A as a practical therapy in humans, we reviewed literature data on vitamin A metabolites, pharmacokinetics (PK) and pharmacodynamics (PD).

Results: Administration of C20-D3-vitamin A resulted in significant reduction in lipofuscin pigments, and lipofuscin granules, and prevented age related visual declines in rodent models. Deuterated vitamin A has previously been used in humans in short-term studies over the past twenty years. Due to the fact that normal metabolism of vitamin A does not seem to affect the C20-H bond, the PK/PD profile of deuterated vitamin A is thought to be identical to vitamin A at natural abundance. Potential therapeutic dose, to impede lipofuscin pigment biosynthesis, should be within safe range of vitamin A intake. Whether retarding lipofuscin pigment biosynthesis would ultimately halt vision loss in Stargardt's disease will ultimately need to be evaluated through clinical testing.

Conclusions: The anticipated safety profile of C20-D3-vitamin A and its understood mechanism of action make a rational case for clinical testing.

Cerebral Cavernous Malformations: Clinical outcomes and lesion burden

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Objective: Familial cerebral cavernous malformations (CCM) are characterized by abnormally enlarged capillary cavities without intervening brain parenchyma involving the central nervous system. Familial CCM cases often present with multiple lesions that increase over time. However, it is not known whether increasing lesion burden is correlated with severity of clinical symptoms and outcome. The purpose of this pilot study was to investigate whether clinical symptoms and outcomes are associated with lesion burden in familial CCM cases with the Common-Hispanic mutation (CCM-CHM).

Methods: We retrospectively reviewed neuroimaging and medical records of 150 CCM-CHM patients in the University of New Mexico database. Clinical outcome at time of MRI was available in a subset of 36 patients, and assessed using the modified Rankin Scale (mRS) score; mRS score of 0–2 (living independently) were considered good outcomes and 3–6 (living dependently or dead) poor outcomes. Lesion number on MRI was determined by a neuroradiologist and log-transformed prior to inclusion in multivariable regression models adjusting for age.

Results: The mean age of 36 CCM-CHM patients was 30±17 years, ranging from 2 to 57 years, and 51% were female. A total of 1,168 lesions were identified for a mean and median of 32.4 and 6 lesions, respectively. The largest lesion was located in the infratentorium in 5/36 patients (14%). The majority of patients had symptomatic presentation 28/36 (78%) and with 13 of those 28 (46%) presented with an acute hemorrhagic event. Seven out of 36 patients (19%) had poor clinical outcome. Higher lesion number was associated with worse mRS scores independent of age at MRI (beta=0.25, P=0.038). The risk of poor clinical outcome was 2-fold higher for every log unit increase in lesion number, adjusting for age (OR=2.03, 95% CI=0.98-4.21, P=0.057). This association remained after further adjusting for symptomatic presentation (OR=2.18, 95% CI=0.98-4.83, P=0.055).

Conclusions: A large proportion of CCM-CHM patients have symptomatic presentation, particularly hemorrhages, in this unique population. Higher lesion burden was associated with worse clinical outcome independent of age and clinical symptoms.

Are Acute Phase Reactants Predictive of Disease Activity in Patients with Wegener's Granulomatosis and Microscopic Polyangiitis?

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Objective: Wegener's granulomatosis (WG) and microscopic polyangiitis (MPA) are rare systemic vasculitic diseases characterized by inflammation of the small and medium sized blood vessels. Disease activity is assessed clinically using the Birmingham Vasculitis Activity Score for Wegener's granulomatosis (BVAS-WG), a standardized and validated instrument. In the BVAS-WG a score of 0 is indicative of remission, with a score of > 1 indicating disease activity. Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are commonly used acute phase reactants that are used as surrogate markers of systemic inflammation. In this study, we investigated whether ESR and CRP are useful in predicting disease activity as gauged clinically by the BVAS-WG when examined within a large scale multi-center cohort.

Methods: We analyzed patients with WG and MPA enrolled in the Vasculitis Clinical Research Consortium (VCRC) database, which contains serial information on disease activity and laboratory parameters. WG and MPA were defined based upon modified American College of Rheumatology Criteria for the classification of WG and the Chapel Hill Consensus Conference criteria for MPA. For purposes of statistical analyses, the BVAS-WG, ESR and CRP were converted into categorical variables (normal/abnormal), with age, gender, hemoglobin, platelet count and serum creatinine were used as continuous variables. A generalized estimating equation (GEE) model was used for the analysis with categorized BVAS-WG as the outcome variable, categorized ESR and CRP as main predictors, and age, gender, hemoglobin, platelet count, serum creatinine as covariates. An exchangeable working correlation structure was used for the calculated correlation matrix of GEE analysis. Statistical analysis was performed using R (version 2.10.1).

Results: There were 295 patients in the VCRC database (158 females and 137 males) with 1381 total observations. Males were older than females [57 (45.0-66.0) vs. 49.5 (35.0 – 62.0) years, median (IQR)]. 175/293 (60%) of patients had experienced a flare during the course of their illness as assessed by the BVAS-WG. Based on the GEE model, patients with normal CRP levels (categorized) appeared to have a 2.46 fold greater odds of a having a BVAS-WG score of 0 (categorized) as assessed by the physician. ESR, hemoglobin levels, platelet counts and creatinine levels were not predictive of BVAS-WG based on this model.

Conclusion: Normal CRP levels correlate with physician assessment of disease remission in patients with WG and MPA.

Clinical and Molecular Features of the Immune Dysregulation, Polyendocrinopathy, Enteropathy, X-Linked (IPEX) Syndrome

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Objective: IPEX Syndrome is a rare inherited disorder of severe autoimmunity characterized by enteropathy, endocrinopathy, and dermatitis, with onset in the first months of life. Other autoimmune features are also common (hemolytic anemia, thrombocytopenia, neutropenia, hepatitis, etc.). IPEX has been linked to mutations in the *FOXP3* gene, which is essential for development of CD4+CD25+ regulatory T-cells (Treg) however <50% of patients with a clinical phenotype suggestive of IPEX have *FOXP3* mutations. The objectives of this study were to determine whether specific clinical or lab features predict a *FOXP3* mutation and to determine if a genotype/phenotype correlation exists relating to severity of disease or degree of regulatory T cell deficiency among patients with *FOXP3* mutations.

Methods: Clinical, laboratory, and molecular features were evaluated in a cohort of 110 patients with IPEX symptoms referred to our lab for *FOXP3* analysis. Clinical and laboratory data were obtained from referring providers via questionnaire. Mutational analysis for *FOXP3* was performed. *FOXP3* mRNA expression was evaluated by quantitative PCR. *FOXP3* protein expression and regulatory T cell numbers were evaluated by Facs.

Results: We identified 26 different pathogenic *FOXP3* mutations in 55 patients from 42 families with IPEX. Pathogenic mutations in *FOXP3* were not found in the remaining 55 patients who were therefore termed "IPEX-like". A similar rate of enteropathy, endocrinopathy, and skin disease were found between IPEX and IPEX-like patients. However, IPEX patients were more likely to have all three features (p=0.045) as well as food allergies (p<0.001). *FOXP3* expression was absent or very low in the IPEX group. However, among IPEX-like patients, only half demonstrated low *FOXP3* expression levels.

Conclusions: Among patients with clinical features of IPEX, a complete triad of enteropathy, endocrinopathy, and dermatitis was the strongest predictors of a *FOXP3* gene mutation. The only other significant predictor was food allergies. *FOXP3*+ Treg numbers were low in patients with *FOXP3* mutations but only half of those patients lacking *FOXP3* mutations had low *FOXP3* Treg numbers. We postulate that some "IPEX-like" patients may have defects in regulatory regions of the *FOXP3* gene or in novel genes not yet linked to Treg development or function.

Prevalence of HHT: Can We Improve Rates of Diagnosis?

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Objective: Experts report that Hereditary Hemorrhagic Telangiectasia (HHT), a rare autosomal dominant disease characterized by arteriovenous malformations, is under-diagnosed though this has not been studied. Our primary objective was to provide evidence of under-diagnosis of HHT in North America. We specifically hypothesized that there would be variation in prevalence across regions in the province of Ontario and across age groups, for example, due to under-diagnosis in certain groups. Our secondary objective was to collect data regarding healthcare utilization amongst HHT patients, and access to appropriate specialists, to help guide potential future diagnostic programs.

Methods: *Primary objective:* 556 patients with definite HHT diagnosis, seen at Toronto HHT Centre, were identified and were geocoded with postal codes. Prevalence rates were calculated using Canadian census data. *Secondary objectives:* A driving network model was developed in ArcGIS. Service area buffers around ENT clinics in Ontario were generated within 30, 60 and 90 minute drives of clinics to determine proportion of Ontarians within the service area.

A survey was sent to the email contact list of HHT Foundation International (N=3036), targeting people with diagnosed HHT, regarding consultation with ENT physicians and timing of HHT diagnosis.

Results: Prevalence rates varied among regions, from 0.16 to 2.36 cases per 5000. There were no significant differences between urban and rural prevalence rates. Variation in prevalence was seen across age groups, with greater prevalence in older adults (50years+) (0.40), compared to the 20-49 age group (0.23). We also determined that 92.7%, 97.8% and 98.6% of Ontarians were within 30, 60 and 90 minute drives, respectively, of ENT clinics.

Plastic Bronchitis: Longitudinal Pathological Assessment and Responsiveness of Casts to Tissue Plasminogen Activator (tPA).

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Objective: Plastic bronchitis (PB) is a rare and most often pediatric disease that is associated with palliated congenital heart disease (CHD) with Fontan physiology. PB is characterized by the production of fibrin or mucin casts in the airways. Presently, PB is anecdotally treated with inhaled tPA with unknown safety and efficacy. Despite the current use of tPA, the concentrations needed to reduce PB cast mass have not been established. These data are essential to ensure the design of safe and effective tPA dosing for the treatment of PB. The objective of our work was twofold: 1) to longitudinally assess the composition of PB casts; and 2) to measure the responsiveness of PB casts to tPA *ex vivo*.

Methods: We obtained spontaneously expectorated PB casts from children (n=4) with Fontan physiology and one adult patient with idiopathic PB over six-months. A minimum of two casts at least one month apart were obtained from each patient. Each cast sample was sectioned in order to assess the same mass to tPA ratio for each patient across a range of different tPA concentrations (0-20µg/mL) that are achievable in the airways. Portions of each cast were fixed and stained (H&E or mucicarmine) to determine cast composition. Drug therapy information was collected from patients at the time of cast expectoration.

Results: All but one of the four CHD patients had hypoplastic left heart syndrome; three were male and the adult patient was female. PB casts from all patients were primarily comprised of fibrin and cast composition did not change over time. tPA treatment was effective in reducing cast mass by up to 70%.

Conclusions: It has been previously reported that PB casts from CHD patients are predominantly mucinous. Our results suggest that they are fibrin and composition does not change over time. This is further evidenced by the ability of tPA to reduce cast mass making inhaled tPA a potentially viable option for symptomatic relief of PB while we work to unravel the complexity of PB pathogenesis.

Auditory Sensitivity and Function in Chromosome 7 Disorders

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Objective: The goal of this research is to study the role of elastin (ELN) in peripheral and central auditory function. The *ELN* gene is located on chromosome 7q11.23. One of the better known *ELN*-disruption disorders is Williams syndrome (WS), which involves a deletion of approximately 28 genes including *ELN*. The *ELN* deletion causes insufficient production of elastin (*ELN* haploinsufficiency). Two other examples of *ELN*-disruption disorders are isolated supravalvar aortic stenosis (*ELN*^{+/-}), caused by a large spectrum of *ELN* mutations and leading to functional *ELN* haploinsufficiency, and cutis laxa (CL), in which a mutation of *ELN* leads to synthesis of a mutant elastin precursor, which disrupts the assembly of elastic fibers in a dominant fashion. *ELN* has been identified in the structures of the middle ear and has been hypothesized to impact function in inner-ear structures.

Methods: We obtained tympanometry (middle-ear function), audiometric thresholds, and distortion product otoacoustic emissions (DPOAEs; inner-ear function) in individuals with WS (n=81), *ELN*^{+/-} (n=10) and CL (n=3).

Results: Genetic testing confirmed group constituency. Sixty-three percent of school-age children and 92% of adults with WS demonstrated mild to moderate-to-severe sensorineural hearing loss (SNHL). These results were also corroborated with the DPOAE conditions. In a group of children with WS with normal hearing (n=14), DPOAEs were significantly decreased, indicating that the auditory systems in this subgroup were not normal. In addition, the DPOAE input/output function at 4000 Hz in this subgroup showed a loss of cochlear compression (in the absence of hearing loss). Individuals with point mutations of a single elastin gene, who do not have WS (*ELN*^{+/-}) have significantly depressed cochlear function (DPOAEs) but relatively preserved hearing sensitivity. Individuals with CL showed normal hearing sensitivity and DPOAEs.

Conclusions: This data is an extension of those reported in Marler et al., (2005). Theoretically, WS ears may be an experimental model for “tender ears” (Maison & Liberman, 2000), or ears genetically predisposed to trauma from normal environmental noise. Further work needs to be conducted to identify the possible role of *ELN* in central auditory system function.

Nontuberculous Mycobacterial Infection in Cystic Fibrosis Patients at a Large Care Center.

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Objective: Nontuberculous mycobacteria (NTM) are environmental organisms with pathogenic potential in patients with cystic fibrosis (CF). The prevalence of positive cultures for NTM infection in CF is increasing, although it is unclear if this represents rising rates of infection or improved surveillance. Currently it is not known what predisposes certain CF patients to asymptomatic "colonization" with NTM and what factors result in progressive NTM disease. The objective of this study was to retrospectively examine a database of CF culture results at the largest CF center in the United States in order to identify clinical characteristics of those with positive NTM cultures.

Methods: Clinical data was obtained from database review of the pediatric CF program at the University of Colorado from 2006 to 2010. Results of all respiratory cultures were reviewed. For those with a positive NTM culture, additional demographic and clinical information was abstracted.

Results: A total of 35 patients with CF have at least one positive mycobacterial culture for a prevalence of 7.8%. Fifty-one percent of the population had a single positive culture while 49% of the population had more than one positive culture. The average age of first positive NTM culture was 15.6 years. The overall male to female ratio was 56% to 46% with patients with *M. abscessus* having a slightly higher male ratio of 60%. Twenty-five patients (71.4%) had *M. avium* complex (MAC) and 10 (28.6%) had *M. abscessus*. Sixty percent of patients with an NTM positive culture had F508del homozygous genotype. The average FEV1% predicted at time of first positive culture was 84% (82% for *M. abscessus*, 85% for MAC).

Conclusions: The prevalence of NTM infection in the pediatric CF population in Colorado was 7.8% and first occurred in patients with an average age of 15.6 years. NTM infection occurred more typically in males and in patients with F508del homozygous genotype. Further retrospective and prospective analysis of this population is planned to help gain insight as to the significance of a first positive NTM culture as well as predictors of disease phenotype and progression of disease in both pediatric and adult patients with CF.

The Shared Transcriptional Network in Nephrotic Syndrome

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Objective: Glomerular filtration barrier failure in nephrotic syndrome (NS) manifests with similar structural and functional alterations irrespective of disease causation. Our underlying hypothesis is that focal segmental glomerulosclerosis (FSGS), membranous nephropathy (MN) and minimal change disease (MCD) presenting with NS share transcriptional networks involved in the conserved alterations of podocyte architecture. Transcriptional signatures from these patients could be used for prediction of the disease course.

Methods: To test this, we used genome-wide gene expression profiles of microdissected glomeruli isolated from FSGS (n=13), MN (n=21), and MCD (n=6). Glomerular gene expression levels were analyzed using the GenePattern pipeline and compared with pre-transplant living donor kidney biopsies. Hierarchical cluster analysis and functional context analysis were employed to define the shared transcriptome, common functional categories (Gene Ontology) and canonical pathways (Ingenuity[®] Software Suite). Glomerular-specific transcripts were detected by comparing glomerular and tubulointerstitial gene expression profiles in the living donor control cohort. The Genomatix[®] Software Suite was used for detection of common transcriptional regulatory elements of glomerular-specific and shared transcriptome genes, followed by ridge regression analysis for prediction of glomerular filtration rate (GFR).

Results: Among the differentially expressed transcripts in disease vs. controls, 302 transcripts were commonly regulated in all three diseases. Hierarchical cluster analysis revealed a homogeneous signature across FSGS, MN and MCD. Functional context analysis showed enrichment for functional categories and pathways known to be involved in the pathogenesis of NS i.e. actin-cytoskeleton signaling, T-cell activation and Notch-signaling. The glomerular-specific genes among the shared transcriptome genes served as a starting point for the detection of a common transcriptional regulatory pattern. This regulatory pattern was able to predict Δ GFR via ridge regression modeling with a cross-validated $r^2 = 0.94$. The same marker set was able to predict Δ GFR in an independent test cohort of 13 FSGS patients with an $r^2 = 0.26$.

Conclusions: Glomerular-specific gene expression profiles of three different diseases with nephrotic syndrome defined common transcriptional networks with shared functional categories known for their involvement in the pathogenesis of nephrotic syndrome. Transcriptional regulatory patterns were able to predict Δ GFR in FSGS.

MECP2-Duplication Disorders in Males: A Comprehensive Approach

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Objective: Males with MECP2 gene duplications present with similar phenotypes that include: moderate to profound mental retardation, hypotonia, developmental delay, absence of or poor speech development, increased susceptibility to infections, facial dysmorphism, feeding difficulties, epilepsy, and subsequent spasticity. In this study, we characterize the common phenotypic presentations and overall phenotypic variability among these males and explore possible phenotype-genotype relationships.

Methods: Data were gathered from literature accessed via a PubMed search with keywords MECP2, gene duplication, males, phenotype, and methyl-CpG-binding protein. Information was also gathered from participants from the Rett syndrome clinics at UAB and the Greenwood Genetic Center. Data were then entered into a database to allow correlation between the duplicated genes and the respective phenotypic presentations.

Results: To date, 143 males have been identified with duplications of varying size involving the Xq28 region that includes MECP2 as well as many other genes of interest. The Core features of the MECP2-duplication phenotypes are mental retardation (136/136), delayed motor development (135/136), absent or limited speech (122/122), abnormal gait (108/108), facial dysmorphism (114/123), infantile hypotonia (96/106), and recurrent infections (92/119). Less common features include autism (20/50), feeding difficulties (46/57), hand stereotypies (31/53), genital abnormalities (39/66), abnormal cranial MRI (48/66), GERD (36/53), and seizures (66/122).

Conclusions: It is clear that males with MECP2-duplication disorder display specific core features. However, it is also clear that significant variability exists. While assertions have been made as to whether other genes involved in the duplication may alter the phenotypic presentations of this condition, no convincing evidence has been developed to support this contention. Further analysis of our database is underway to determine whether or not this is a possibility.

Biomarkers for Global and Renal Disease Activity in Juvenile Systemic Lupus Erythematosus (jSLE)

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Objective: Preliminary data suggest that the Lupus Nephritis Renal Panel (LNRP), composed of transferrin, ceruloplasmin, acid-1-glycoprotein, neutrophil gelatinase-associated lipocalin, prostaglandin-D synthetase and monocyte chemoattractant protein 1, is associated with worsening lupus nephritis in jSLE. Cell-bound complement activated products (CB-CAP) which consist of erythrocyte, reticulocyte and platelet complement components (E-C4d, E-C3d E-fBb, E-CR1, R-C4d, R-C3d, R-fBb and P-C4d) have been shown to reflect ongoing global disease activity in studies limited to adults with SLE. Therefore, the objective of this study is to evaluate the association of the LNRP and CB-CAP with both global/extra-renal and renal disease activity in jSLE.

Methods: Clinical, laboratory and biomarker data were collected from 12 jSLE patients during initial and follow-up visits. Levels of LNRP standardized to urine creatinine and CB-CAP were measured by enzyme-linked immunosorbent assay and flow cytometry respectively. Physician-rated change in patient's disease course between visits (global/renal disease worsening: yes/no) served as the criterion standard.

Results: Using Wilcoxon Rank Sum Test and Spearman's correlation, statistically significant association was seen between levels of specific urine biomarkers and the renal domains of disease activity indices and the criterion standard (*see Table A*). Levels of urine biomarkers were seen to be elevated three months prior to renal worsening with the change in the biomarker level associated with the change in the renal disease activity scores. No significant correlation between extra-renal disease activity and urine biomarkers was seen. Of the CB-CAP, E-CR1, E-fBb and R-C4d were shown to be associated with current global/extra-renal disease activity. Traditional biomarkers for global and renal worsening correlated poorly with disease course and activity.

Conclusions: LNRP is associated with renal disease activity and CB-CAP with extra-renal disease activity in jSLE. Further studies assessing the predictive properties of combining these biomarkers are underway.

MYB-NFIB Gene Fusion in Salivary Adenoid Cystic Carcinoma

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Objective: Adenoid cystic carcinoma (ACC) of the salivary gland is a rare malignancy, comprising 1% of all head and neck tumors. Recently, the *MYB-NFIB* fusion gene, resulting from the t(6;9)(q22-23;p23-24) translocation, was detected in total 11 cases of breast and head and neck ACCs. The aim of this comprehensive analysis was to determine the incidence, transcript variants and the clinicopathological significance of the *MYB-NFIB* gene fusion in salivary ACCs.

Methods: We undertook an extensive analysis involving a total of 123 salivary carcinomas, including primary ACCs of the salivary gland, metastatic ACCs, non-ACC salivary carcinomas and normal salivary gland tissue. The *MYB-NFIB* fusion transcripts were detected by RT-PCR and sequencing analysis. MYB expression was determined by Quantitative RT-PCR and immunohistochemistry (IHC) analysis. FISH was performed to identify the *MYB/NFIB* rearrangement.

Results: We identified the *MYB-NFIB* fusion gene in 28% of primary and 35% of metastatic ACCs, but not in any of the other salivary carcinomas. Sequencing analysis of the fusion transcripts were verified by the different exons in both *MYB* and *NFIB*. FISH analysis revealed that fusion negative ACCs by RT-PCR have no *MYB/NFIB* rearrangement. Our quantitative RT-PCR using 2 sets of primers for *MYB* indicated that *MYB* expression of exon 2-3 in the fusion-positive tumors was over two-fold higher than in fusion-negative ACCs and 100-fold higher than in non-ACCs, but the expression of exon 15 was much higher in fusion-negative tumors than in fusion-positive and non-ACCs salivary carcinomas. The clinicopathological correlation of MYB IHC analysis showed that the presence of the chimeric fusion gene was significantly associated ($p = 0.03$) with patients older than 50 years of age.

Conclusion: The *MYB-NFIB* fusion gene defines a subset of ACCs and contributes to *MYB* overexpression. These findings suggest that *MYB* may be a novel target for tumor intervention in patients with ACC. Comparative genomic analysis of fusion positive and negative ACCs are being done to identify the clinical pathway of biological significance.

Using the i2b2-SHRINE Software Platform as a Modular, Shareable Evidence Base for Chronic Disease Registries and Comparative Effectiveness Studies in Pediatric Rheumatology and Gastroenterology

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Objective: Disease registries represent high-quality, carefully curated sources of longitudinal clinical data, and may be linked to claims-based data sources, but historically have not been designed with widespread, intra- and inter-registry data sharing in mind. Using the i2b2-SHRINE software platform as an open-source, federated semantic data warehouse, we enable permissioned data sharing within two newly established chronic disease registries.

Methods: The Childhood Arthritis and Rheumatology Research Alliance network (CARRANet) is a 60-site consortium funded by a U.S. Grand Opportunities grant (RC2AR058934, NIH/NIAMS) to build a national observational clinical research data framework for childhood rheumatic disease. In addition to a conventional chronic disease registry, this framework is envisioned to support multiple additional studies among different subsets of investigators, including a drug post-marketing surveillance network. Similarly, the Harvard-affiliated hospitals Inflammatory Bowel Disease network seeks to link the major IBD centers affiliated with Harvard Medical School in a research data collection and sharing network. For our needs, we adapted the existing i2b2 (Informatics for Integrating the Biology and the Bedside, <http://i2b2.org>) and SHRINE (Shared Health Research Information Network) open-source software platforms, which have been designed for federating Electronic Health Record data for research purposes across the Harvard-affiliated hospitals.

Results: A peer-group architecture based on i2b2/SHRINE satisfied the design requirements, supporting permissioned query delivery, processing, and data aggregation. We augmented the SHRINE peer-to-peer network software to support (1) peer group assignment and (2) user- and peer-specific authorization, thereby enabling permissioned, federated queries within peer groups. In this design, each peer member (typically a single registry site) in a group comprises a single, site-specific i2b2 data warehouse which contributes data to the larger research consortium.

Conclusions: A software architecture which enables permissioned, peer group-based access to federated data sources appears to be a promising strategy for promoting data availability and access within and across chronic disease registries.

Developing rare disease resources in developing countries

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Objectives: To identify existing scattered rare disease and disorder (RDD) registries as well as patient support groups and coordinate them to develop a comprehensive online RDD registry, develop disease specific information, develop RDD biospecimens repository and make available these resources for RDD patients, healthcare professionals, basic and clinical researchers and policy planners in developing countries.

Methods: Pioneered by the United States, a handful of countries have targeted laws to comprehensively assist RDD sufferers. But lack of such laws in most developing countries compounds sufferings of generally neglected RDD sufferers there. India as a model country with its vast pool of scientific knowledge, established information technology prowess and relatively high number of published RDD case reports can bring about a change without awaiting major government initiatives. Existing multiple RDD information resources in the US cater to the world. Though comprehensive these resources do not sufficiently address geo-restricted RDD such as Handigodu syndrome, Kyasanur forest disease or Madras motor neuron disease in India or Mseleni joint disease in South Africa, to mention a few. The three basic resources necessary for RDD research are 1) disease specific information 2) disease registries and 3) biospecimens for scientific research. It is practical to develop the former two using internet tools at relatively low cost with volunteer participation. Once this is established, biospecimens from RDD patients can be gathered, which however will require substantial financial and physical infrastructures.

Results: A small online information database is being developed as a resource for RDD community by volunteer participants involving a handful of scientists, clinicians and information technology professionals by employing 'cloud computing' approach. Hardware infrastructure is not owned or maintained and also volunteers are not required to cluster under a single roof, thus minimizing capital expenditure. At the outset this experimental disease information resource at www.rarediseasesindia.org will serve RDD patient community, students and non-specialist readers and could potentially progress to include registry setup and ultimately could help scientific researchers and government policy planners.

Conclusions: Developing viable internet-based country-specific resource databases can help highlight RDD and bring related information together for patients and researchers and in due course can help recruit patients for clinical trials. Such databases along with activism from patient advocacy groups may help evolve specific laws benefiting RDD community in developing countries.

Eye Disease in Behcet's Syndrome Patients in a North American Cohort

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Background: Ocular disease has been reported in up to 75 % of patients with Behcet's Syndrome (BS) in endemic regions where permanent visual loss is common. The prevalence of eye disease in North American BS patients is unknown, but felt to be lower than in endemic regions. More prevalent and severe eye disease is expected in North American populations with an ethnic background in those regions.

Methods: A BS center was established in New York City in 2004. Patients at the center completed an MDHAQ, Behcet Syndrome Activity Score, questionnaires about past medical history, medication use, Behcet's specific history, ethnic and demographic information. These data were prospectively collected over 5 years. Patients fulfilling the International Study Group (ISG) criteria were analyzed as one cohort and in 2 groups: Group A= ethnic background in Europe, North America and self declared Caucasians without Mediterranean or Middle Eastern background; Group B= Mediterranean, Middle Eastern, North African, or Far Eastern ethnic background. These groups were compared for their prevalence, type and outcome of ocular disease.

Results: 471 patients were seen. 296 (62.8%) fulfilled ISG criteria and were included in the present study. Of those, 121 (40.9%) patients had eye disease, which included 56 (18.9%) with uveitis, 8 (2.7%) with retinitis, 11 (3.7%) with episcleritis, and 42 (14.2%) with other eye disease. There was no statistically significant difference between Groups A (n=163) and B (n=133) regarding the prevalence of eye disease (41.1% vs. 40.6%, p<0.93), types of involvement: uveitis (19.6% vs. 18.0%, p<0.729), retinitis (1.8% vs. 3.8%, p<0.311), episcleritis (3.1% vs. 4.5%, p<0.514), baseline disease activity and use of immunosuppressive medications. None of the patients presented with or developed blindness during the study period.

Conclusions: Eye disease was less prevalent and seemed to have better outcomes in this North American cohort of BS patients than in cohorts studied in endemic BS regions. There was no significant difference in prevalence or outcome between North Americans of non-Mediterranean European ancestry compared to individuals of Mediterranean, Middle- or Far Eastern descent living in the US. These findings could suggest a role of environmental factors in the phenotypic expression of BS in general, and in the pathogenesis of Behcet's eye disease in particular.

Circadian Hemodynamic Changes in Patients with Autonomic Failure and Supine Hypertension

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Objective: Blood pressure (BP) normally decreases by 10-30% of daytime values during the night (“dipping”) and increases on awakening (“morning surge”). The autonomic nervous system is thought to play a role in mediating this phenomenon. Accordingly, we previously reported that most patients with autonomic failure (AF) and supine hypertension have a nondipping pattern of BP. In about 1/3 of AF patients, however, BP “dipped” at night and returned to their elevated baseline values on awakening despite their autonomic impairment. The purpose of this study was to determine the circadian hemodynamic changes underlying the dipping and morning surge phenomena in AF patients with supine hypertension.

Methods: Seven dippers with AF underwent a 24-hr BP monitoring. Supine BP, cardiac output (CO), stroke volume (SV) and systemic vascular resistance (SVR) were measured at 5pm, 8pm, 4am and 8am. Urine and body weight were measured from 8am-8pm and 8pm-8am.

Results: Nighttime systolic BP (12am-6am; 127 ± 9 mm Hg) decreased by $20 \pm 3\%$ of daytime values (157 ± 6 mm Hg). This was associated with a $28 \pm 7\%$ decrease in CO at 4am (from 5.2 ± 0.4 during the day to 3.7 ± 0.4 l/min). SV and heart rate (HR) significantly decreased from daytime to 4am by $24 \pm 7\%$ (71 ± 4 vs. 55 ± 8 ml, $p=0.028$) and $6 \pm 2\%$ respectively (74 ± 4 vs. 69 ± 3 bpm, $p=0.028$). Body weight decreased significantly during the night (-1.9 ± 0.2 Kg, $p=0.018$), but we could not document a significant increase in nocturnal diuresis or natriuresis. The morning BP surge was 28 ± 5 mm Hg and was associated with a non-significant increase in CO and SV from 4am to 8am ($21 \pm 9\%$ and $19 \pm 9\%$, respectively). SVR and HR remained unchanged ($-5 \pm 6\%$ and $2 \pm 2\%$, respectively).

Conclusion: Dipping was associated with a decrease in CO and SV, which are likely secondary to nocturnal volume loss. The morning BP surge was not associated with increases in SVR or HR; redistribution of body fluid and an increase in SV are possible mechanisms underlying this phenomenon.

Harnessing Sympathetic Activity in the Treatment of Autonomic Failure. The Synergistic Effect of Norepinephrine Transporter Blockade and Alpha-2 Antagonism

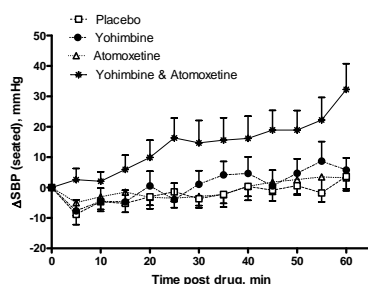
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Objective: Patients with autonomic failure (AF) have disabling orthostatic hypotension due to impaired sympathetic activity. Norepinephrine transporter (NET) blockade with atomoxetine raises blood pressure in AF by increasing synaptic norepinephrine concentrations in peripheral sympathetic neurons. This effect, however, requires tonic release of norepinephrine and is not seen in patients with low sympathetic tone. We hypothesized that increasing residual sympathetic outflow with the alpha-2 antagonist yohimbine, would potentiate the pressor effect of NET blockade with atomoxetine and improve orthostatic tolerance in AF.

Methods: Seventeen patients were given a single oral dose of either placebo, yohimbine 5.4 mg, atomoxetine 18 mg and the combination yohimbine and atomoxetine on separate mornings in a single blind, crossover study. Blood pressure and heart rate were assessed while patients were seated and standing for up to 10 minutes before and 1 hour after the intervention.

Results: Neither yohimbine nor atomoxetine produced a significant increase in seated systolic blood pressure (SBP) or orthostatic tolerance (AUC of standing SBP) compared to placebo. Only the combination of atomoxetine with yohimbine increased seated SBP and orthostatic tolerance significantly (Figure; $p < 0.001$ and $p = 0.016$, respectively). The maximal increase in seated SBP seen with this combination was 32 ± 9 mmHg at 60 minutes post-drug.

Conclusion: The combination yohimbine and atomoxetine has a synergistic pressor effect in peripheral AF, which may be explained by an increased release of norepinephrine in peripheral sympathetic neurons by alpha-2 antagonist combined with a reduced norepinephrine clearance by NET blockade. This pharmacologic approach was useful to improve orthostatic tolerance in these patients.



Are Patients with Wegener's Granulomatosis (WG) or Microscopic Polyangiitis (MPA) Entered in Clinical Trials Representative of Those Followed in Observational Cohorts? Comparison of Characteristics and Outcomes of Patients in the WEGENT and WEG91 Trials Versus Those Included in the Observational FVSG Patients' Database

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Objective: Clinical trials in patients with rare diseases typically have small sample sizes. Whether patients entered in trials are representative of those followed in observational cohorts is unclear.

Methods: We compared the clinical characteristics at the time of diagnosis and global outcomes (relapse and death) of patients with systemic WG or MPA with FFS_{≥1} pre-enrolled in the WEG91 or WEGENT trials to those followed in the FVSG observational cohort. All patients had to fulfill ACR and/or Chapel Hill nomenclature criteria.

Systemic WG was diagnosed in patients with either renal disease, involvement of 1 organ and constitutional symptoms, or ≥2 organs involved.

Results: In comparison to the 182 patients from the observational cohort, the 220 trial patients (159 WEGENT, 61 WEG91; including 214 newly diagnosed) were older and had more severe disease at diagnosis, as attested by their higher mean activity score (BVAS) and lower GFR (See table in Poster). They had a higher mortality (22.3% versus 6.6%; p<0.001), mainly during the first 2 years, but a comparable relapse rate (45.9% versus 44%; p=0.77).

Conclusions: WG and MPA trial patients differ from those followed in observational cohorts in their initial disease severity, but not relapse risk. We are planning to similarly study other trials and observational cohorts as well as other vasculitides.

Adeno-Associated Viral Vector Mediated Liver-Specific Transgene Expression in Patients with Hemophilia B and Galactosialidosis

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Objective: Severe hemophilia B is an X-linked recessive bleeding disorder affecting about 1 in 30,000 males. Factor IX (FIX) gene mutations cause a clotting factor deficiency which results in frequent hemorrhages into joints or soft tissue and a high risk of intracranial bleeding. Galactosialidosis is a rare lysosomal storage disease due to a combined deficiency of β-galactosidase and neuraminidase, secondary to a deficiency of Protective Protein/Cathepsin A (PPCA). Patients with the attenuated, late infantile form of galactosialidosis have hepatosplenomegaly, growth retardation and cardiac involvement, but rarely neurological abnormalities. Both conditions are single gene disorders with a direct cause and effect relationship and can be treated by liver-specific gene expression. In both, tightly regulated control of gene expression is not essential since a wide range of protein activity is expected to be beneficial and non-toxic. A novel self-complementary adeno-associated viral vector (scAAV2/8-LP1) with liver-specific expression successfully delivered either the FIX or the PPCA gene via peripheral vein in mice and nonhuman primates. A phase I/II clinical trial for severe hemophilia B patients was recently opened. A different clinical trial for PPCA gene transfer in patients with late infantile galactosialidosis is currently submitted to the regulatory agencies.

Methods: Two patients with severe hemophilia B were peripherally infused with the FIX gene-containing vector scAAV2/8-LP1-hFIX_{co}.

Results: The vector infusion was well tolerated without acute or delayed adverse effects. Stable expression of low levels of FIX has been achieved in the first participant for more than 4 months. If no safety concerns arise in both participants in the next several weeks, the vector dose will be escalated in the third participant.

Conclusions: Adeno-associated viral vector-mediated liver-specific transgene delivery via peripheral vein, using the scAAV2/8-LP1 vector construct, appears safe and potentially efficacious in hemophilia B according to early results of the ongoing trial. These results are promising for plans using the same vector construct for PPCA gene transfer in patients with late infantile galactosialidosis and for exploring application in other single gene disorders with liver-specific expression.

The Diagnostic Yield of an Extended Sleep EEG in Angelman Syndrome (AS)

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Objective: The goal of this study was to define the optimal EEG recording (length and montage--full head vs. limited) needed to identify ictal, interictal and benign abnormal rhythms in AS.

Methods: Overnight sleep EEGs in conjunction with PSG from 16 patients with AS between the ages of 3 -16 years old enrolled in the Angelman Natural History protocol study were reviewed. A blinded electroencephalographer reviewed the first hour of each EEG using 6- and 21-channel montages. Data was first reviewed in the 6-channel montage and then in the 21-channel montage at least 72 hours later. Each EEG received a score based on finding found on the EEG. The categories scored included: overall EEG impression, EEG background appearance, presence of an occipital rhythm, rhythmic theta or delta, epileptiform abnormalities, and sleep. A second epileptologist independently reviewed the studies to confirm inter-rater reliability of the scores. After each EEG was scored in the different montages, a chart review was done to identify clinical history of seizures and any previous epilepsy monitoring unit (EMU) admissions.

Results: Although 81% of patients had a clinical history of seizures and were on anti-epileptic drugs (13/16), only 18% had IED and/or ictal discharge on prolonged EEG (3/16). These proportions were similar on both the 6- and 21-lead EEGs. Only 1 of the 18 patients had an admission to the EMU. Both the limited 6-lead EEG and the full 21-lead EEG were able to distinguish benign abnormal rhythms, in particular the diffuse rhythmic delta pattern. There was 100% concordance in identifying rhythmic delta and/or theta patterns. Otherwise, the background abnormalities, presence or absence of sleep patterns, and overall background appearance were often misrepresented on the 6-lead EEG. The 6-lead EEG also missed an ictal discharge emanating from the frontal lobe.

Conclusions: A limited 6-lead EEG is able to detect some common abnormal rhythms seen in AS but is sub-optimal in detecting overall background appearance and seizures in AS. In contrast to a general epilepsy population, IEDs are less likely to be detected on a prolonged EEG in AS patients, even when sleep is captured. Thus, an EMU admission may be needed to characterize and localize seizures in this special population.

Screening for Respiratory Ciliary Dysfunction in Autosomal Recessive Polycystic Kidney Disease

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Objective: Autosomal recessive polycystic kidney disease (ARPKD) is a rare sensory ciliopathy characterized by asymmetrically enlarged kidneys due to renal cysts and associated congenital hepatic fibrosis (CHF). Recent data have suggested a possible connection between sensory ciliopathies and respiratory ciliary dysmotility. *In vivo* measurement of nasal nitric oxide (nNO) production has been used as a sensitive screen for primary ciliary dyskinesia (PCD). We screened for respiratory ciliary dysfunction by assessing nNO production and looked for evidence of respiratory tract abnormalities in ARPKD patients.

Methods: Records of patients enrolled in a natural history study of ARPKD/CHF at the NIH were reviewed for a history of respiratory symptoms and/or respiratory tract infections, prior pulmonary function test results, and chest imaging. Sampling of nNO was performed through a foam nasal probe during exhalation through a resistor and was measured in real-time via a NiOx (Aerocrine) chemiluminescence analyzer. Values from ARPKD patients were compared to the nNO measurements from healthy volunteers.

Results: The average age and nNO of the ARPKD patients (n=16) were 12±(SEM)2 years and 198±17 nL/min respectively, compared to 32±2 years (p<0.000001) and 289±15 nL/min (p<0.0005) in the controls (n=25). Five ARPKD patients (31%) had a history recurring respiratory tract infections including 3 with otitis media requiring myringotomy tubes (nNO: 115, 132, 182 nL/min). Air trapping on lung volume measurements was noted in 5 (n=13, 38%) and abnormal diffusion capacity was noted in 7 (n=11, 64%) patients. Three patients had high resolution chest CT scans that revealed no evidence of bronchiectasis.

Conclusion: None of the ARPKD patients had nNO levels in the range seen with PCD (<100 nL/min). However, the values were significantly lower than non age-matched controls (values from healthy younger children may be lower) with lower levels in those with more respiratory problems. Respiratory infections and respiratory tract abnormalities in ARPKD indicate further investigation of respiratory ciliary dysfunction is warranted.

Supported in part by intramural funds of the NIAID and NHGRI; Genetic Diseases of Mucociliary Clearance Consortium by NIH 9 U54 HL096458-06 funded by Office of the Director, and supported by ORDR and NHLBI, NIH

A Review of Investigational Treatments for Stargardt's Disease

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Objective: Stargardt's disease, often referred to as the juvenile form of macular degeneration, affects approximately 30,000 people in the U.S. and is the most prevalent cause of inherited blindness for which there is no cure or treatment. The disease is thought to originate from genetic defects in the *ABCA4* gene resulting in dysfunction of a RimP protein. Several approaches have been suggested for the treatment of Stargardt's disease and clinical trials should soon follow. The objective of this study is to present and evaluate current investigational treatments for Stargardt's disease.

Methods: We reviewed the literature and summarized various treatment strategies based on mechanism of action, mode of treatment delivery, whether the approach was prophylactic or curative, and whether it could potentially halt or reverse vision loss. We presented the pros and cons of each approach.

Results: We evaluated five approaches: (1) gene therapy such as lentiviral vector containing the human *ABCA4* gene (Stargen™ gene therapy); (2) stem cell based therapy using differentiated retinal pigment epithelium (RPE); (3) retinol binding protein antagonist (Fenretinide); (4) visual cycle antagonist (Ret-NH₂, ACU-4429, RPE65 inhibitor); and (5) compound hindering the aberrant reactivity of vitamin A (C20-D₃-vitamin A). Gene or cell-based therapies have the potential to cure Stargardt's disease and might lead to long-term benefit, however there remain several safety and technological hurdles hindering their implementation. Due to their longer history and well-accepted evaluation protocols, small molecules based therapies raise fewer concerns and could be rapidly implemented. While such approaches are likely to have prophylactic benefits and may even prevent vision loss, it is not known if such therapies will reverse vision loss.

Conclusions: The dynamic activity of investigational treatment strategies for Stargardt's disease highlights the tremendous progress made since the discovery of the link between the *ABCA4* gene and the disease, thirteen years ago. The existence of various therapeutic approaches discussed in this review bode well for the future treatment for Stargardt's disease.

Genotype-Phenotype Correlation Study in Phelan-McDermid Syndrome

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Objective: This investigation examined the association between size of deletion and phenotype in Phelan-McDermid Syndrome. This syndrome, also known as 22q13 Deletion Syndrome, is a rare and underdiagnosed syndrome characterized by global developmental delay, hypotonia, mildly dysmorphic features, and occasional autistic-like characteristics. The syndrome is caused by deletions ranging from 0.1 to 9.0 million bases on the distal long arm of chromosome 22. SHANK3, located in the subtelomeric region, is generally assumed to be the primary cause of neurologic phenotypes. The severity and expression of the syndrome are highly variable suggesting other genes in the deletion region may be important.

Methods: A custom oligo array comparative genomic hybridization (CGH) technique was used to interrogate 22q12-qter to pinpoint deletion breakpoints to a resolution of 100 base pairs. This information was coupled with physical exam and medical history information to statistically assess the association between deletion size and clinical phenotypes for 97 affected individuals.

Results: Preliminary analysis suggests that up to 10 different phenotypes are associated with larger deletions. Further, specific genes and gene regions most associated with these phenotypes will be presented.

Conclusion: While SHANK3 may be responsible for the majority of the neurologic phenotypes, other genes present on the long arm of chromosome 22 are important contributors to the syndrome and may help explain physical findings in other syndromes.

Rescue of Purkinje Neuron Depolarization Block Improves the Motor Phenotype in a Mouse Model of Spinocerebellar Ataxia Type 3

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Objective: To determine if alterations in Purkinje neuron physiology can explain some of the motor deficits in Spinocerebellar ataxia type 3 (SCA3).

Background: Most degenerative ataxic disorders are associated with prominent loss of cerebellar Purkinje neurons. Although ataxia is a significant feature of SCA3, the most common dominantly inherited ataxia, in many affected persons with SCA3 only modest loss of Purkinje neurons is present.

Methods: We employed whole cell recordings from acute cerebellar slices of SCA3 transgenic mice and littermate controls to determine the intrinsic firing properties of Purkinje neurons. SCA3 transgenic mice express the full human *ATXN3* gene with an expanded CAG repeat that encodes a polyglutamine tract with 84 residues.

Results: In cerebellar slices from SCA3 mice, a significant fraction of Purkinje neurons underwent depolarization block and lost the ability to sustain spontaneous repetitive firing. Depolarized neurons had a normal resting membrane potential and could sustain repetitive firing following depolarization from a negative holding potential, arguing against a nonspecific loss of membrane integrity. Voltage-activated potassium currents in somatic patches from SCA3 neurons showed increased inactivation compared to neurons from wild-type littermates. Administration of an activator of calcium-activated potassium channels, SKA-31, partially corrected abnormal Purkinje cell firing and improved motor function in symptomatic SCA3 mice.

Conclusions: We demonstrate early changes in Purkinje cell physiology in a mouse model of SCA3 and identify a defect in voltage activated potassium channels as the likely cause. These functional changes in Purkinje neurons in SCA3, even in the absence of neuronal loss, likely explain some of the ataxia seen in this disorder and represent an important target for the symptomatic treatment of this disorder.

Clinical Symptoms Associated with Primary Ciliary Dyskinesia – Results of a Multi-Center Study

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Objectives: Primary Ciliary Dyskinesia (PCD), a genetic disorder of cilia resulting in impaired mucociliary clearance, presents as chronic oto-sino-pulmonary disease. Identifying people with PCD can be difficult. Disease-specific clinical criteria for PCD would be useful to direct earlier testing of appropriate patients.

Methods: Our Consortium assessed symptomatic people with suspected PCD using systematic clinical symptom data entry, nasal nitric oxide (nNO) measurement, ciliary electron microscopy, and genetic testing. After diagnostic evaluation, patients were defined as definite PCD (clinical phenotype + PCD specific cilia defects and/or 2 known PCD gene mutations), Probable PCD (clinical phenotype in a child >5 years + nNO<100nl/min, but no cilia defect or gene mutation), Possible PCD (same as probable PCD but <5 years, so nNO less reliable), or Unlikely PCD (nNO>100nl/min and no cilia defect or genetic mutation).

Results: Of 444 patients (ages 0.08 to 79 years, median 12 years) referred for PCD evaluation, 174 (39%) have PCD, 83 (19%) have Probable PCD, 23 (5%) have Possible PCD, 164 (37%) are Unlikely PCD. Clinical features more prevalent in subjects with PCD+Probable PCD versus Unlikely PCD are neonatal respiratory distress (70% versus 12%, p<.0001), year-round wet cough (94% versus 60%, p<.0001), year-round nasal congestion (93% versus 64%, p<.0001), and chronic otitis media (89% versus 63%, p<.0001). Median age of onset for wet cough is significantly younger in patients with PCD+Probable PCD (0.08 years, interquartile range (IQR) 1.9) versus Unlikely PCD (4.0 years, IQR 12.8, p<.0001). Median age of onset for nasal congestion is also significantly younger in patients with PCD + probable PCD (0.08 years, IQR 0.4) versus unlikely PCD (1.0 years, IQR 6.2, p<.0001).

Conclusions: In a population with suspected PCD, PCD-specific clinical features include: neonatal respiratory distress, year-round wet cough, year-round nasal congestion, and recurrent otitis media. Typically, onset of cough and nasal congestion occurs in the first month of life. This early onset with the above disease-specific clinical criteria should prompt PCD evaluation.

NIH Grant-9-U54-HL096458-06, funded by Office of the Director, supported by ORDR/NHLBI/NIH

Evaluating Respiratory Cilia Dysfunction in Two Heterotaxy Populations

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Objectives: Heterotaxy is a rare disorder of organ laterality defects, often involving congenital heart disease. At least 6% of patients with primary ciliary dyskinesia (PCD), a disease of chronic sino-oto-pulmonary symptoms from respiratory cilia dysfunction, also have heterotaxy; however, the prevalence of PCD in heterotaxy is unknown. We evaluated the prevalence of respiratory cilia dysfunction in two heterotaxy populations.

Methods: Nasal nitric oxide (nNO), a surrogate marker for PCD, was measured in two heterotaxy populations (using tidal breathing in children <5 years or resistance plateau techniques); one referred for chronic sino-pulmonary disease and one not previously referred for sino-pulmonary disease. nNO was also measured in healthy controls and primary ciliopathies (Polycystic Kidney Disease, Retinitis Pigmentosa, Bardet-Biedl Syndrome). Referred participants were diagnosed with PCD if they had appropriate clinical phenotypes and classic electron microscopic cilia defects or 2 known disease-causing genetic mutations. Probable PCD was diagnosed if participants had appropriate phenotypes and nNO resistor levels <100nl/min, but no cilia defect or genetic mutations.

Results: Of 42 participants referred for sino-pulmonary disease, 36 have complete diagnostic studies for PCD. Of these, 16 (44%) have definite PCD, 6 (17%) have probable PCD, 14 (39%) are unlikely PCD. Median nNO resistor levels are 22 ±14nl/min in PCD, 23.9 ±16nL/min in Probable PCD, and 280 ±96nL/min (P<0.001) in unlikely PCD. Symptoms are more prevalent in PCD+Probable PCD versus unlikely PCD for year-round, wet cough (96% vs 31%, P<0.0001), neonatal respiratory distress (94% vs 55%, P=0.02), bronchitis/pneumonia (83% vs 57%, P=0.13), year-round nasal congestion (87% vs 64, P=0.22), and recurrent otitis media (91% vs 79%, P=0.36).

In the non-referred population, nNO resistor measurements in heterotaxy were significantly lower (N=14, median 197 ±61nL/min, p<0.001) versus healthy controls (N=77, median 287 ±118nL/min) and primary ciliopathies (N=12, 308 ±66nL/min). nNO tidal breathing measurements were similar in heterotaxy (N=8, median 117 ±61 nL/min, p=0.53) and healthy controls (N=123, median 93 ±60 nL/min). One heterotaxy participant has nNO in a range suspicious for PCD (<60nL/min).

The Prevalence of Primary Ciliary Dyskinesia in Heterotaxy: Evaluation Through Internet Based Survey

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Objectives: Heterotaxy is a rare disorder involving organ laterality defects that often include congenital heart disease. Primary ciliary dyskinesia (PCD), a disease of chronic sino-oto-pulmonary symptoms from respiratory cilia dysfunction, is associated with laterality defects – approximately 50% have situs inversus totalis and at least 6% have heterotaxy. The prevalence of PCD within the heterotaxy population is not known. We evaluated prevalence of respiratory symptoms associated with PCD within members of a heterotaxy organization through an Internet based member survey.

Methods: An online survey was created with Qualtrics software and the internet link was distributed to 200 members of the Heterotaxy Hope Organization, an international non-profit group of self-referred individuals with heterotaxy or parents of children with heterotaxy. Respondents participated voluntarily, and information was collected on laterality defects, symptoms of sino-oto-pulmonary disease, past respiratory disease investigations, and personal/family history of other ciliopathies. Respondents were designated at increased risk of PCD if they had at least two symptoms of chronic sino-oto-pulmonary disease, diagnosed bronchiectasis, or respiratory cultures positive for pseudomonas or nontuberculous mycobacterium.

Results: After one month, 32 survey responses were received (mean participant age 9 years). 13 participants have right heart isomerism, 5 have left heart isomerism, 11 have polysplenia, and 21 have asplenia. Sweat chloride testing has been performed in 4 people, and none have Cystic Fibrosis. Nasal ciliary biopsies have been done on 4 respondents, and none have been diagnosed with PCD. No respondents have other ciliopathies, and 3 respondents have family members with heterotaxy. Bronchoscopy has been performed on 8 participants.

Copy Number Variation and Candidate Gene Analysis in PHACE syndrome.

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Objective: PHACE syndrome (posterior fossa brain malformations, hemangiomas, arterial anomalies, coarctation of the aorta and cardiac defects, and eye abnormalities) is a rare multisystem developmental disorder with significant morbidity due to congenital heart disease, congenital brain malformations and developmental delay as well as disfigurement due to a large vascular birthmark on the face (infantile hemangioma). We hypothesize that copy number variation (CNV) in one or more regions of the genome may play a role in the etiology.

Methods: Genomic DNA samples from peripheral blood have been collected from 92 patients, 45 parents and 1 sibling at Oregon Health and Sciences University (OHSU) and 19 PHACE patients and their parents at the Medical College of Wisconsin/Children's Hospital of Wisconsin (MCW). DNA from 91 subjects has been assessed using the Affymetrix Gene Chip 6.0 single nucleotide polymorphism (SNP) array at MCW.

Results: SNP CNV analysis for the preliminary data consisted of 16 subjects from the PHACE genetic repository who are of Northern European descent and for whom parental 6.0 SNP data was also created. These trios were evaluated using the PennCNV protocol for high-resolution copy number variation detection. There were 561 rare CNV regions across all subjects. There were a total of 218 deNovo CNV events, one of which was not in the Database of Genomic Variants. In the second phase of the analysis, all 91 subjects were analyzed using 3 different algorithms; Aroma Affymetrix, Birdsuite and PennCNV. We are currently scanning for CNV containing genes that are important in development, vascular biology and congenital heart disease.

Conclusions: Identified CNV regions in PHACE patients may contain defects in a limited number of genes that may explain the constellation of abnormalities in PHACE syndrome. Identification of a causal genes in PHACE syndrome would contribute to a better understanding of in hemangioma pathophysiology and molecular pathways involved in cerebral artery, brain and cardiac development.

Porphyria cutanea tarda relapse as related to susceptibility factors and type of treatment

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Objective: There is limited information on relapse of Porphyria cutanea tarda (PCT) after complete remission is achieved with accepted forms of treatment. We report on relapse rates as related to susceptibility factors in an ongoing study comparing treatment by phlebotomy (PHB) or low-dose hydroxychloroquine (HCQ).

Methods: Patients with documented PCT were characterized for known susceptibility factors and treated with either PHB (450 mL usually every 2 weeks until a target ferritin of <20 ng/mL) or HCQ (100 mg twice weekly until normal plasma porphyrins for \geq 1 month). Patients were randomized unless they had contraindications to either treatment. Remission was defined as normalization of plasma and urine porphyrins. Fisher's exact and t-tests were used to compare proportions and means respectively. Life table and Cox proportional hazard regression analyses performed to assess time to relapse and factors predicting relapse respectively.

Results: Response rates were comparable with both treatments. To date, 21 patients (mean age 51 \pm 6 yrs, 62% males, 91% Caucasians) achieved remission and then been followed for at least 30 days and up to 3 years. Of these, 11 were treated by PHB and 10 by HCQ. Susceptibility factors were alcohol abuse in 95%, hepatitis C in 81%, smoking in 76%, HFE mutations in 76%, estrogen use in 25% of females, and inherited UROD deficiency in 9.5%. Nine patients have relapsed and 12 have remained in remission during a median follow up period of 359 (35-799) and 251 (56-1199) days, respectively. Relapse occurred in 6 of 10 treated with HCQ, and 3 of 11 treated by phlebotomy, but this difference is not significant (Chi-Sq P=0.13). Time to relapse was similar with PHB and HCQ treatment (Log Rank P=0.13). Those relapsed were more likely to have experienced a more delayed response to treatment, and not achieved remission within 6 months (89 vs. 42%; p=0.027). Individual susceptibility factors noted above, which were multiple in each patient, were not associated with more frequent relapse.

Conclusions: Phlebotomy and low-dose HCQ are associated with comparable response and relapse rates in this interim analysis. Long term follow up of larger numbers of patients is needed to determine influence of individual susceptibility factors on relapse of PCT.

The New Childhood Liver Disease Research and Education Network (ChiLDREN): A Cooperative Endeavor Between NIDDK, Academic Centers and Patient Advocacy Groups.

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Investigation of rare pediatric liver diseases benefits from multi-centered collaboration in order to impact diagnosis and treatment in a relatively short period of time. Such issues as optimal study design, use of emerging technologies, careful use of resources and research subject participation are facilitated through this collaborative approach. In recent years, the NIH has funded two clinical research networks investigating pediatric cholestatic liver diseases, the Biliary Atresia Research Consortium (BARC) and the Cholestatic Liver Disease Consortium (CLiC). Involving 10 academic pediatric centers and a data coordinating center, BARC and CLiC have studied biliary atresia (BA), idiopathic neonatal hepatitis, alpha-one antitrypsin deficiency, Alagille syndrome, progressive familial intrahepatic cholestasis, bile acid synthesis defects, mitochondrial hepatopathies and cystic fibrosis liver disease. In June, 2009, BARC and CLiC were combined and expanded by NIDDK to form ChiLDREN, which will include the addition of 6 more clinical centers, investigators and patient enrollment capabilities. Seven Patient Advocacy Groups that address the needs of families and affected children participate in the activities and decisions of the network. Longitudinal studies are ongoing for the 8 cholestatic diseases, with the goal to improve diagnostics, understand genetics and pathogenesis and develop and test new treatments. For example, a randomized, controlled, double-blinded study of post-hepatoportoenterostomy corticosteroid therapy is ongoing for BA. ChiLDREN will continue to collect and store tissue specimens, serum, urine and DNA on study participants, utilizing NIDDK biorepositories. Much of the exploratory science is performed through ancillary studies which require additional funding. Ancillary studies can be proposed by ChiLDREN investigators or others in collaboration with a ChiLDREN investigator. ChiLDREN also provides up-to-date web-based educational material for the public and for professionals. A unique aspect of ChiLDREN is the ability to perform long-term longitudinal studies and understand natural history, clinical and developmental outcomes and predictors of outcome measures, which can all be linked to the biospecimens. ChiLDREN desires to work with pediatric gastroenterologists across the U.S. to allow their patients to participate in these essential studies.

Neurofibromatosis I, Women of Color and Stigma

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Objective: Neurofibromatosis 1 is a genetic disorder in which affected individuals develop predominantly benign tumors. Features of NF1 include multiple skin tumors, café au lait spots, optic gliomas, cranio-facial anomalies, scoliosis, etc. Irving Goffman (1963) argued that stigma is an attribute that is extremely discrediting. Stigmatization is a social process of global devaluation of individuals who possess an undesirable attribute. NF1 is a decidedly stigmatizing attribute because it can be highly visible and calls into question the affected individual's ability to fully participate in a culture that values physical perfection. Non-affected individuals may harbor anxiety and fear of contagion towards individuals with NF1. Mahajan et al. (2008) described a theoretical conceptualization of compound stigma when inter-related components converge as result of one or more devalued attributes (distinguishing and labeling of human differences; devaluation, isolation, loss of social status and subsequent discrimination). A woman of color with NF1 must negotiate a culture that views her as imperfect because of her gender, race and compromised appearance.

Method: (1) Terminology used when referring to adults with NF 1 and others with *facial distinctions* (e.g. handicapped, abnormal, aberrant and disfigured) will be discussed. (2) Reports of women of color with NF1 who participated in a study to describe their experience of living with a *facial distinction* will be presented.

Results: Limited data focuses on adults with NF1 and scarce data exists on under-represented groups, particularly women with NF1 or other facial distinctions. The language used in the literature to describe people with NF 1 or others with *facial distinctions* (e.g., deformed, disfigured, deviant, abnormal, etc.) does little to address the appearance related stigma that is experienced by affected individuals. Ninety-six percent of the women of color with NF1 did not describe themselves in terms of their stigmatizing attribute; however, 69% of the women reported that others viewed them unfavorably (e.g., weird, contagious) or felt sorrow or pity for them.

Conclusions: As researchers, teachers and medical and mental health care providers it is important to consider the language we use to describe the people we serve. Language shapes research, practice and intervention. NF1, a rare and overlooked disorder in the psychological literature, particularly with regard to adult populations and more so for people of color, presents a unique opportunity to examine stigma and resilience.

Cardiac and Skeletal Muscle Deficiencies in a shRNA-Mediated Knockdown Model of Barth Syndrome

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Objective: Barth Syndrome is an X-linked mitochondrial disease that is associated with mutations within the Tafazzin (TAZ) gene and is characterized by cardiomyopathy, neutropenia, and muscle weakness. Much of the molecular mechanism behind TAZ-deficiencies has been gained from the study of Barth patient cells, and from manipulation of yeast, fruit fly, and zebrafish models of BTHS. While these models have been valuable tools in understanding the effects of tafazzin mutations on cardiolipin metabolism, the cardiovascular and immune systems of these models differ vastly from that of mammals. Previous attempts have been made to generate a knockout mouse model; however no mutated mice have been viable. Recently, Taconic Artemis generated a doxycycline inducible shRNA-mediated Tafazzin knockdown mouse model; however, these mice have yet to be characterized. Our goal is to evaluate the TAZ-deficient mouse model on a molecular scale and to test its clinical relevance to the patient disorder by looking for the presence of the major hallmarks of the disease, cardiac and skeletal myopathy.

Methods: Females were started on doxycycline chow (200mg/kg) prior to pregnancy. Upon weaning, mice were maintained on doxycycline chow. At 2 months of age, mice were sacrificed and Real Time-PCR was used to measure Tafazzin and β -actin mRNA levels. Cardiac mass and ejection fraction were measured using magnetic resonance imaging. Soleus contractile function was measured *ex vivo* at increasing frequencies.

Results: Using real-time quantitative RT-PCR analysis, we report that induced shRNAs result in >90% knockdown of TAZ mRNA in both heart and skeletal muscle tissue. In addition, cardiac mitochondria exhibit a reduction in cardiolipin and an accumulation of monolysocardiolipin species. We observed a reduction in soleus contractile strength in TAZ-deficient mice at 2 months of age and preliminary cardiac measurements made via magnetic resonance imaging revealed that TAZ deficient mice have lower ejection fractions and increased left ventricular mass when compared to control mice at 6 months of age.

Conclusions: Preliminary observations suggest this inducible TAZ-deficient murine model exhibits the clinical phenotypes of Barth Syndrome patients and may ultimately help to improve our understanding of BTHS-related cardioskeletal myopathy.

Phase 3 Trial of Dichloroacetate for Pyruvate Dehydrogenase Deficiency

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Objective: Pyruvate dehydrogenase (PDH) is a common cause of congenital lactic acidosis and an ultimately fatal disease of neurological and neuromuscular degeneration for which no proven treatment exists. We aim to organize and implement a N. American, multi-center, randomized clinical trial (RCT) of chronic, oral dichloroacetate (DCA) in approximately 40 young children with biochemically proven defects in any component of the PDH complex. DCA represents targeted therapy for PDH deficiency because of its ability to increase both the catalytic activity and stability of the enzyme complex (Mitochondrion 6:126, 2006).

Methods: Under the auspices of a Planning Grant from NICHD, investigators from >20 institutions in the U.S. and Canada are undertaking the process of developing the infrastructure, design and methodologies to conduct the first RCT of any therapy for PDH deficiency. Clinical treatment centers are being identified to accept patients from throughout N. America. The collaborative network for this trial leverages the support of the United Mt Disease Fdn, the N. American Mt Disease Consortium (a member of the Rare Diseases Clinical Research Network) and participating CTSA institutions.

Results: Although DCA is a potential reversible peripheral neurotoxin, especially in adults, children generally tolerate chronic administration well (Pediatrics 117:1519, 2006; *ibid* 121:e1223, 2008). Working Groups have been established to address issues of study design; diagnostic criteria; safety measures; tissue banking; nutritional support; disease biomarker discovery; and application of appropriate neuroimaging, neurobehavioral and neurophysiological techniques.

Conclusions: The proposal to conduct a 5-year RCT of DCA in PDH deficiency will be submitted October, 2011, with earliest commencement of patient recruitment in mid-2012. For further information, contact Peter Stacpoole at pws@ufl.edu.

Growth Charts for Rett Syndrome from Birth to 18 Years of Age: Correlation Between Genotype and Anthropometric Values

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Objectives: 1) To develop growth charts for Rett syndrome (RTT) which meet US National Center for Health Statistics (NCHS) standards. 2) To compare growth in RTT to growth in unaffected children. 3) To compare patterns of growth among RTT subgroups with different genetic and phenotypic characteristics.

Methods: To understand growth patterns in RTT, participants were recruited through the Rare Diseases Clinical Research Network RTT natural history study. Cross-sectional and longitudinal growth data were collected along with comprehensive clinical information. A reliability study confirmed consistency within and among anthropometrists. Reference curves for height, weight, head circumference, and body mass index (BMI) were generated using a semi-parametric model with goodness-of-fit tests. These references were compared to the normative population using Student's t-test. Subgroups of RTT based on genotype and phenotype were compared using ANOVA and linear regression.

Results: From 9175 observations of 791 female participants, growth charts of classic and atypical RTT were created with excellent fidelity to the empirical data. Mean growth for classic RTT decreases below the normative population at 1.5 months for head circumference, 6 months for weight, and 17 months for length. However, BMI was similar in RTT compared to the normative population. Normal pubertal increases in height and weight were absent in RTT. Classic and atypical RTT populations showed no significant differences in growth. Early gross and fine motor development were associated with significantly better growth later in life, but language ability was not. Type of genetic mutation was not significantly associated with growth failure, although too few participants were available in all mutation categories.

Conclusion: High quality reference charts meeting NCHS standards can be created for specialized populations allowing clinicians to monitor nutritional interventions more effectively and researchers to examine novel treatments in RTT with greater precision. Growth failure in RTT occurs predominantly in girls who experienced poor early gross and fine motor development. Although no pattern emerged between growth and certain mutations or mutation types, larger longitudinal studies are needed to further examine this association.

Vaccine Antibody Responses in Severe Combined Immunodeficiency (SCID) Patients Receiving Immunoglobulin Supplementation Post-Hematopoietic Stem Cell Transplant

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Background: Current guidelines recommend the administration of inactivated vaccines 6-12 months after hematopoietic stem cell transplant (HSCT). In SCID patients, development of specific antibody responses post-HSCT may be delayed. Usually, they receive immunoglobulin supplementation (IVIg) from diagnosis and parameters of safe IVIg discontinuation have not been established. We sought to demonstrate antibody responses to common vaccines in 10 SCID patients who were receiving IVIg.

Methods: Pneumococcal, tetanus and diphtheria immunizations were initiated at least 12 months after HSCT and administered two weeks after IVIg infusions. The frequency of IVIg infusions were decreased from every 4 weeks to every 6 weeks, while maintaining the same dose for each patient (300-500 mg/Kg/dose). We measured serum immunoglobulin and specific antibody concentrations before and after immunizations. Positive response to immunization was defined as at least 4-fold increase of specific antibody concentrations above pre immunization baseline.

Results: Six B+ SCID and four B- SCID patients received HSCT. Chimerism studies revealed 100% donor T and B cells in 5 patients; 100% donor T cells and 1.5 to 95% donor B cells in 4 patients; and 85% donor T cells and 45% donor B cells in 1 patient. Nine of 10 patients demonstrated responses to immunizations. Geometric means (baseline and peak post-immunizations) of specific antibody concentrations in the responders were 1.00 and 12.73 ug/mL respectively for tetanus ($p < 0.001$); 0.17 and 7.79 ug/mL for diphtheria ($p < 0.001$); 1.08 and 8.49 ug/mL for pneumococcal serotype 14 ($p < 0.001$). Based on these responses and clinical assessment, IVIg was discontinued in those 9 patients who demonstrated specific antibody responses. They have maintained both normal serum Ig levels and protective specific antibody responses to date (range = 8-46 months). The non-responder patient was an X-linked SCID, received a conditioned, HLA-haploidentical (mother) HSCT, did not develop GvHD, and had a 61% donor B cell chimerism. His IgG, IgA and IgM levels were within normal range.

Discussion: Appropriately timed immunization with inactivated vaccines was helpful in determining when IVIg replacement could safely be discontinued in post-HSCT SCID patients. It also identified a SCID patient with immune dysfunction despite normal serum immunoglobulin levels.

Immune Response to Intrathecal Enzyme Therapy in Mucopolysaccharidosis I Patients

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Objective: The immune response to exogenous proteins is a well known confounder of therapies including enzyme replacement for lysosomal storage diseases. The majority of patients who develop serum antibodies to intravenous recombinant human alpha-L-iduronidase (rhIDU) for MPS I do not suffer related adverse events. Currently, intrathecal enzyme replacement therapy (IT-ERT) is being studied in clinical trials as a novel potential treatment for central nervous system disease in MPS I. The goals of this project are to characterize the immune response in CSF to IT-ERT, study the response of serum and CSF cytokines in ERT, and correlate these with adverse event reports to determine the safety of this important new treatment modality.

Methods: Serum and CSF have been collected from 10 subjects with severe or attenuated MPS I receiving IT rhIDU at 30 to 90 day intervals over a 1-year period in clinical trials (NCT-00215527, NCT-00786968, NCT-00638547, NCT-00852358). Samples were collected immediately prior to IT-ERT injections. Antibody titers of anti-rhIDU IgG, IgM, IgE, and IgD are measured by ELISA as OD units per μL and converted to absolute concentrations using a purified human immunoglobulin standard curve. Inflammatory cytokines are assayed by ELISA (CSF) or Luminex (serum) using commercially available kits. Cytokines assayed include IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IFN γ , TNF α , and GM-CSF. Adverse events (AE) are categorized by type, severity, duration, and relation to rhIDU. These will be correlated with concentrations of anti-rhIDU IgG, IgE, and the above cytokines.

Results: CSF anti-rhIDU IgG transiently developed in 3 of 10 MPS I subjects at very low levels (188-845 picograms/ μL) compared to a positive serum control (average 197,402 picograms/ μL). One subject with prior exposure to intravenous ERT had low serum levels of anti-rhIDU IgG but did not develop antibody in the CSF. Additional antibody and cytokine assays are pending. The most commonly reported AE was headache. There was no clinical evidence of allergic or other immune response to rhIDU.

Conclusions: The MPS I patients undergoing IT-ERT studied thus far have very low levels of anti-rhIDU IgG antibody in CSF. Our study will characterize the immune response in CSF to IT-ERT including the serum and CSF cytokine response, and will establish a validated CSF anti-rhIDU antibody assay.

Discovering the Diagnosis of 47,XXY and 48,XXYY

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Objective: The prevalence of Klinefelter syndrome or 47,XXY is between 1 in 581 to 1 in 917 males. Approximately, 64% of males with 47,XXY are never diagnosed. 10% are identified prenatally, and 26% are diagnosed postnatally when they present with developmental delay, behavioral problems, gynecomastia, or infertility. 48,XXYY syndrome occurs in an estimated 1:18,000-1:50,000 males. Herein, we determine families' experiences in discovering the diagnosis of 47,XXY and 48,XXYY and explore the potential factors associated with the timeliness of diagnosis.

Methods: Study participants (n = 89) were parents of children with 47,XXY or males with 47,XXY, and parents of males with 48,XXYY (n = 76).

Results: In the postnatally diagnosed cohort with 47,XXY, 59% presented with developmental delay, with mean first age of parental concern at 5.2 years and mean age of diagnosis at 9.8 years. 41% presented with endocrinologic issues with mean first age of concern at 19.1 years and mean age of diagnosis at 20.9 years. In the cohort presenting with endocrinologic issues, 15/18 patients were unaware of their lack of pubertal development, and routine physical examination by their physician led to genetic testing. In the 48,XXYY group, 93% presented with developmental delay, with mean first age of parental concern at 1.8 years and mean age of diagnosis of 7.4 years.

Conclusions: There is considerable variability in the age of diagnosis for males with 47,XXY. For those presenting with developmental delay, parental concerns arose ~ 4.6 years before a professional ordered a karyotype. In contrast, diagnosis was delayed by only 1.8 years for those presenting with endocrinologic issues. In males with 48,XXYY, parents expressed concern of developmental issues at an earlier age (1.8 years) than the 47,XXY group (5.2 years). Despite being concerned earlier than the 47,XXY group, there is a gap of 5.6 years from the time of concern to diagnosis. The process of discovering the diagnosis of 47,XXY and 48,XXYY takes too long for most patients who are not identified in the prenatal period. As a result, children may not benefit from early intervention and parents may be stressed that their concerns are not addressed appropriately. It is important to value parents' concerns and incorporating regular developmental screening in pediatric practice.

Neurofunctional Characteristics of Childhood Disintegrative Disorder (CDD)

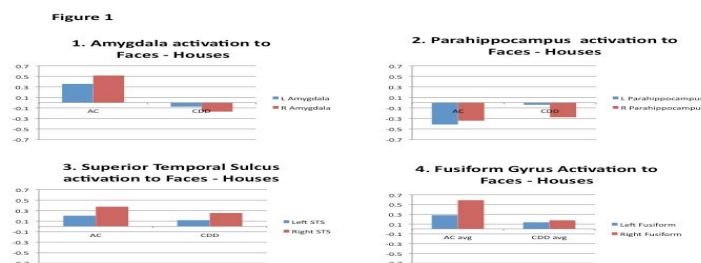
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Objectives: CDD, the most rare and poorly understood Autism Spectrum Disorder (ASD), is characterized by a dramatic regression of adaptive skills after at least two years of normal development. We have previously shown that CDD and Autistic Disorder (AD) share a common clinical phenomenology when IQ is taken into account, raising the question of whether they represent common neuropathology. Using functional magnetic resonance imaging (fMRI) and a passive viewing task which probes brain areas identified as abnormal in AD, we explore the neurofunctional characteristics of CDD.

Methods: Two subjects who met criteria for an ASD as measured by the ADOS, ADI-R, and clinical exam, and were diagnosed with CDD by DSM-IV-TR criteria were selected. They watched alternating blocks of images of houses and fearful faces (from the NimStim set of facial expressions) presented over a 2 ½ minute period while undergoing fMRI. Based on our *a priori* hypothesis of amygdala, fusiform gyrus (FFG), and superior temporal sulcus (sts) abnormalities dysfunction, we conducted a *region of interest* analysis comparing them to six typical subjects.

Results: The results are shown below in Figure 1. Charts 1-4 depict brain activity (beta values) of 2 CDD subjects (CDD) relative to four controls (AC) in the STS, FFG, and amygdala ($q < 0.001$, $k = 12$).



Conclusions: The STS, FFG, and amygdala, play a central role in social information processing. These suggest that in contrasts to other ASDs, where the FFG, STS, and amygdala are abnormal, in CDD, only the amygdala is affected. If this set of findings is is repeated across multiple subjects it would suggest that although the phenomenology of CDD and the other ASDs converge, the neural underpinnings are distinct.

Purkinje Cells loss characterizes the brain pathology of Spinocerebellar Ataxia 10

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Objectives: Spinocerebellar Ataxia 10 (SCA10) is a rare, dominantly inherited cerebellar degenerative disorder manifested by ataxia and seizures from expansion of a pentanucleotide (ATTCT) repeat in the 9th intron of ATAXN10 gene. Imaging studies revealed dramatic cerebellar atrophy but no clinical pathology has been reported. Here, we present a case of autopathy study in a SCA 10 patient.

Methods: Brain from a 42 year-old Mexican male with SCA 10 was harvested 3 hours postmortem. The expansion of pentanucleotide repeat contained 2350 ATTCTs. Gross pathology, microscopy, immunohistochemical studies of Neu N, GFAP, Calbindin, Rmbdo 20, Ubiquitin, TUNEL, apoptosis markers, autophagy markers and Transmission Electron Microscopy were conducted.

Results: Gross pathology reveals cerebellar atrophy affecting both the vermis as well as the cerebellar hemisphere. Microscopically, the most striking changes are extensive Purkinje cell loss with Bergmann glial proliferation. Vermis is relatively less affected. In contrast, the internal granular layer is spared. Calbindin and Rmbdo 20 stainings reveal aberrant arborization and loss of dendrites of residual Purkinje cells, indicating that dendritic regression precedes the PC loss. Ubiquitin stainings which present as positive foci or aggregates were detected in PCs body and aberrant dendrites, raising the possibility of inclusion body formation. Ultrastructure analysis by electron microscopy indicates autophagocytosis. Different stage and pattern of autophagy were found in degenerated PCs. There appear increased lysosomes. Double-membrane bound structures that contain mitochondria were detected. Strong positive staining of LC3, an autophagy marker, was found in residual Purkinje Cells. TUNEL analysis is negative. The rest of the brain does not present obvious abnormality

Conclusions: Brain pathology of SCA 10 is characterized by profound Purkinje cell loss. The aberrant arborization, loss of dendrite tree and downward displacement of residual Purkinje cells suggest Purkinje cell loss starts from dendritic regression. EM analysis reveals dysregulated autophagy activation and mitophagy, which may contribute to the Purkinje cell degeneration. Further exploration of the pathway of the expanded ATTCT repeats-induced autophagy will shed light on our general understanding of spinocerebellar degeneration and further treatment planning.