

1. Is the MPS I Medical Phenotype Associated with Specific Causative Factors?

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Objectives: Mucopolysaccharidosis type I (MPS I), a lysosomal disease caused by deficiency of alpha-L-iduronidase affects multiple organ systems. We seek to find factors that are associated with differing medical phenotypes. We hypothesize that age at visit, treatment (enzyme replacement therapy ERT or hematopoietic cell transplant HCT), and genotype affect medical phenotypes.

Methods: 45 patients from MPS Longitudinal study of the Lysosomal Disease Network included, 28 Hurler treated with HCT and 17 attenuated treated with ERT. The HCT group was divided into an older and younger group (6 years and over and under 6 years of age). Patients 6 years and over were compared between HCT and ERT groups. Genotypes were classified as 1) Homozygous nonsense-NH, 2) Nonsense/deletion or splice site-NDS, 3) Nonsense/missense-NM, 4) Other combinations of missense, deletion, splice site abnormalities-MDS. The HCT group included all of the NH and NDS groups and 2 patients from the NM group. The ERT group included NM and MDS groups. Medical histories were scored by a system of frequency and severity.

Results: Effects of age: Corneal clouding, orthopedic and carpal tunnel syndrome are more evident in older patients. Hearing loss is present from the beginning but also worsens over time. Respiratory and cardiac problems are unaffected by age. Effects of treatment and genotype: More orthopedic problems were found in those in the HCT group. Fewer orthopedic problems were found in the MDS/ERT group. All patients had carpal tunnel and hepatosplenomegaly. Vision, hearing, cardiac, and respiratory abnormalities were unrelated to type of mutation. Number of surgeries was high in all groups but more in the HCT group especially NH.

Shunted hydrocephalus and cord compression were not found in the HCT group, except for one patient. These symptoms were also absent in the MDS/ERT group. In the NM group, 4 patients had hydrocephalus with shunts and 3 had cord compression. The L238Q together with a nonsense or deletion was associated with hydrocephalus, cord compression, as well as psychiatric disturbance.

Conclusion: Medical outcomes vary by age, treatment, and mutation. ERT does not apparently help to prevent hydrocephalus and cord compression in the NM group. Specificity of mutation and outcome were found in a single mutation L238Q-NM group; more specificity may be found with a larger patient sample.

2. Integrated systems biology approach for targeted therapy in cholangiocarcinoma

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Objective: Cholangiocarcinoma (CCA) is a rare and heterogeneous biliary tract cancer with dismal outcome. Previously, we have classified 104 CCAs into four genomic subgroups with distinct molecular and clinical characteristics, providing the basis for developing targeted therapies.

Method: We have shown a significant increase in receptor tyrosine kinase (RTK) expression associated with poor survival. To assess RTKs as potential drugable targets, we applied a translational genomics-driven approach by integrating data from our patient cohort and 7 human CCA cell lines to investigate optimal treatment options for subgroups of CCA. The CCA lines were exposed to TKIs, trastuzumab and lapatinib, and the anti-inflammatory COX2 inhibitor, celecoxib, and AKT inhibitor (MK2206). Targets were validated by transcriptomics, Western and immunostaining. The effects of celecoxib and trastuzumab co-administration or targeting AKT alone were examined in vitro and in TKI-resistant xenografts (30mg/kg celecoxib gavage bid, 0.45mg/dose trastuzumab i.p. twice weekly or 120mg/kg MK2206).

Results: To assess EGFR and HER2 as potential drugable targets, we integrated the cell lines with our cohort and established a concordance between the lines and patient subsets. The cell lines were exposed to trastuzumab and lapatinib for 7-days. Lapatinib was significantly more effective showing 50-80% growth inhibition compared to 15-20% inhibition achieved by trastuzumab. Classification was confirmed by transcriptomics, separating the lines based on drug-response. TKI-resistance was observed in 3/7 lines showing an increased AKT and MAPK activity. Each cell line was analyzed for mutations by whole-exome sequencing with disruption in EGFR, HER2 and KRAS. A combination of celecoxib and trastuzumab suppressed COX2 and AKT activity and inhibited growth of TKI-resistant lines in vitro ($P < 0.0001$). In the TKI-resistant CCA xenografts, co-administration of celecoxib and trastuzumab significantly ($P < 0.004$) reduced tumor growth with a steady increase in the inhibition rate ($IR = 0.75$, $R2 = 0.95$, $P < 0.02$) and overall survival ($HR = 6.26$, $P < 0.02$) compared to mono-therapies or control mice. The effect of celecoxib was associated with inhibition of AKT both in vitro and in vivo. Similarly, inhibition of AKT with MK2206 for three weeks decreased the relative tumor volume (RTV, $P < 0.0006$) as compared to control mice in a TKI-resistant xenograft model ($P < 0.0001$).

Conclusion: Our study demonstrates that TKI-resistance can be surmounted by co-administration of TKIs with celecoxib. Targeting AKT downstream of KRAS sensitized TKI-resistant CCA to TKIs. These data emphasize the implication of translational genomics for precision therapy.

3. Do Anti-Phospholipase A2 Receptor Antibodies Predict Recurrence of Membranous Nephropathy After Transplantation?

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Objective: Primary Membranous Nephropathy (MN) recurs in 10-45% of cases after transplantation. Early diagnosis of recurrent MN should prompt treatment as graft loss occurs in a high proportion of cases. Previous studies have not disclosed any pre-transplant variables that differentiate patients that will recur from those that will not. We analyzed if the presence of circulating Anti-Phospholipase A2 Receptor Antibodies (anti-PLA2R) at the time of transplantation predicts recurrence and assessed the prevalence of anti-PLA2R soon after recurrence.

Methods: We conducted a retrospective analysis of anti-PLA2R by western blotting of sera obtained at or before transplantation (pre-TP), when available, and within 4 months of recurrence or non-recurrence as diagnosed by protocol biopsy or proteinuria. Anti-PLA2R level was semi-quantitated by band intensity.

Results: Pre-TP sera were available from 34 patients. MN has recurred in 17 of the 23 patients that were anti-PLA2R positive (74%); however 8 (32%) of the patients that recurred were negative for pre-TP anti-PLA2R. Of 16 patients in which serum was available at the time of recurrence, anti-PLA2R was detected in 11 (69%) and was negative in 5 (31%). The median time for recurrence in 9 patients that were strongly positive for anti-PLA2R pre-TP was significantly shorter than in 7 patients with undetected pre-TP anti-PLA2R [11 (1- 45) vs 51 (12 – 270) weeks, p=0.0127].

Conclusions: We found that positive anti-PLA2R pre-TP predicts a high risk for recurrence but not all seropositive cases have recurred to date. The absence of pre-TP anti-PLA2R does not rule out future recurrence, which might be explained by post-TP reappearance of anti-PLA2R or other as yet unidentified antibodies. The prevalence of anti-PLA2R at the time of recurrence is similar to that seen in primary MN. Our results also suggest that high pre-TP anti-PLA2R levels predict early recurrence. Thus we propose that the presence of anti-PLA2R before or after transplantation merits close monitoring for recurrent MN.

4. Presenting Characteristics of Dent Disease Patients

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Objective: Dent disease is a rare X-linked disorder characterized by low molecular weight proteinuria, hypercalciuria, kidney stones, and chronic kidney disease. The presentation of patients as well as clinical course is variable, making diagnosis challenging. The Rare Kidney Stone Consortium founded a voluntary registry for Dent disease. Collected data was analyzed to improve understanding of the disease.

Methods: Presenting characteristics of patients enrolled in registry for Dent disease were queried. Data are presented as mean (SD) or % affected.

Results: 71 patients are currently enrolled including 43 from North America, 9 from Europe, 9 from Asia, and 10 unknown/other locations. Disease type was confirmed Dent 1 (*CLCN5* mutation, n=44), Dent 2 (*OCRL1* mutation, n=1), neither Dent 1 nor Dent 2 (n=4), or unknown (n=22). Age at diagnosis was 13.6 (11.7) yrs; 9 patients (13%) were diagnosed after age 30. Mean followup is 3.8 yrs. 14 patients had kidney stones at an age of 17.2 (10.2) years; 3 patients had their first stone a mean age of 16.7 (6.8 years after diagnosis). For those with data low molecular weight proteinuria (95%), hypercalciuria (87%), and hematuria (44%) were common, while kidney stones (27%) and bone disease (11%) were less common. Total protein excretion was 1.8 (1.6) g/24-hr. Random urinary calcium:creatinine ratio was 0.61 (0.60) mg/mg. Among those with normal kidney function and >16 yrs old (n=8), 24-hr urine calcium was 361 (174) mg. First available serum creatinine was 1.1 (1.9) mg/dl at an age of 14.1 (11.6) yrs. 6 patients progressed to ESRD at a mean age of 30.5 (13.2) yrs; 4 currently have functioning renal allografts.

Conclusions: Patients with Dent disease are most often diagnosed in their early teens and only a minority ever experience clinical kidney stones. Moderate proteinuria, hypercalciuria, and hematuria are more commonly present. Clinicians should maintain a high index of suspicion for Dent disease in younger males with unexplained proteinuria and CKD.

5. Characterization of a novel recessive ataxia gene in a consanguineous Turkish family

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The cerebellar ataxias are a heterogeneous group of neurological disorders with onset of symptoms occurring between early childhood and late adulthood. While the genetic causes of several ataxias have already been identified, many more, especially rare recessive forms, are still unknown. Heterogeneity, or the large number of genes that can cause recessive ataxias, is the major reason why many genes for recessive forms are still unknown. Individual families with independent mutations are too small to positively identify a chromosomal region by genetic linkage.

Objective: We are studying a consanguineous Turkish family with a non-progressive, congenital ataxia of unknown etiology. Symptoms include hypotonia, developmental delay, mental retardation, nystagmus, truncal and extremity ataxia and cerebellar hypoplasia that do not progress with age. In this study, we aim to elucidate the genetic cause of a rare recessive ataxia.

Methods: We utilized a combination of exome sequencing with homozygosity mapping and expression analysis to identify candidate genes. Sanger sequencing was used to verify damaging variants. Molecular assays, such as RT-PCR and immunoblotting, were used to determine the functional consequences of gene variants.

Results: We have identified a variant in an obligatory splice sequence (the first base of an intron is changed from GT to AT). This mutation is absent in >10,000 American control DNAs (Exome Variant Server: <http://evs.gs.washington.edu/EVS/>) and 200 Turkish control chromosomes. RT-PCR demonstrates that this splice mutation causes skipping of an exon that is present in all isoforms and expression of the gene is lower in patients than controls, probably due to nonsense-mediated decay. Immunoblot demonstrates loss of protein in the affected individuals.

Conclusion: Our results suggest that we have identified a novel gene causing recessive ataxia. Experiments to determine whether mutations in this gene are present in US samples of recessive or sporadic congenital ataxia are ongoing. Additionally, we are developing a zebrafish animal model, utilizing morpholino-mediated knockdown, to study this disorder. Our research may help identify causes of other ataxias and may lead to novel therapies to treat ataxias.

6. Clinical Factors Associated with Lesion Count in Familial Cerebral Cavernous Malformation Type 1 Patients with the Common Hispanic Mutation

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Objective: Cerebral Cavernous Malformations (CCMs) are clusters of abnormally enlarged, capillary cavities that can lead to hemorrhages, seizures, neurological deficits and death. Familial CCM type 1 (CCM1) is caused by mutations in *KRIT1* and is characterized by multiple lesions that vary widely among patients. The factors that influence variability in lesion count within patients with the same gene mutation are not known. The purpose of this study was to identify clinical factors associated with lesion count among familial CCM1 patients who all carry the common Hispanic mutation (CCM1-CHM).

Methods: As a part of the Brain Vascular Malformation Consortium (BVMC) study, we reviewed detailed clinical and neuroimaging data on 137 CCM1-CHM patients from 34 families with at least two members and 35 singletons. We performed a cross-sectional analysis of clinical factors and lesion count using linear regression analysis, adjusting for covariates and standard errors for clustering within families.

Results: Sixty-three percent of patients were symptomatic at presentation with a mean age \pm standard deviation (SD) at CCM diagnosis of 30.8 ± 18.7 years (range: 0.32-70.9). The main clinical symptom leading to diagnosis was hemorrhage (24%); others included seizure (21%), headache (24%), and focal neurologic deficit (4%). Lesion count (range: 0-713; mean \pm SD: 63.7 ± 124.4) was highly variable among CCM1-CHM patients and positively correlated with increasing age ($P < 0.001$). Prevalence of cardiovascular co-morbidities was: 40% for obesity, 11% for diabetes, 20% for hypertension and 19% for hyperlipidemia. Obesity was significantly associated with a 47% lower lesion count ($P = 0.006$) in multivariate analysis adjusting for age ($P < 0.001$), male gender ($P = 0.024$), symptomatic presentation ($P = 0.144$) and other cardiovascular co-morbidities.

Conclusions: Hispanic familial CCM1-CHM patients present with a wide range in lesion count. Older age, male gender and absence of obesity may be predictors of higher lesion count. Further work is needed to elucidate how these factors influence lesion burden and determine associations with clinical outcomes.

7. Designing a Rare Disease Network for Chromosome 18 Abnormalities

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Objective: Design a rare disease network (RDN) to accelerate treatment-oriented research for chromosome 18 conditions.

Methods: In order to inform the organization of our own research infrastructure, we investigated models of existing rare disease networks (RDNs). We focused on the methods used by RDNs to describe and address the medical and developmental needs of the population as well as the relationship of the RDN with researchers actively investigating the condition.

Results: Based on our review, the majority of RDNs were designed for conditions that predominantly affect a single organ system and that already have a body of literature as well as multiple study sites and investigators. In contrast, the chromosome 18 conditions involve multiple body systems. In addition, the existing medical literature was limited and predominantly focused on dysmorphology rather than therapeutics. In order to meet the needs of our population, we designed a new model that is fundamentally different from previous models of RDNs. Rather than creating a network of investigators who are then linked to their own network of participants, we created a clinical science core housed at one institution that acts as a hub for both participants and scientists interested in aspects of these conditions. The Chromosome 18 Clinical Research Center includes 21 investigators from 18 different medical specialties, supported by seven research staff members. Having one research center enables us to maximize the number of participants while minimizing administrative and infrastructure spending. The multidisciplinary nature of the clinical science core allows us to conduct comprehensive evaluations on-site, maximizing data collection while minimizing participants' effort. Families have a single point of contact for all questions relating to the research project and are not asked to participate in competing studies.

Conclusions: To date, the Center has enrolled 460 individuals and collected innumerable medical records and developmental surveys. We have performed comprehensive clinical evaluations on 183 participants over the last 19 years. We have generated 41 scientific publications on a broad variety of topics, including molecular genetics, growth and endocrine issues, neuroimaging, cognitive phenotypes, auditory and ophthalmologic features, psychiatric concerns, and long term outcomes. We have also established collaborations with five other institutions with whom we share de-identified information and samples. Our model has been effective in advancing scientific knowledge of the chromosome 18 conditions.

8. Idiopathic Bronchiectasis and Connective Tissue Fibrillinopathies: Dural Ectasia as a Marker of a Distinct Bronchiectasis Subgroup

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Objective: Idiopathic bronchiectasis (IB), or bronchiectasis without a known cause, occurs in >100,000 Americans. The genetics underlying IB are unknown, but IB patients often have body morphotypes that are similar to patients with fibrillinopathies that involve the lung such as Marfan's syndrome. Dural ectasia, dilatation of the fluid filled dural sac surrounding the spinal cord, is common in patients with fibrillinopathies, and can be detected using MRI or CT imaging of the lower spine, but quantitative methods have not been standardized. We hypothesized that IB patients will have dural ectasia, and developed a standardized method to quantitate dural sac size, and assess for increased prevalence of dural ectasia in IB, as compared to healthy and disease controls with bronchiectasis due to cystic fibrosis (CF) and primary ciliary dyskinesia (PCD).

Methods: A total of 109 subjects were studied, including 40 healthy controls, 28 IB patients, and 41 disease controls (CF and PCD). Ages ranged from 18-82 years. The thoraco-lumbar spine was examined using a 1.5T MRI scanner. Anterior-posterior dural sac diameter (DSD), dural sac volume (DSV), and vertebral body height (VBH) were measured between L1 and S1 by three individuals blinded to diagnosis. Additional data included arm span, height, and presence of scoliosis and pectus abnormalities.

Results: DSD and DSV (corrected for VBH) were not different between healthy males and females, there was no influence of age, and average DSD and total DSV/VBH were well correlated (R-squared = 0.86). When compared to healthy controls, patients with IB have a significantly larger DSD and DSV/VBH at each vertebral level ($p \leq 0.01$) and across the entire L1 to S1 region ($p < 0.0005$). Bronchiectasis controls (CF + PCD) have dural sac values intermediate between healthy subjects and IB.

Conclusion: Dural ectasia can be quantified using DSD or DSV/VBH. Patients with IB have larger dural sacs as compared to healthy controls, suggesting some shared genetic predisposition in connective tissue fibrillinopathy genes causing dilatation of the dural sac and bronchi. Further research is ongoing to determine the relationship between dural ectasia and other body morphotypes.

Supported by CTSA UL1TR000083 and U54 HL096458-06, funded by the Office of the Director, and supported by ORDR and NHLBI, NIH.

9. Heterogeneous pulmonary phenotypes associated with *NKX2-1* mutations

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Rationale: Mutations in the gene encoding thyroid transcription factor (*NKX2-1*) result in neurologic abnormalities, hypothyroidism, and neonatal respiratory distress (RDS) known as the “Brain-Thyroid-Lung syndrome.” We identified individuals with mutations in *NKX2-1* whose primary manifestation was respiratory disease.

Objective: To characterize the spectrum of pulmonary phenotypes associated with mutations in *NKX2-1*.

Methods: Retrospective and prospective approaches identified infants and children with unexplained diffuse lung disease for *NKX2-1* sequencing abnormalities. Histopathology, electron microscopy and immunohistochemical analysis for surfactant associated proteins were assessed in a subset of 10 children for whom lung tissue was available.

Results: We identified 16 individuals with heterozygous missense, nonsense and frameshift mutations and 5 individuals with heterozygous whole gene deletions of *NKX2-1*. Neonatal RDS was the presenting pulmonary phenotype in 16 (76%), interstitial lung disease in 4 (19%), and pulmonary fibrosis in 1 adult family member. Altogether, 11 individuals (52%) had the full triad of neurologic, thyroid, and respiratory manifestations, but 6 (29%) had only pulmonary symptoms at the time of presentation. Recurrent respiratory infections were a prominent feature in 9 subjects. Lung histopathology demonstrated evidence of disrupted surfactant homeostasis in the majority of cases and at least 5 cases had evidence of disrupted lung growth. Five individuals died from their lung disease, four underwent lung transplantation, and the remaining individuals are alive with varying degrees of respiratory dysfunction.

Conclusions: Patients with mutations in *NKX2-1* may present with pulmonary manifestations in the newborn period or childhood in the absence of thyroid or neurologic abnormalities. Surfactant dysfunction and, in more severe cases, disrupted lung development, are likely mechanisms for the respiratory disease. *NKX2-1* mutations may be an under recognized type of rare disease with major morbidity and mortality.

10. Investigating an early brain development gene within the critical region of 1q deletion syndrome

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Objective: Submicroscopic terminal 1q deletion results in a syndrome consisting of severe psychomotor delays, aphasia, hypotonia, microcephaly, corpus callosum abnormalities, and facial dysmorphism. Though previous studies have narrowed the critical region for this phenotype to 1q43-1q44, the deleted genes responsible for the syndrome remain unknown. A potential contributor to this syndrome is the uncharacterized gene *SCCPDH*, which lies within the 1q44 critical region. To investigate whether *SCCPDH* deletion may contribute to an abnormal neurological development, we examined the effects of *SCCPDH* knockdown in zebrafish.

Methods: RT-PCR analysis was performed to determine the level of *SCCPDH* expression at various stages of development. The *SCCPDH* homologue was knocked down in zebrafish by post-fertilization embryo injection of synthetic antisense oligomers, or morpholinos (MO), designed to bind and block either the exon 4/intron 4 (E4I4) splice site or the 5'-untranslated region (UTR) within *SCCPDH* mRNA. The phenotype rescue experiment was conducted by co-injection of the E4I4 MO and wild type *SCCPDH* mRNA. Touch response behavioral tests were performed manually on un-injected, mismatch control MO-injected, and the E4I4 MO-injected embryos at 48 hours post fertilization.

Results: We determined that zebrafish *SCCPDH* homologue is expressed throughout development with its highest expression during the earliest stages. At 24 and 48 hours post fertilization, both the E4I4 MO-injected and the 5'-UTR MO-injected embryos had a dose-dependent defect in midbrain-hindbrain boundary formation and hydrocephalus, respectively. Demonstrating that the effects of the MO are specific to *SCCPDH* knockdown, co-injection of *SCCPDH* mRNA and the *SCCPDH* splice-site targeting MO rescued the phenotype; over 70% of the co-injected embryos were morphologically similar to the un-injected control embryos. Interestingly, the overexpression of *SCCPDH* mRNA alone caused cyclopia and poor brain development. Furthermore, approximately 77% of E4I4 MO-injected embryos were found to have an obvious reduction in the defined, stereotypical touch response.

Conclusions: These results indicate that the level of *SCCPDH* expression is highly regulated and that alterations beyond a threshold can result in abnormal brain development. The observed morphological changes in the *SCCPDH* morphant brain seem to also lead to a psychomotor delay or defect. This work suggests that *SCCPDH* plays an important role in early brain development and merits further investigation.

11. The Rare and Orphan Disease Center at The Jackson Laboratory

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Objective: The Rare and Orphan Disease Center at The Jackson Laboratory focuses on bringing together global mouse resources and the expertise of research scientists to develop and distribute mouse models which closely recapitulate human disease. The Center's Mission is to a) join with experts from around the world, including foundations, researchers and pharmaceutical developers for innovative partnerships to facilitate research into treatments of these less common diseases; b) engineer new solutions by creating, refining and expanding the variety of rare disease preclinical models available to the scientific community; c) accelerate drug discovery and efficacy testing via worldwide distribution of over 6,000 mouse strains.

Methods: We genetically engineer new mouse models, from construct creation and microinjection, to evaluation and characterization. We enhance existing models through phenotypic evaluation, quality assurance and genetic background standardization. We distribute validated preclinical mouse models for drug discovery to the worldwide biomedical community.

Results: Ongoing rare disease research projects involve partnerships with foundations, companies and principle investigators. To date, our primary focus has been on model creation and genetic standardization for rare neurological disorders, such as spinal muscular atrophy (SMA), amyotrophic lateral sclerosis (ALS or Lou Gehrig's disease), Friedreich's ataxia, and Duchenne muscular dystrophy (DMD). We currently distribute over 1,800 strains for the study of rare and orphan diseases, including translational models harboring patients' mutations.

Conclusions: With over 7,000 human rare diseases and approximately 350 million people affected worldwide, to develop treatments or cures, partnerships and collaborations across diverse areas of expertise are needed. We are interested in partnering with new groups across all therapeutic areas of research. Visit our website at www.jax.org/rare to learn more.

12. Short telomeres in multiple hematopoietic lineages define a subset of patients with autoimmunity and dysgammaglobulinemia

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Objective: Diseases of telomere shortening arise from premature telomere erosion at the ends of chromosomes. In patients with impaired telomere maintenance, highly proliferative cell types, including hematopoietic stem cells, lymphocytes and some epithelial cells, are induced to undergo cell senescence or apoptosis once telomeres reach a critical length resulting in a varied clinical presentation including aplastic anemia, bone marrow failure, malignancy and pulmonary fibrosis. Premature erosion of telomeres in lymphocytes likely results in impaired proliferation and function leading to immune dysregulation and immunodeficiency in these patients. We identified a subset of patients (7 males, 1 female, ages 15 months to 21 years old) that presented with clinical features more consistent with combined variable immune deficiency (CVID), autoimmune lymphoproliferative syndrome (ALPS) or Immunodeficiency, Polyendocrinopathy, and Enteropathy, X-Linked Syndrome (IPEX), but with negative traditional diagnostic work-ups for these diseases. Our aim was to further define the clinical and immunological characteristics of this cohort.

Methods: We retrospectively reviewed clinical information of patients referred for immune evaluation and were subsequently found to have prematurely shortened telomeres in peripheral blood leukocytes.

Results: The most common (7/8) clinical finding in this cohort was multilineage cytopenias. Autoimmunity, including antibodies directed against leukocytes, was prevalent (7/8) in this cohort as well. These patients were subsequently found to have low to very low median telomere length in at least four of the six leukocyte subsets, including naïve T lymphocytes. Immune studies showed a low percentage of naïve CD4+ cells in a majority (6/7) of cohort subjects. Median telomere length in patients of similar clinical phenotype and age with confirmed diagnoses was normal in a majority of the leukocyte subsets.

Conclusions: Premature shortening of telomeres in our cohort is not a consequence of immune dysregulation, but instead is a defect that results in their clinical phenotype. Genetic work-up of these patients to identify possible defects that result in telomere shortening is in progress. Further studies are needed to define the mechanism of the immune dysregulation that leads to autoimmunity that is prevalent in this cohort.

13. **Reasons for failure of botulinum toxin treatments in cervical dystonia.**

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Objective: Cervical dystonia (CD) is perhaps the most common and readily diagnosed of the focal dystonias. It is characterized by abnormal neck control and neck muscle pain. The bothersome symptoms of the disorder can be treated with local injections of botulinum toxin (BTX), which significantly reduce symptoms and improve quality of life. However, a small proportion of patients derive little to no benefit. The reasons for poor responses in some patients are not well understood. Therefore, we analyzed BTX treatment records of patients who have not had beneficial responses to BTX treatments.

Methods: We considered all patients with a diagnosis of CD referred to an outpatient clinic at a tertiary care academic center from January 1, 2010 to the present. We methodically extracted data using an interview and questionnaire developed for this study regarding prior evaluation and treatments and outcomes following referral.

Results: A total of 25 patients were referred to Emory due to BTX treatment failure. 17 (68%) of these patients were interviewed and had their data collected during their first visit at Emory. Data from the other 8 (32%) patients was collected retrospectively from the medical record. Following re-evaluation, 4 (16%) patients were considered to have diagnoses other than CD. All 4 had disorders not typically responsive to BTX. The other 21 (84%) patients had CD, and for 20 (80%) of them repeat BTX treatment led to 50% or greater improvement of the movement disorder according to the physician and patient. In 1 (4%) case the patient did not want repeat treatments due to extreme pain from the previous treatment. In 14 (56%) of the patients the reason for initial apparent treatment failure was improper BTX dosing and/or improper muscle selection. In 2 (8%) cases the patient's perception of benefit was discordant with the provider's assessment of benefit. In 4 cases (16%) the reason for initial apparent failure was not readily identifiable with the information provided. In 2 of those 4 cases, the patient was responsive to one formulation of BTX but not to two others.

Conclusions: True BTX treatment failures in CD are not common. The main reasons for apparent BTX failure point to the need for further education regarding the diagnosis and treatment of CD.

14. **Pilot Study to Identify Factors that Determine Recurrence of Porphyria Cutanea Tarda**

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Background: Porphyria cutanea tarda (PCT) is the most common and readily treated type of the human porphyria, but recurs unpredictably. PCT results from inhibition of hepatic uroporphyrinogen decarboxylase (UROD), the fifth of eight enzymes in the heme biosynthetic pathway, leading to accumulation of porphyrins that are photosensitizing and cause blistering skin lesions. Susceptibility factors include alcohol, smoking, estrogens, hepatitis C, human immunodeficiency virus, heterozygous *UROD* mutations and hemochromatosis gene (*HFE*) mutations. These factors are multiple in individual patients, but none is essential in causing PCT or relapses of the disease. In this pilot study we are examining susceptibility factors in a large cohort of PCT patients followed at UTMB to determine which might be more likely to be associated with relapse.

Design/Methods: We reviewed records on patients who a) had well documented PCT, b) were characterized for the susceptibility factors noted above, c) were treated with either phlebotomy or low-dose hydroxychloroquine, d) achieved biochemically proven remission and e) were followed for 2 years or longer after remission to assess whether or not relapse occurred. The aim is to determine if demographic features, susceptibility factors or the type of treatment influence recurrence of PCT, and to assess sample size that might be needed for a larger and more definitive study.

Results: To date we have identified 72 patients (60% male) who meet the above criteria. Their mean age when first seen was 55 years, and age of onset of PCT was 42.5 ± 9.2 years (mean \pm SD). Alcohol, estrogen and smoking are characterized in detail in terms of exposure before and during treatment and remission. Between-group analysis will be performed, comparing patients who relapsed to nonrelapsers.

Conclusions: What determines a durable or temporary remission is a major unanswered question in this disease. This pilot study will be the first large cohort study of determinants of relapse in patients with PCT, and utilizes the substantial information collected on a large cohort of PCT patients at this center. The results will guide the design of a larger prospective study planned for the Porphyrias Consortium, which will include patients from at least 6 centers who are followed prospectively.

15. Upper Airway Gene Expression Profiling in Granulomatosis with Polyangiitis

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Objective: Nasal disease occurs in the majority of patients with granulomatosis with polyangiitis (Wegener's, GPA). The objectives were to use gene expression profiling techniques to understand the biology of upper airway disease in GPA and explore the potential utility of genome-wide gene expression signatures as measures of nasal disease activity.

Methods: Nasal brushings of the inferior turbinate were obtained from 32 subjects with GPA (n=10 active nasal disease, n=13 prior nasal disease, n=9 never nasal disease) and 35 comparator subjects with and without inflammatory nasal disease (n=12 healthy, n=15 sarcoidosis, n=8 allergic rhinitis). RNA extracted from the brushings was hybridized to Affymetrix Human Gene 1.0 ST Arrays. Gene expression changes associated with nasal disease activity were identified with a linear mixed effects model controlling for microarray batch and use of immunosuppressant medications. Significant differences were defined at a threshold false discovery rate (FDR) <0.1 and fold change > 1.5. Functional enrichment of biologic pathways was determined using Gene Set Enrichment Analysis (GSEA) ($FDR_{GSEA} < 0.25$). The relationship of nasal gene expression profiles to peripheral blood gene expression levels associated with GPA (*Cheadle et al A&R 2010*) was determined using GSEA.

Results: The expression levels of 452 genes were associated with active nasal disease, 309 with prior nasal disease, and 0 with never nasal disease in subjects with GPA. GSEA revealed enrichment of several biologic pathways among genes associated with nasal disease activity. The 20 most significantly enriched pathways among subjects with active nasal disease were also significantly enriched among subjects with prior nasal disease and included pathways related to immune response (eg HSA04679 Leukocyte Transendothelial Migration) and thrombosis (eg HSA04610 Complement And Coagulation Cascade). Peripheral blood gene expression levels associated with GPA were similarly altered in the nasal gene expression profiles of subjects with active or prior nasal disease, but were not significantly enriched in subjects with never nasal disease.

Conclusions: Nasal gene expression profiles are associated with nasal disease activity in subjects with GPA. The biologic functions of genes altered in subjects with active nasal disease are similar to subjects with prior nasal disease. Upper airway gene expression profiles are similar to patterns of gene expression changes derived from peripheral blood in GPA, suggesting that nasal gene expression profiles in GPA may reflect systemic disease activity.

16. Accelerated Clinical Supplies Platform for Translational Research in Rare Disease

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Objective: The National Institute for Pharmaceutical Technology and Education (NIPTE) is a non-profit organization consisting of thirteen major university schools and colleges of pharmacy and chemical engineering. NIPTE was created to promote research and education in pharmaceutical technology, engineering and product development. Its capabilities include the knowledge, expertise and facilities to synthesize drug (API), develop a formulation, and prepare clinical supplies of that formulation. The objective of its Accelerated Translation Program (*via* Fast Clinical Supplies Preparation) is to speed up drug development and accelerate translation of new discoveries into new medicines.

Methods: A clinical trial cannot proceed without the availability of high quality clinical supplies. Developing processes and formulations and ensuring availability of high quality clinical supplies in a timely manner is a key component of bringing new drugs to the market.

Manufacture of clinical supplies requires 3 steps:

1. Development of a manufacturing process and synthesis of the drug (API)
2. Conversion of the API to a bioavailable formulation
3. Manufacture of clinical supplies and placebo.

As an integrated consortium, NIPTE can help to commercialize drugs developed in academic institutions. NIPTE has all of the modern platforms for drug synthesis and inventing new medicines including non-interacting formulations, amorphous formulations, cocrystalline formulations, nano formulations, and others. NIPTE's goal is to ensure that the clinical supplies are never on the critical path in conducting clinical trials.

Conclusions: The NIPTE program combines development, preparation and/or sourcing of the drug substance (API), formulation design, and clinical supplies manufacture in a fast development regime. In this way clinical supplies can be rapidly prepared for clinical trial.

17. Using an N-linked tetrasaccharide as a biomarker for the diagnosis of ALG1-CDG (CDG-Ik)

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Objective: Mannose is a key component of glycans on glycoproteins and glycosylphosphatidylinositol lipid anchors. In eukaryotes, a majority of the secretory and membrane-bound proteins are modified by N-glycosylation on selected Asn residues (N-glycans). Mannose residues comprise a key portion of the scaffold for all the N-glycans on glycoproteins to form di-antennary and multi-antennary structure. Primary deficiencies in mannosylation of N-glycans represent over 60% of the patients with congenital disorders of glycosylation (CDG) including CDG Ia, Ib and Ik. This study is to describe a biomarker for the deficiency in mannosylation of N-glycans for the diagnosis of this group of CDG subtypes.

Method: The N-linked glycan were released from total glycoprotein in plasma or serum using PNGase F digestion. The N-glycan profiling was achieved by MALDI-TOF analysis of permethylated glycans. The quantification of one of the abnormal glycans was measured by LC-MS/MS method using 20 normal controls, 15 sera from PMM2-CDG (CDG-Ia), one serum from a MPI-CDG (CDG-Ib) and 10 sera from ALG1-CDG (CDG-Ik). All the molecular studies were done by whole gene Sanger sequencing.

Result: We discovered a group of abnormal N-glycans on glycoproteins from the plasma and cultured skin fibroblasts from CDG-Ik, CDG-Ia and CDG-Ib patients that are completely deficient in mannose but contain many of the other major sugar moieties. We have been able to identify 15 ALG1-CDG (CDG-Ik) and 3 PMM2-CDG patients in the United States by measuring these unique N-glycans in the past two years. The diagnoses of all these ALG1-CDG (CDG-Ik) cases were also confirmed by molecular studies. We have demonstrated the clear segregation between normal, PMM2-CDG (CDG-Ia) or MPI-CDG (CDG-Ib), and ALG1-CDG (CDG-Ik) by the levels of this biomarker.

Conclusion: Our study here provides evidence that this mannose deficient N-glycan should be used as a diagnostic biomarker for ALG1-CDG (CDG-Ik) and as an additional biomarker for deficiencies in neighboring biosynthetic steps in N-linked protein glycosylation.

18. Pediatric versus Adult AVM Angioarchitecture: Are Children Really Just Small Adults?
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Objective: To determine if clinical presentation and angioarchitectural features differ between children and adults with brain arteriovenous malformations (AVMs).

Methods: Under an IRB approved protocol, an institutional brain AVM database collected prospectively since 2001 was queried. Patients with nidus AVMs were included for analysis; those with vein of Galen malformations, dural AV fistulas, or non-Galenic pial AV fistulas were excluded. Demographic and angioarchitecture information was abstracted and analyzed with univariate and multivariable models.

Results: Children were more likely to present with AVM hemorrhage than adults (Table 1). Hispanic children were overrepresented. Although the size of AVM nidus did not differ between children and adults, location and venous drainage did. Children were more likely to have exclusively deep venous drainage. Venous ectasia and feeding artery aneurysms were more prevalent in adults.

Table 1. Clinical and Angioarchitectural Characteristics at Presentation

Characteristic	Children (n=170)	Adults (n=583)	Total (n=753)	P Value
Male Gender	92 (54%)	280 (48%)	372 (49%)	0.162
Age at Diagnosis (years)	12±5	42±15	35±18	<0.001
Ethnicity				0.006
<i>Non-Hispanic Caucasian</i>	74 (44%)	320 (55%)	387 (53%)	
<i>Black</i>	9 (5%)	42 (7%)	51 (7%)	
<i>Hispanic</i>	54 (31%)	135 (23%)	182 (25%)	
<i>Asian</i>	29 (17%)	73 (13%)	102 (14%)	
<i>Native American</i>	1 (1%)	8 (2%)	9 (1%)	
Initial Presentation with Hemorrhage	100 (59%)	239 (41%)	339 (45%)	<0.001
Nidus Size (cm)	2.90±1.5	3.16±1.8	2.95±1.5	0.090
Venous Drainage				<0.001
<i>Superficial</i>	50 (35%)	297 (55%)	347 (51%)	
<i>Deep</i>	48 (33%)	79 (15%)	127 (19%)	
<i>Superficial and Deep</i>	46 (32%)	158 (30%)	204 (30%)	
Venous Ectasia	30 (31%)	204 (52%)	234 (48%)	<0.001
Lobar Location	96 (69%)	404 (79%)	500 (76%)	0.013
Central Location	62 (44%)	182 (35%)	244 (37%)	0.054
Feeding Artery Aneurysm Related to Shunt Flow	17 (13%)	127 (27%)	144 (24%)	0.002

Conclusions: Although children with brain AVMs were more likely to come to clinical attention due to hemorrhage than adults, high risk features that may take time to develop (venous stenosis, feeding artery aneurysms) were underrepresented in children. AVMs were also more likely to be located deep within the brain in children, raising the possibility that centrally-located AVMs may arise earlier in development or be more likely to come to clinical attention early in life.

19. Community Partnership Approaches For Advancing Research in Rare Genetic Conditions: a Process Report from the MUSC/Alpha-1 Community Research Partnership Model

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Objective: To describe the community based participatory research partnership (CBPR) process involving a community of individuals living with the rare genetic disease alpha-1 antitrypsin deficiency (AATD), and interdisciplinary academic research scientists at the Medical University of South Carolina (MUSC). This process description includes several stages: formative steps of partnership capacity assessments, partner training, designing an early research agenda, including success in obtaining internal (CTSA), community (Foundation) and external PCORI Patient Centered Outcomes Research Institute (PCORI) funding. This abstract highlights the lessons learned and ongoing work where rare disease community leaders and interdisciplinary research scientists apply community partnership approaches to advancing research. We will describe how the collective of families with AATD functions as a “virtual community”, overcoming geographical dispersment through innovative use of social media, information technology and cyberspace.

Methods: Using CBPR strategies, this community/academic scientist partnership employs mixed methods, currently framed in three studies to: 1) explore social media, cell phone mobile applications and information technology to raise awareness of their social burdens (CTSA funded) 2) test the hypothesis there is disparity in social burden caused by the rarity and genetic etiology of their lung disease experience (foundation funded) and 3) develop a psychosocial scale advancing measurement of their social burden (PCORI funded).

Results: The results of the described partnership processes will include: informed strategies to maximize rare disease community use of information technology in research and policy advocacy; pilot data to begin documentation of health disparities in the AATD community and a deliverable, theory driven, validated and reliable psychosocial scale that includes rare disease social burdens. We project scale instrument testing targeting generalizability for other rare disease communities. Measureable outcomes from the described partnership processes will inform future research partnerships in other rare disease communities.

Conclusions: The CBPR process with the AATD community demonstrates success in forming a research agenda and funding capacity to advance study of patient centered outcomes of interest to them. The CBPR approach and this rare disease partnership model have potential for other academic research centers and rare disease communities.

20. Treatment of Resistant Primary Focal Segmental Glomerulosclerosis (FSGS) with Adrenocorticotropic Hormone Gel

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Background: Immunosuppression-resistant FSGS often leads to ESRD. ACTH has shown efficacy in resistant nephrotic syndrome, but data are limited with ACTH treatment of FSGS.

Methods: In this retrospective case series, 12 patients with primary FSGS and nephrotic syndrome resistant to previous immunosuppression were administered ACTH gel (median dose 80 units subcutaneously SC twice weekly) for a median of 26 (range 12-56) weeks of therapy. Complete remission was defined as stable/improved serum Cr and a sustained fall in proteinuria to <500 mg/day by last follow-up, and partial remission as stable/improved serum Cr with 50% reduction proteinuria to 500-3500 mg/day by last f/u.

Results: Median age was 41 (range 18-66) years and median time from diagnosis to treatment was 30 (range 2-276) months. Median eGFR was 33 (range 17 to 116) mL/min/1.73m² and median proteinuria 6100 (range 1600 to 16,800) mg/day. FSGS subtypes were tip lesion (n=6), NOS (n=4) and cellular (n=2). All patients had previously received prednisone and had failed or relapsed after at least 2 (median 3) prior immunosuppressive therapies, and 10 patients had failed at least 3 therapies. Five of 12 patients experienced partial remission at last follow-up (median follow-up time 58 (range f/u 4-82) weeks after stopping ACTH).

Serum Creatinine and Proteinuria for Patients Experiencing Partial Remission with ACTH

Pre-ACTH Cr (mg/dL)	Pre-ACTH Proteinuria (mg/day)	Post-ACTH Cr (mg/dL)	Post-ACTH Proteinuria (mg/day)	Cr at last f/u (mg/dL)	Proteinuria at last f/u (mg/day)	F/U (wks) after stopping ACTH
2.3	4000	1.3	963	1.3	1297	11
1.1	5200	0.7	1500	0.7	960	28
1.2	2100	1.4	272	1.3	641	82
1.6	10,300	1.2	5380	1.6	1277	56
3.6	15,200	1.3	891	1.1	1810	4

The median time-to-remission was 6 (range 6-24) weeks. No severe adverse events were reported. No clinical or biopsy characteristics predicted response to ACTH therapy.

Conclusions: ACTH gel is a useful treatment option for some patients with nephrotic syndrome due to immunosuppression-resistant primary FSGS, with partial remissions observed in 5 of 12 patients.

21. Patient Choice Early Escape to the Alternative Treatment Arm in a Cross-over Double Blind, Placebo-Controlled Trial of Rare Diseases: Design and Analytical Considerations

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Background and Aim: In a randomized, double-blind, placebo-controlled trial of rilonacept, an interleukin-1 inhibitor, for familial Mediterranean fever (FMF) in patients resistant or intolerant of colchicine, we implemented a modified cross-over trial design where patients decided on their own whether to escape to the alternative blinded treatment (Rilonaceptor Placebo), or remain on the original randomized treatment. We examined the feasibility and utility of this approach.

Methods: Eligible patients were randomized to 1 of 4 treatment sequences that include 2 rilonacept and 2 placebo 3-month treatment courses. Patients who developed ≥ 2 attacks during any treatment course could elect to escape to the other treatment arm until the end of that course (remaining blinded) and then resume their assigned sequence. Statistical reason and simulation studies were used to compare two designs: early escape by protocol vs. early escape by patient choice. Bayesian modeling used 3 pre-specified priors: non-informative, enthusiastic and skeptical prior; considering escape and cross-over effects. The primary outcome was the rilonacept/placebo risk ratio of FMF attacks; the secondary outcome was the escape rate. Primary analyses excluded data from escape courses.

Results: Results are summarized in the Table 1.

Conclusions: We demonstrated the successful application of this design in this rare disease trial of FMF. The design offers several advantages over conventional designs: 1) minimizes patient dropout; 2) allows treatment effect evaluation using clinical endpoints; 3) improves trial efficiency.

Table 1: Summary of Study Results

Number of Patients	Course 1	Course 2	Course 3	Course 4
Total Completed Course	14	12	11	11
No Escape	10	6	6	9
Escape from placebo to rilonacept	2	4	3	2
Escape from rilonacept to placebo	2	2	2	0
Escape after 2 attacks	1	4	1	1
Escape after 3 attacks	1	2	3	0
Escape after 4 attacks	1	0	1	0
Escape after 5 attacks	1	0	0	1

1. The rilonacept/placebo risk ratio of FMF attacks: 0.59 (SD=0.12), 95% credible interval 0.39-0.85. The results were consistent for the 3 different priors and whether or not escape courses were analyzed. 2. Odds ratio of placebo to rilonacept/rilonacept to placebo escape: 3.10 (SD=0.47), 95% credible interval 2.35-4.19. 3. Cross-over effect of rilonacept was not significant.

22. Beyond Brittle Bones: The Osteogenesis Imperfecta Adult Natural History Initiative

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 5)Osteogenesis Imperfecta Foundation

Objective: Adults with osteogenesis imperfecta (OI), a heterogeneous, rare disorder most commonly affecting Type I collagen, face the possibility of complications in nearly every organ system in the body. There is little information about the natural history of OI beyond childhood. The OI Adult Natural History Initiative (OI ANHI) was developed in collaboration with adults with OI, the Osteogenesis Imperfecta Foundation (OIF), and members of the medical community in order to address this data and care gap. The team established a web-based portal within the existing OIF website to create a cost-effective outreach and data management clearinghouse with which to survey the OI community and capture a first-ever “snapshot” of the health status, needs, and priorities of adults with OI.

Methods: The survey included questions on general health concerns and health behaviors plus a review of systems, with scale-based rankings of respondents’ priorities, concerns, and the impact of common impairments and conditions within each medical system. The survey was created using the National Institutes of Health (NIH)-sponsored tool PROMIS[®] (Patient-Reported Outcomes Measurement Information System) and was conducted over 3-months.

Results: More than 1,100 individuals responded to the ANHI survey. Over 93% of respondents completed the survey on the web. 70% of respondents were women and 93% were white. 31% of respondents reported they did not know their OI type. Survey analyses suggest that adults with OI have more pulmonary and cardiovascular issues than the general US population.

Conclusions: The OI ANHI survey identified numerous opportunities for future research. As our next step, we plan to pilot a system-specific health module to determine whether a patient reported outcomes tool can measure discernible differences in pulmonary function in individuals with OI. The marked heterogeneity of OI also demands a clearer methodology for stratifying patients; therefore we plan to use survey measures to explore the development of a new classification/stratification system for OI that might allow both patients and clinicians to assign risk for both skeletal and non-skeletal complications with greater confidence. It will be essential to recruit more men and minorities in future surveys.

23. Recommended Tools for Joint Chronic Graft-versus-Host Disease: The Chronic GVHD Consortium

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Objective: Assessing joint chronic graft-versus-host disease (GVHD) needs to be accomplished reliably, simply and in a clinically meaningful way. To determine the optimal tool for assessing joint GVHD, we evaluated two NIH recommended joint tools and a photographic range of motion (P-ROM) scale.

Methods: Patients with systemically treated chronic GVHD were eligible for a prospective multicenter observational study. At follow-up visits every 3-6 months, the clinician (MD) and patient (PT) rated their perception of change in joint GVHD on an 8-pt scale, which was collapsed into improved, stable or worse categories. Linear mixed models were used to correlate change in each tool with MD or PT-perceived change (improved vs. stable or worse vs. stable) in joint GVHD.

Results: Nine sites in the Consortium enrolled 567 participants through December 2011. Joint involvement, as defined by NIH joint/fascia score ≥ 1 , was present at enrollment in 164 (29%) patients and included wrists (64%), ankles (47%), shoulders (35%) and elbows (30%). Change in joint GVHD status was examined for 652 paired visits when joint involvement was documented in the previous or current visit. In the later visits, both MDs and PTs more often reported improvement than worsening. All three tools correlated with MD-perceived improvement, and estimated change in the Hopkins score was slightly smaller than the other tools. The NIH and Hopkins scores correlated with PT-perceived improvement, and estimated change was similar between them. In contrast, all three tools correlated with both MD and PT-perceived worsening, but estimated change for the P-ROM score was significantly larger than in the other tools.

Conclusions: Joint GVHD is frequent. Our results support the combined use of NIH joint/fascia score and P-ROM scale to assess joint GVHD in clinical trials. The NIH score better reflects joint improvement and the P-ROM scale better reflects joint worsening. The more objective P-ROM scale is insensitive to PT-perceived joint improvement possibly because it does not incorporate tightness with or without activities of daily living.

24. Neurodevelopmental and Molecular Characteristics in Children with a new Sterol Metabolism disorder-SC4MOL deficiency.

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Objective: Sterol C4 Methyl Oxidase (SC4MOL) deficiency is a rare recently reported sterol metabolism disorder, confirmed in 4 patients worldwide to date with mutations in the *SC4MOL* gene (OMIM# 607545), located on chromosome 4q32.3. Elevations of 4, 4'-dimethylsterols and 4 α -monomethylsterols in plasma are also diagnostic apart from low cholesterol. In the cholesterologenesis pathway, Sterol C4 methyl oxidase is involved in sequential removal of two C4 methyl groups catalyzing 4, 4'-dimethylcholesta-8(9),-en-3 β -ol to 4 carboxyl monomethylsterol and 4 α -monomethylsterol to 4 carboxyl sterol. Molecular genetics and biochemical analysis are available readily and current research endeavors focus on immunological markers and treatment. Full extent of this disorder remains yet to be characterized.

This study aims to highlight the clinical, specifically neurodevelopmental and molecular characteristics seen in these patients.

Method: Patient chart review and clinical information exchange with genetics/metabolic clinicians who evaluated these patients at their first visit (prior to biochemical or molecular testing) to characterize the clinical presentation.

Results:

Patient#	SC4MOL/MSMO I gene mutations	Age seen by genetics clinic	Age diagnosed	Sex	Congenital cataracts	Microcephaly	Developmental delay	Speech/ Expressive language delay	Ichthyosis	FTT
Patient 1	T519A and A731G	13 yrs	15 yrs	Female	+++	+++	+++	+++	+++++	++
Patient 2	G115R and G115R	20mo	5yrs	Female	+++	+++	+++++	++++	+/-	++
Patient 3	K167N and R270P	10 yrs	10 yrs	Male	-	-	++++ Autismspectrum	++++	+++	++
Patient 4	K167N and R270P	6.5 yrs	6.5 yrs	Female	+++	-	+/-	+/- selective mutism	+++	+

Conclusion: Clinical characteristics and molecular genetic analysis of all 4 SC4MOL patients highlight the associated spectrum of neurodevelopmental and behavioral involvement including developmental delay specifically speech/expressive language delay and possibly intellectual disability, congenital cataracts (only in females), microcephaly in this cholesterologenesis defect. It would be important to evaluate for severity of intellectual disability and autism spectrum disorder, as well as, correlate any cholesterol or statins treatment intervention outcomes. Additional work is needed to identify mechanism of underlying neurodevelopmental and behavioral manifestation especially developmental delay, expressive language delay, and possibly autism spectrum disorder as well as congenital cataracts noted only in females.

25. N-acetylcysteine Therapy in Children with Cerebral Adrenoleukodystrophy undergoing Hematopoietic Stem Cell Transplantation

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Objective: Childhood cerebral adrenoleukodystrophy (CCALD) is effectively treated by hematopoietic stem cell transplantation (HSCT) when diagnosed early; however, it is ineffective in late-stage disease. Administration of N-acetylcysteine (NAC), an antioxidant that reduces free radicals and facilitates glutathione biosynthesis, in combination with HSCT improves long-term survival of boys with late-stage disease. The objective of this study was to characterize NAC pharmacokinetics and mechanisms of action in CCALD patients, in order to optimize NAC therapy for late-stage CCALD. Further we investigated the levels of cytoprotective proteins, heme oxygenase-1 (HO-1) and ferritin, as potential biomarkers for the antioxidant effects of NAC.

Methods: This was an open-label study of hospitalized, CCALD patients (n=11-15) undergoing HSCT. NAC was administered IV at 70mg/kg over one hour, every six hours. Blood samples were collected prior to and 7 and 21 days after HSCT at: pre-dose, 1.5, 2, 3, 4, and 6 hours post-dose. Total NAC, cysteine (Cys) and glutathione (GSH) concentrations in plasma and red blood cells and GSH redox status (GSH/GSSG) in whole blood were analyzed using HPLC-MS methods. Expression of HO-1 and downstream ferritin were determined by ELISA in paired samples (prior to and after NAC dosing) from 11 patients.

Results: Mean maximum plasma NAC concentrations were 100-120 ug/mL with elimination half-lives ranging from 1.3 to 1.6 hours. NAC also entered RBCs with maximum concentrations approximately 20% that of plasma. Despite measurable NAC concentrations in plasma and RBCs, we observed either no change or a transient decrease in plasma Cys and GSH concentrations. Cys concentrations were also unchanged in RBCs, while GSH concentrations increased, with significantly greater GSH exposure at 21 versus 7 days post transplantation. An increase in GSH redox status in whole blood was also observed following NAC administration. Further there was significant induction of antioxidant proteins, HO-1 (3.2-fold) and ferritin (4.6-fold) in plasma with a positive correlation ($r=0.74$; $p<0.0001$) between HO-1 and ferritin levels.

Conclusions: NAC may exert its effects by 1) increasing intracellular GSH concentrations, 2) altering GSH redox status and 3) inducing cytoprotective proteins such as HO-1 and ferritin. Future studies will evaluate the effect of NAC on brain GSH levels, and further elucidate mechanisms contributing to the observed increases in GSH, HO-1 and ferritin.

26. Study of body composition variables and bone mineral density in patients with Prader-Willi syndrome

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Objective: Prader-Willi syndrome (PWS) is a genetic imprinting disorder resulting from loss of the paternally expressed genes on chromosome 15q11-q13. About 70% of subjects with PWS have paternal deletion (Del) of this region, and 30% have maternal uniparental disomy (UPD) of chromosome 15 or an imprinting center defect. Some individuals with PWS show evidence of low bone mineral density (BMD). Previous studies reported improved body composition variables and BMD in children with PWS who are treated with growth hormone. There is limited consensus regarding the management of bone mineralization problems in individuals with PWS.

Methods: Using dual-energy X-ray absorptiometry (DEXA), we evaluated bone mineralization in 74 individuals with PWS (44 from previously published study at UC Irvine (Galassetti et al. 2007), and 30 from the UC Irvine RDCRN cohort (males=35, females=39, of these individuals 43 had a deletion, 25 had UPD, 6 were unclassified, and 47 were on growth hormone).

Results: Individuals with UPD had a lower weight, BMI, whole body fat %, and higher whole body BMD (g/cm^2) than those with deletion ($p\leq 0.05$). Individuals on growth hormone treatment were taller, had a lower BMI and whole body fat mass compared to untreated individuals. 8 /74 (11%) of the entire cohort had had a hip or spine Z-score < -2 , Del:UPD=6:1) and 24/74 (32%) had a Z-score of -1 to -2 SD (Del:UPD=8:15).

Conclusion: This is the largest study that compares BMD and body composition variables in deletion and UPD subclasses of PWS. In UPD there was a lower BMI, however BMD was higher compared to individuals with a deletion. Individuals treated with growth hormone had favorable body composition variables. Osteopenia or osteoporosis was seen in 45% of individuals with PWS, suggesting the importance of evaluating bone mineralization status regularly and supplementation with calcium, vitamin D and/or bisphosphonates to prevent fractures. Larger longitudinal studies are required to evaluate the effects of growth hormone and genetic subtype on bone mineralization in individuals with PWS.

27. Pulmonary Lymphangiomyomatosis (LAM): Therapeutic Targeting of Airspace Enlargement and TSC2-Null Lung Tumors Using LAM Mouse Model

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Objective: Pulmonary lymphangiomyomatosis (LAM) is a rare genetic disease characterized by neoplastic growth of atypical smooth muscle-like LAM cells, destruction of lung parenchyma, obstruction of lymphatics, and formation of lung cysts leading to spontaneous pneumothoraces. The disease affects predominantly women of childbearing age and is associated with mutational inactivation of tumor suppressor genes *tuberous sclerosis complex 1 (TSC1)* or *TSC2*. The mechanism of alveolar destruction and airspace enlargement in LAM, and whether these processes can be ameliorated therapeutically have not been investigated.

Methods: We developed and characterized a TSC2-null mouse LAM model. This model was used to develop potential therapeutic targeting of TSC2-null lesions and alveolar destruction seen in human pulmonary LAM.

Results: TSC2-null mouse LAM model is characterized by multiple random TSC2-null lung tumors, increased VEGF-D expression and lymphangiogenesis, destruction of lung parenchyma and decreased survival similar to human LAM. The mice show enlargement of alveolar airspaces that is associated with progressive growth of TSC2-null lesions in the lung, upregulation of proinflammatory cytokines and matrix metalloproteinases (MMPs) which degrade extracellular matrix, and destruction of elastic fibers. TSC2-null lesions and alveolar destruction were differentially inhibited by the macrolide antibiotic, rapamycin (which inhibits TSC2-null lesion growth by a cytostatic mechanism), and an HMG-CoA reductase inhibitor, simvastatin (which inhibits growth of TSC2-null lesions by a predominantly pro-apoptotic mechanism). Treatment with simvastatin markedly inhibited MMP-2, MMP-3 and MMP-9 levels in lung and prevented alveolar destruction. The combination of rapamycin and simvastatin prevented both growth of TSC2-null lesions and lung destruction by inhibiting MMP-2, MMP-3 and MMP-9.

Conclusions: Our findings demonstrate a mechanistic link between loss of TSC2 and alveolar destruction in LAM and provide preclinical proof-of-concept that combined rapamycin and simvastatin treatment could provide clinical benefits to patients with LAM by targeting cells with TSC2 dysfunction and preventing airspace enlargement.

28. Airway inflammation in a large cohort of children with primary ciliary dyskinesia

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Objectives: Primary ciliary dyskinesia (PCD) is a rare genetic disease characterized by abnormal ciliary structure and function that leads to impaired mucociliary clearance and chronic sinopulmonary disease. Little is known about the extent and role of airway inflammation in PCD lung disease. We characterized airway inflammation in sputum from a cohort of children with PCD, examined relationships between sputum measures of inflammation and lung function in these children, and compared these measurements to those from an age-matched cohort of children with cystic fibrosis (CF).

Methods: Spontaneous sputum collection and pulmonary function tests were performed in 34 children with PCD (15 males, mean age 12.2 years, range 5-18 years, mean FEV₁% pred 82.9, mean FVC%pred 92.5) at an annual study visit. Subjects with confirmed (n=23) and probable PCD (n=11) were included. Probable PCD was defined as an absence of ciliary structural abnormality or known genetic mutation, but with a compatible clinical phenotype and low nasal nitric oxide. Inflammatory cytokines (IL-1 β , IL-6, IL-8, TNF- α), proteases (free neutrophil elastase activity, MMP-9 activity), and antiproteases (SLPI, TIMP-1) were measured in sputum. These markers were correlated with lung function using nonparametric testing. Due to lack of normative values, these data were compared to measurements in induced sputum samples from an age-matched cohort of children with CF (18 males, mean age 12.2 years, range 6-15 years).

Results: Among PCD subjects, sputum neutrophil elastase correlated with poorer lung function [FVC (r=-0.32), FEV₁ (r=-0.38), FEF₂₅₋₇₅ (r=-0.38)]. Compared to those with CF, subjects with PCD had higher IL-1 β , IL-6, IL-8, TNF- α , neutrophil elastase, MMP-9, and TIMP-1 (p<0.02 for each). PCD subjects had significantly worse lung function than CF subjects (p<0.01 for FEV₁, p=0.04 for FVC).

Conclusions: Airway inflammation in PCD is characterized by the presence of pro-inflammatory cytokines, proteases, and anti-proteases. The negative correlation between neutrophil elastase and lung function suggests that neutrophil elastase may mediate lung injury in PCD. The difference in lung function between disease groups may be due in part to greater inflammation in the PCD cohort compared to the CF cohort.

Supported by the CF Foundation (KUPFER10B0) and NIH/NHLBI #9 U54 HL096458-07

29. Hyperghrelinemia Begins Early in Prader-Willi syndrome and May Promote Fat Mass Deposition

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Objective: Obesity is the major cause of morbidity and mortality in Prader-Willi syndrome (PWS) however the mechanism(s) behind it is unclear. Ghrelin is an orexigenic hormone that increases food intake while decreasing energy expenditure and fat catabolism. It is significantly elevated in PWS adults, however its level in young PWS children is controversial and its role in obesity and hyperphagia in PWS is unclear. In this study, we measured ghrelin levels in individuals with PWS from early infancy to 36 years of age. Individuals with non-PWS early-onset morbid obesity (EMO) and normal weight sibling controls were used as comparison groups.

Methods: Fasting serum ghrelin was measured using a fluorescent Enzyme-linked Immunosorbent Assay (ELISA) kit from Phoenix Pharmaceuticals, Inc., California, USA. We analyzed a total of 334 fasting serum samples: 136 PWS samples, 55 EMO samples, and 143 normal control samples.

Results: Serum ghrelin was significantly elevated in young PWS children (0-2 years) relative to normal controls ($p=0.0057$) but not in older PWS children (2-5 years, 5-12 years). Older EMO children (5-12 years) had significantly less serum ghrelin than older PWS children of same age range ($p=0.0095$), but not in the 2-5 year age range ($p=0.0631$). PWS teenagers and adults (12-36 years) had significantly higher ghrelin levels than their counterparts in the EMO and normal control groups. Ghrelin was significantly elevated in PWS children well before the onset of obesity and hyperphagia ($p<0.0001$) and nutritional phase was more prognostic of ghrelin levels in PWS than was age.

Conclusions: It is unlikely that elevated ghrelin levels are causing the switch to the hyperphagic phases of PWS as ghrelin was significantly elevated beginning in early infancy in PWS, well before the onset of obesity and hyperphagia. Ghrelin, however, can act to increase fat mass independent of its effect on appetite (Perez-Tilve et. al, *FASEB J.* 25:2814, 2011). It is therefore likely that the elevated ghrelin levels are causing the increased fat mass seen in infants with PWS compared to normal infants with similar body mass indices (BMI). Because nutritional phase was more prognostic of ghrelin levels in PWS than was age, consideration of nutritional phase is critical when analyzing ghrelin levels in PWS individuals.

30. Stimulant Use in Patients with Sturge-Weber Syndrome: Safety and Efficacy

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Objective: Sturge Weber Syndrome (SWS) is characterized by a facial port-wine birthmark, vascular eye abnormalities, and a leptomeningeal angioma. Attention and behavioral issues are common in SWS; literature evidence for stimulant treatment is primarily derived from a previous survey. This study evaluates stimulant medication safety and efficacy in SWS patients.

Methods: The research database of the Hunter Nelson Sturge-Weber Center (n = 210 subjects with SWS brain involvement) was reviewed for stimulant use. Thirteen subjects (mean age 10.7 years, ages 4 to 21) on stimulants were seen between 2002 and 2012. A retrospective chart review obtained co-morbid diagnoses, stimulant type and dosage, medication side effects, vital signs, and medication efficacy.

Results: All thirteen subjects had disease brain involvement (unilateral - ten; bilateral – three). Subjects were diagnosed with attention issues by neurologist (six), neuropsychological testing (five), or had prior diagnoses (two). Additional co-morbidities included epilepsy (thirteen), hemi-paresis (nine), headaches (eight), and vision deficits (seven). Eight subjects reported side effects, primarily appetite suppression (four) and headaches (three). There were no statistically significant changes in weight or blood pressure six months after medication initiation. A majority of patients (78%) had good seizure control (no seizures or seizures within the last six months but not monthly) and stable seizure clinical neurological scores (71%) while on the medication. Medication efficacy was reported in twelve subjects. Eight patients remained on stimulants at their most recent follow up visit.

Conclusions: This study evaluates stimulant medication use in a group of SWS patients. Stimulants were tolerated and effective in most subjects and did not negatively impact seizure control. Side effects were mostly minor and medication did not negatively impact growth or vital signs. Stimulant medication may be a safe and effective intervention for SWS children with attention issues/ADHD.

31. Distribution of Vasculitides in CARRA-Affiliated Pediatric Rheumatology Centers in the United States

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Background/Purpose: The epidemiology of pediatric vasculitis is poorly described. Two studies in the 1990s described the frequency in pediatric rheumatology centers in the United States (US) and Canada (1.2-2.4% of rheumatologic diagnoses). Subsequently, consensus classification criteria for pediatric vasculitis were developed, yet disease patterns have not been reassessed. Although many immunomodulatory regimens are used, treatment patterns are not known. Recently, the Childhood Arthritis and Rheumatology Research Alliance developed a registry of children treated at US pediatric rheumatology centers (CARRAnet). The purpose of this study was to delineate the distribution and treatment of vasculitides during CARRAnet's first 24 months.

Methods: This is a retrospective observational cohort study. 55 centers submitted data on patients with defined rheumatologic disease (vasculitis, 35 centers) (2010-2012). Not all patients with these diagnoses at each study site were included in CARRAnet. Demographic, diagnostic evaluation, disease manifestation, and treatment data were collected using standardized forms and entered into an electronic database. This cohort includes children with vasculitis.

Results: 129 children, 1.8% of the registry, had vasculitis (95% confidence interval 1.5-2.1): 50% granulomatosis with polyangiitis, 14% Behçet's disease; 12% Takayasu arteritis; 9% microscopic polyangiitis; 5% central nervous system vasculitis; 3% cutaneous PAN; 2% Churg-Strauss syndrome. The median age of onset was 12.1 years; the median time to diagnosis was 2.8 years. There was a significant difference in the distribution of the age at onset ($p < 0.01$), but not in the distribution of sex, race, or ethnicity ($p = 0.14$, $p = 0.28$, $p = 0.68$), between vasculitides. Most children were treated with glucocorticoids (96%) and a non-biologic disease modifying anti-rheumatic drug (DMARD) (91%); 60% were treated with cyclophosphamide. 33% were treated with a biologic DMARD: anti-tumor necrosis factor- α (14%); anti-CD20 (15%).

Conclusion: While the frequency of vasculitis in CARRAnet is similar to that in earlier clinic-based registries, the distribution of diagnoses differs; a greater percentage of children had GPA and TAK and a smaller percentage MPA. Compared to a recent pediatric GPA cohort, despite the increased use of anti-CD20, children were not spared exposure to cyclophosphamide. Among centers, treatment regimens varied considerably. This study demonstrates CARRAnet's usefulness for studying even rare conditions. Expanded enrollment, and follow-up of cases, will provide important observational data not obtainable by single-center studies.

32. Hyperacetylation of Histone H4 in Systemic Lupus Erythematosus

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Objective: Epigenetic processes, such as histone modifications, regulates gene expression without altering the genomic sequence and represents important disease mechanisms that have had little attention in systemic lupus erythematosus (SLE) to date. We previously reported that histone H4 acetylation (H4ac) is globally increased across the genome in monocytes of SLE patients as compared to controls using a tiling array approach. In order to further characterize H4ac, we looked for imbalances in histone acetyltransferases (HATs) and histone deacetylases (HDACs). We utilized flow cytometry to identify specific lysine residues hyperacetylated in SLE.

Methods: Peripheral blood monocytes were obtained from 7 controls and 7 SLE patients. The patients had low SLEDAI score (mean < 2) and were on no immune suppressive medications other than low-dose prednisone. Flow cytometry for H4 lysine acetyl groups: K5, K8, K12 and K16 were run on a FACS Calibur instrument using appropriate isotype controls. RNA-Seq studies were performed on monocytes from a different set of 8 controls and 8 SLE patients and examined the differential gene expression of HATs and HDACs between the groups to identify enzymes involved in increased H4ac in SLE.

Results: Analysis of HATs gene expression found that PCAF-KAT2B was significantly increased in SLE monocytes as compared to controls. PCAF-KAT2B can associate with IRF1 and acetylates H4K5, H4K8, and H4K16. Further examination of the RNA-Seq data revealed increased expression of genes regulated by IRF1. Also, when compared to the control group, the SLE group had significantly decreased gene expression of HDAC3, which normally deacetylates all H4 lysine acetyl groups, but preferentially H4K5 and H4K12. HDAC11 expression in SLE monocytes was also significantly reduced as compared to controls. HDAC11 normally represses antigen-presenting cells' production of IL-10, which is known to be increased in SLE patients. Finally, using flow cytometry, we found H4K5, H4K8, and H4K16 acetylation increased (but not significant statistically) in SLE monocytes.

Conclusion: These data demonstrate that in addition to specific gene sets being dysregulated in SLE, global alterations to H4 acetylation occur as well. These findings parallel studies that examined CpG DNA methylation and discovered evidence of global gene demethylation. The identification of a candidate HAT provides for a potential therapeutic target.

33. Erythropoietic Protoporphyrins: Frequency of *FECH* Gene Mutations Causing Autosomal Recessive Erythropoietic Protoporphyrin and *ALAS2* Gene Mutations Causing X-Linked Protoporphyrin

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Objective: Erythropoietic Protoporphyrin (EPP) is an autosomal recessive disorder caused by “loss of function” (LOF) mutations in the *FECH* gene, which lead to reduced activity of the enzyme ferrochelatase. Recently, an X-linked form of EPP (XLP) was described due to “gain of function” deletions in the *ALAS2* gene, encoding erythroid-specific 5'-aminolevulinic synthase 2. EPP and XLP are characterized by painful cutaneous photosensitivity and elevated erythrocyte protoporphyrins. While free and zinc-bound protoporphyrins are equally elevated in XLP, free protoporphyrins are predominant in EPP. So far, 162 *FECH* mutations and a common low-expression allele, IVS3-48T>C (present in about 10% of Caucasians), have been described. In XLP, two *ALAS2* deletions have been reported.

Methods: To determine the frequency of *FECH* and *ALAS2* mutations causing EPP or XLP, we performed mutation analysis in 107 unrelated patients with biochemical and/or clinical evidence of an erythropoietic protoporphyrin.

Results: *FECH* mutations, 20 of them novel, were identified in 85 patients. Two individuals had two LOF mutations and 83 carried one LOF mutation and the low-expression allele. Testing of 43 family members revealed nine additional affected individuals. Of the 19 subjects with no detectable *FECH* mutations, 10 carried *ALAS2* deletions: c.1734delG was present in one and c.1706_1709delAGTG in 9 subjects. In addition, a novel missense mutation, Q548X, was identified in one patient with XLP. Testing of 43 XLP family members identified seven affected males and 14 heterozygous females with generally milder manifestations.

Conclusions: These findings indicate that XLP occurs in about 10% of biochemically confirmed erythropoietic protoporphyrin patients, suggesting that *ALAS2* mutation analysis should be undertaken in patients with symptoms of EPP and no *FECH* mutations. Finally, there was significant clinical variability in heterozygous XLP females and these patients should be counseled appropriately.

34. Identification of a novel autosomal dominant adult-onset distal myopathy

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Introduction: Distal myopathies are genetically heterogeneous diseases. Known associated disease genes encode proteins in the sarcomere (titin, myosin), plasma membrane (dysferlin, caveolin), or cytoskeleton (desmin, myotilin, α B-crystallin, ZASP, filamin C, nebulin). Despite this progress, the majority of cases remain undefined and a systematic explanation for a disease mechanism in this ubiquitously expressed group of muscle proteins remains unresolved. Most of the distal myopathies involve muscles of the anterior compartment of the leg, except for the autosomal recessive dysferlinopathy that distinctly involves the gastrocnemius muscles. We define a new posterior distal myopathy in a two-generation family due to a defect in an uncharacterized enzyme.

Method: The proband was admitted under the Undiagnosed Disease Program. SNP and whole exome sequencing were done on genomic DNA extracted from blood. MRI was done on patient's lower extremities. Routine histopathology was performed on her muscle biopsy slides. Cellular and biochemical characteristic of the putative causative gene was studied.

Results: Affected individuals have progressive weakness of distal muscles beginning late in the third decade of life without evidence of neuropathy or increased serum creatine kinase, respiratory or cardiac symptoms. MRI studies showed fatty replacement of the posterior compartments of the legs, mainly of the medial gastrocnemius. Muscle pathology showed variation of fiber size due to fiber atrophy. Eosinophilic cytoplasmic inclusions, which were devoid of desmin, plasma membrane proteins and oxidative activity, were seen without associated rimmed vacuoles. Ultrastructural studies showed non-membrane bound fibrillary deposits 6-12 microns in diameter in the Z-bands. Pathological findings suggest a new form of aggregate myopathy. Whole exome and Sanger sequencing excluded mutations in known genes associated with distal myopathy and identified an intronic splice site mutation in *HINT3* causing exon 4 skipping. The resulting frameshift mutation removes the putative active-site nucleophile, His145, from the gene's protein product, HINT3, an enzyme that has an acyl-adenylate hydrolase activity. Based on the dominant inheritance and comparable expression of the mutant and wild type mRNAs, the truncated enzyme is an antimorph or neomorph. Biochemical measurement of HINT3 activity in patient's fibroblasts is being undertaken. We are using several cell and animal models for functional analysis. In cultured patient dermal fibroblasts, fibroblast-derived myoblasts, HeLa cells and C2C12 cells, HINT3 localizes predominantly to the mitochondria and to a lesser degree the nucleus. When overexpressed in C2C12 cells, the mutant HINT3 protein is mistargeted and trapped in the endoplasmic reticulum. For *in vivo* analysis, we are studying the effect of the mutant HINT3 on murine muscle strength, performance, and pathology following sonoporation and the effect of the mutant HINT3 in transgenic zebra fish.

Conclusion: In summary, we have identified a mutation of *HINT3* as a novel cause of a dominant distal myopathy. Our findings suggest that disease arises via an antimorphic or neomorphic mechanism.

35. Whole-exome sequencing combined with functional validation in zebrafish identifies novel disease genes for cortical malformations

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Objectives: α -dystroglycanopathies are a group of recessive disorders severely affecting the development of the muscle, eye and brain, causing an array of cortical malformations including cobblestone lissencephaly, hydrocephalus and polymicrogyria. While mutations in eight genes are known to cause these disorders, they explain less than half of the cases, displaying extreme genetic heterogeneity, which has hindered the identification of additional disease genes. Our objective was to use a combination of whole-exome sequencing (WES) and functional validation in the zebrafish to identify new disease genes for these disorders.

Methods: We utilized WES in a large cohort of patients (n=35) and then knocked-down candidate genes in the zebrafish embryo and larva by injecting morpholino oligonucleotides in the fertilized oocyte, to study the effect of the candidate mutations on brain, eye and muscle development.

Results: We filtered WES results for variants which were rare, likely pathogenic and inherited in a recessive fashion in the family of the affected individuals, leading to the identification of an average number of five candidate mutations in each individual. In several families we found severe loss of function mutations in two novel candidate genes *GTDC2* and *B3GALNT2*. Morpholino knock-down of the zebrafish orthologs for these two genes recapitulated brain, eye and muscle phenotypes consistent with those observed in the human dystroglycanopathy patients.

Conclusions: The combination of WES and functional validation in the zebrafish embryo is an effective approach to identify novel disease genes for severe recessive disorders. We identified two novel genes for dystroglycanopathies, *GTDC2* and *B3GALNT2*, and reduced the number of candidates in other families to five or fewer.

36. Cholesterol pool size in Smith-Lemli-Opitz syndrome children receiving cholesterol supplementation alone or combined with simvastatin

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Objective: Smith-Lemli-Opitz syndrome (SLOS) is caused by defective 7-dehydrocholesterol Δ 7-reductase activity, resulting in low plasma cholesterol but high 7-dehydrocholesterol (DHC) and 8-DHC levels in tissues and plasma. Therapeutic interventions in SLOS patients include strategies to maximize whole body cholesterol pool size (WBCP) while down-regulating biosynthesis to decrease the buildup of potentially toxic precursors including 7-DHC and 8-DHC. The study aim was to assess changes in blood isotope cholesterol enrichment over time to measure WBCP in SLOS patients receiving supplemental dietary cholesterol alone or combined with simvastatin.

Methods: SLOS subjects receiving a high cholesterol diet alone (n=14; mean age: 7 \pm 2 yr) or combined with simvastatin (n=4; mean age: 9 \pm 2 yr) were given intravenous injections of [¹⁸O]-cholesterol (1.0-1.4 mg/kg bodyweight) or D₇-cholesterol (0.9-1.4 mg/kg). Blood samples were collected at baseline and serially over a 10 wk period; cholesterol extracted from red blood cells, derivatized with piconyl ester and analyzed using a sensitive liquid chromatography-tandem mass spectrometry (LC/MS/MS) method. The [M+H]⁺>[M- picolinic acid]⁺ transition was chosen for monitoring, with ¹⁸O-cholesterol at m/z 494>369 and D₇-cholesterol at 499>376. Plasma sterols were measured by gas chromatography. Treatments were compared using unpaired t-test and reported as mean \pm standard error mean.

Results: SLOS patients receiving high cholesterol diet combined with simvastatin showed greater WBCP (546.8 \pm 143.6 mg/kg bodyweight) compared to those on cholesterol only (331.6 \pm 30.1 mg/kg bodyweight); p<0.05. Similarly, the patients on the combination therapy had lower plasma level of 7-DHC (2.9 \pm 0.5 mg/dL) compared with those given high cholesterol only (10.4 \pm 1.8 mg/dL); p<0.05. Plasma cholesterol level was not related to WBCP (p>0.05), but inversely related to 7DHC and 8DHC (p<0.05).

Conclusions: These results suggest that whole body cholesterol pool size in SLOS patients may be enhanced by dietary cholesterol supplementation combined with simvastatin. This is the first reported study to utilize stable isotope technique to assess cholesterol pool size in SLOS patients. Employment of isotopes for tracer studies *in vivo* provides a powerful tool to investigate cholesterol metabolism, particularly in disease states such as SLOS.

37. Incidence of Pseudomonas Aeruginosa Infection and Association With Lung Function In A Pediatric Cohort With Primary Ciliary DyskinesiaKathleen Nelligan BSc^{1,2}, Annie Dupuis PhD^{1,2}, Yvonne Yau MD^{1,2}, Sharon Dell MD^{1,2}¹ The Hospital for Sick Children; ² University of Toronto; Toronto, Ontario, Canada

Background: Primary ciliary dyskinesia (PCD) is a recessive genetic disorder in which structural ciliary defects result in impaired mucociliary clearance and vulnerability to respiratory tract infections. The opportunistic pathogen, *Pseudomonas aeruginosa*, is known to be a strong predictor of lung deterioration in patients with cystic fibrosis, yet its incidence and impact on lung disease progression remain poorly understood in the PCD population.

Objective: Describe the incidence of *P. aeruginosa* infection and associated lung disease in patients with PCD.

Methods: A retrospective cohort study design was used. Data was collected from health records of patients aged 0 to 18 years attending the PCD clinic at SickKids Hospital between 2000 and 2012 who also had ≥ 1 respiratory culture. Yearly and cumulative incidence of *P. Aeruginosa* infection was calculated based on the number of participants at risk per year. Outcomes included bronchiectasis reported on CT scans and forced expiratory volume in one second (FEV₁ % predicted). The association between *P. aeruginosa* infection and radiographic or functional measures of lung disease was determined using logistic regression or hierarchical linear models, respectively.

Results: A cohort of 68 patients were followed for an average of 4.88 ± 2.85 yrs and had 2 to 3 clinic visits with respiratory culture and lung function measures per year. Twenty (29.4%) had at least one respiratory culture positive for *P. aeruginosa*. Average age of first acquisition was 11.1 years. Average yearly incidence of *P. aeruginosa* was 10.2% (95%CI: 8.6-11.8). Bronchiectatic changes occurred at a similar frequency in those with *P.aeruginosa* (88.9%) compared to those without (80%). FEV₁ tended to decline with age (-0.6255% per year, p=0.0013) but *P. aeruginosa* infection, either intermittently or chronically, was not statistically associated with this decline.

Conclusions: *P. aeruginosa* may occur in nearly one third of patients with PCD before adulthood. In Toronto, *P. aeruginosa* infection was not a major predictor of either radiographic or functional lung disease. However, small sample sizes, limited follow-up time and treatment effect may have limited or confounded the analysis. Multi-centre studies with long-term follow-up and randomization of treatment strategies must be completed to assess the full impact of *P. aeruginosa* infection.

38. Brain Arteriovenous Malformations associated with Hereditary Hemorrhagic Telangiectasia: Gene-Phenotype CorrelationsTakeo Nishida,^{1,2} Marie E. Faughnan,^{2,3,4,5} Timo Krings,^{2,6} Murali Chakinala,^{2,7} James R. Gossage,^{2,8} William L. Young,^{2,9,10,11}Helen Kim,^{2,9,12,13} Tony Pourmohamad,⁹ Katharine J. Henderson,^{1,2} Stacy D. Schrum,^{2,7} Melissa James,^{2,8} Nancy Quinone,^{2,9}Aditya Bharatha,¹⁴ Karel G. terBrugge,^{2,6} Robert I. White Jr.,^{1,2}

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Objective: Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant genetic disease with a wide spectrum of vascular malformations involving multiple organs. Nine-16% of patients with HHT harbor brain arteriovenous malformations (AVMs), which can cause intracranial hemorrhage. Our objective was to study clinical manifestations of brain AVM in patients with HHT and correlate these with the specific gene mutated.

Methods: We reviewed records of 171 patients with HHT and brain AVMs.

Results: A history of intracranial hemorrhage was found in 27% (41/152) patients, with a mean (range) age of 26 +/- 18 (0-68) years. All patients with intracranial hemorrhage were neurologically asymptomatic prior to intracranial hemorrhage. Multiple brain AVMs were found in 23% (170/39) of patients on initial examination. Genetic test results were available in 109 (64%) patients. Mutations in *ENG*, *ACVRL1*, and *SMAD4* were present in 75 (69%), 18 (17%) and 2 (2%), respectively. A history of intracranial hemorrhage was reported in 24% of patients with an *ENG* mutation and 27% of patients with an *ACVRL1* mutation, with a mean (range) age of 26 +/- 16 (2-50) and 18 +/- 21 (0-48) years, respectively. No statistically significant differences in age at first brain AVM diagnosis, prevalence of intracranial hemorrhage history, age at intracranial hemorrhage, or other manifestations of brain AVMs were observed among gene groups.

Conclusions: No evidence for differences in brain AVM characteristics was observed among HHT gene groups, although we cannot exclude clinically important differences. Larger studies are needed to further guide brain AVM screening decisions in patients with HHT.

39. Cells from Chediak-Higashi Syndrome subjects exhibit an altered response to immunogenic challenge

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Objectives: Chediak-Higashi Syndrome (CHS) is a rare autosomal recessive disease characterized by mutations in the lysosomal trafficking regulator gene (*LYST*), leading to generalized immunodeficiency, and broad range of infections including reports of severe periodontal disease. We hypothesized that cultured skin fibroblasts obtained from CHS patients would exhibit an altered response to immunogenic challenge.

Methods: Cells were obtained from skin biopsies, enzymatically digested, and frozen until use. PCR-arrays were used to profile the expression of inflammation-related genes in control and CHS fibroblasts cultured with or without *Escherichia coli* LPS (10ng/mL for 3 h) (n=3). Quantitative PCR was used to further assess mRNA levels of interleukin (IL)-6, IL-1 β , and cyclooxygenase (Cox-2). Statistical analysis was performed by Student's t-test for inter-group comparisons.

Results: Of 84 genes evaluated by PCR-array, 15 were up-regulated and 6 down-regulated (fold-change ≥ 2) in CHS cells compared to control cells at baseline. In control cells, LPS treatment up-regulated 28 genes (fold-change ranging from 2.1 to 4,700) compared to untreated cells ($p \leq 0.05$). In contrast, LPS-treated CHS cells presented 8 (C3AR1, CCL-4, CCL-7, CXCL-1, CXCL-2, CXCL-6, IL-1a, and IL-8) up-regulated genes (fold-change ranging from 2.4 to 75.7), compared to untreated CHS cells ($p \leq 0.05$). In general, CHS and control cells exposed to LPS demonstrated that 9.5% and 33.3% of the genes, respectively, were significantly affected by LPS treatment (fold-change ≥ 2). By qRT-PCR, IL-1 α , IL-6, and COX2 mRNA levels were significantly higher in CHS versus control cells at baseline, however, control cells presented a more dramatic response (4,000-fold change) to LPS treatment than CHS cells (30-fold change).

Conclusion: Surprisingly, CHS cells presented higher baseline mRNA expression of immune markers, but a blunted response to LPS challenge. Although the function of *LYST* remains incompletely understood, these findings support an important role in modulating key factors directing inflammatory responses.

40. Allogenic bone marrow transplantation (BMT) ameliorates muscle atrophy, degeneration, and weakness in mice with GNE myopathy.

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Objective: GNE myopathy, previously known as distal myopathy with rimmed vacuoles (DMRV), or hereditary inclusion body myopathy (hIBM), is an autosomal recessive myopathy characterized by progressive weakness and atrophy involving the distal muscles, and the presence of myofiber vacuolation and degeneration. GNE myopathy results from biallelic mutations in the GNE gene, which encodes the bifunctional enzyme which catalyzes the crucial steps in sialic acid biosynthesis. No therapy has been approved for this debilitating disorder and the pathomechanism has remained elusive. We and others have shown that hyposialylation has a major role in pathomechanism as myopathy in the DMRV/hIBM mouse model was prevented by giving oral sialic acid metabolites or modified ManNAc conjugates despite the fact that sialic acids given orally are rapidly excreted, requiring frequent or almost continuous dosing.

Methods: In this study, we established allogenic bone marrow transplantation (BMT) as a rational therapy as it can provide a continuous source of hematopoietic-derived sialic acid-producing. Bone marrow cells from CAG-GFP mice and depleted of mature T cells were injected intravenously into DMRV mice 30 weeks old after a single dose of sublethal irradiation. After chimerism, the mice were phenotyped to check for response to treatment.

Results: There was a clear increment in cell surface sialylation that correlated with chimerism. GFP-positive donor cells were engrafted into skeletal muscles and other organs of DMRV/hIBM mice. DMRV/hIBM mice subjected to BMT had a marked improvement in lifespan and motor performance. Analysis of muscle physiologic contractile properties and pathology revealed an improved muscle phenotype. Evaluation of specific glycoproteins in the muscle demonstrated a remarkable recovery of cellular sialylation. Measurement of GNE activity and sialic acid levels in several organs were remarkably increased compared to non-treated DMRV/hIBM mice.

Conclusions: Our results provide proof of a concept for cell based therapies in DMRV/hIBM. Extant protocols for bone marrow transplantation can be adapted for DMRV/hIBM treatment. We envision that with this strategy, GNE myopathy patients can have a lasting supply of sialic acid that may enhance disease recovery.

41. Post transplant Recurrence of Oxalosis in Primary Hyperoxaluria type 1: A study of protocol biopsies

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Objective: Primary hyperoxaluria (PH) accounts for 1% of pediatric ESRD in US and European registries. After transplant (Tp) recurrent oxalosis in the allograft remains a problem. Protocol biopsies were studied to assess the frequency and characteristics of recurrence and its effect on renal allograft function.

Methods: Of 330 patients enrolled in Rare Kidney Stone Consortium PH Registry 26 patients who received 27 transplants [7kidney alone (K), 20 simultaneous liver and kidney (L/K)] and had at least one protocol biopsy (4, 12, 24 or 60 months) were included. Median age at Tp was 33 years. Crystal score in kidney tissue was estimated as number of crystals /total glomeruli in the tissue slide. Spearman's correlation was used to test associations.

Results: 73 protocol biopsies were obtained from 27 allografts. Calcium oxalate (CaOx) crystals were found in at least one biopsy in 63% (17/27) of allografts. No associations were found between CaOx crystals and donor age, time on dialysis before Tp and Tp type (K or L/K). Crystals were more often seen in 4 month biopsy (55%) than at 12 and 24 months (15% and 31%, respectively). 15% of implantation biopsies showed crystals 20 min after graft placement. The crystal/glomerulus score (mean 0.08, range 0-1.41) correlated with 24 hour urine oxalate ($r=0.41, p=.0007$), calcium ($r=-0.42, p=.001$), and magnesium ($r=-0.45, p=.003$) and with plasma oxalate at time of biopsy ($r=0.44, p=.0002$). Crystal score at 4 months ($n=19$) correlated with eGFR at 12 and 24 mos after Tp [Fig1]. After 24 months 87.5% grafts survived with a mean eGFR of 60.2 ml/min/1.73m². 2 grafts were lost to oxalosis and 1 to rejection. At 5 years follow up ($n=12$) overall graft survival rates are 66.7% whereas the death censored graft survival was 83%. Graft loss was due to recurrent oxalosis in one, chronic allograft nephropathy in one and death with functioning allograft in two of them. Comparison of graft survival rates among those with and without recurrence is shown in Fig2.

Conclusions: Recurrent oxalosis is seen in 63% of allografts. Intensity of crystal deposition correlates with concurrent urine analytes and has a negative impact on 2yr graft function. There was a trend towards poor graft survival in allografts with recurrence compared to those without recurrence even though it did not reach statistical significance probably because of small numbers.

42. Automating objective, video-based evaluation of blepharospasm symptoms from multicenter clinical examinations

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Objective: Blepharospasm is a form of focal dystonia impairing voluntary control of eyelid and periocular musculature. Symptom severity is typically characterized by observation during clinical examination. Recent advances in computerized video processing offer an adjunct means to quantify symptom severity. The objective of this study was to develop and test a centralized system for computerized processing of blepharospasm clinical exam videos.

Methods: Given the relatively low prevalence of focal dystonias such as blepharospasm (12-133 cases per million), the Dystonia Coalition has developed a central clearinghouse for collecting patient exams from more than 15 participating clinical centers. In this project, we have designed and implemented a system whereby video processing – including quality validation, segmentation, and analysis with CERT (the Computerized Expression Recognition Toolbox) – is jointly conducted between the biorepository site (Washington University) and the analysis site (UCSD). We have tested the system by comparing CERT's eye closure measure with manually identified eye closure annotations.

Results: In addition to implementing the hardware and software elements of the system, we have developed and documented a multi-step, multi-party procedure for video processing. The procedure screens videos for viability, identifies the appropriate template for segmenting videos, creates streamed versions of the video for secure remote viewing, and produces CERT outputs from the video. CERT's eye closure outputs demonstrate high correlation with manually identified eye closure annotations.

Conclusions: Our results demonstrate the viability of a centralized, streamlined system and procedure for computerized analysis of patient face videos. Our approach also minimizes IRB concerns by providing to researchers at the analysis site access to only streamed versions of the videos. The procedure should help ensure a maximally efficient and standardized procedure for analyzing future patient data, particularly timely given the currently superlinear growth rate in the Dystonia Coalition's patient cohort.

Acknowledgments: The Benign Essential Blepharospasm Research Foundation and the NIH (in the form of NIMH 5T32-MH020002 and ORDR/NINDS NS065701 to the Dystonia Coalition, which is part of the NIH Rare Diseases Clinical Research Network).

43. Interleukin 8 (*IL8*) Expression in Cystic Fibrosis (CF) Nasal Epithelia is Correlated with a Genetic Variant in the *IL8* Promoter Associated with CF Lung Disease Severity

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Objective: Cystic fibrosis (CF) is an autosomal recessive genetic disorder caused by mutations in *CFTR*, with pulmonary disease being the leading cause of mortality. Non-*CFTR* modifier genes are expected to account for much of the variability in lung disease severity observed, even amongst F508del homozygotes (heritability 0.54-0.89; Vanscoy et al, 2007 *AJRCCM*). Candidate-gene studies demonstrate that a single nucleotide polymorphism (SNP; rs4073) in the *IL8* promoter is associated with CF lung disease severity: In 1,112 CF patients, 2 copies of the rs4073 risk allele (TT) associated with lung disease severity ($p=0.02$). This effect was pronounced in males ($n=608$; $p=0.001$). A luciferase reporter assay showed differential *IL8* expression associated with rs4073 genotype in a human airway cell line (9HTEo) (Hillian et al, 2008 *Genes and Immunity*). We hypothesize that differential *IL8* expression is correlated with rs4073 genotype *in-vivo*.

Methods: A multicenter collaboration was developed with a goal of obtaining nasal epithelial samples from 150 CF subjects. In 119 subjects, our techniques have yielded high-quality RNA, and we have begun to measure *IL8* expression via real time RT-PCR, testing correlations with rs4073 in an initial 54 CF patients (27 males).

Results: Subjects with 2 copies of the risk allele (TT; $n=15$) demonstrate a 2.82 fold increase in *IL8* expression over subjects with 1 copy (AT; $n=26$) and a 2.11 fold increase over patients with no copies (AA; $n=13$) ($p=0.012$, $p=0.0053$). Analysis of males showed 2 copies of the risk allele (TT; $n=8$) is associated with a 4.24 increase in *IL8* expression over males with 1 copy (AT; $n=13$), and 6.47 fold increase over males with no copies (AA; $n=6$) ($p=0.011$, $p=0.003$).

Conclusions: These findings validate previous SNP association and *in-vitro* expression studies (Hillian et al, 2008), demonstrating that differential *IL8* expression occurs *in-vivo* in association with risk genotype in rs4073, particularly in males. Understanding the association of genetic variation and differential *IL8* expression may provide further insight into mechanisms of lung disease severity, add ability to predict underlying non-*CFTR*-related genetic risk, and aid in development of therapeutic strategies.

Funding: CFFPOLINE09FO; NIHHL095396

44. An International Disease Registry for Susac Syndrome: A Report on 88 Patients

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Objective: Susac Syndrome (SS) is a rare autoimmune disease that affects the microvasculature of the brain, retina, and inner ear, causing ischemic injury to these tissues. It is characterized by the clinical triad of encephalopathy, retinal vasculopathy, and hearing loss. It represents an autoimmune endotheliopathy and responds to immunosuppressive medication. Fewer than 250 cases have been reported in the medical literature. To collect basic data on patients with Susac's syndrome from around the world, we established an International Disease Registry (IDR).

Methods: An IRB approved International Collaborative Study (ICS) of Susac's Syndrome was established. The ICS includes an International Disease Registry (IDR). Basic demographic (age, gender, country) and clinical data (components of the clinical triad experienced and presence/absence of characteristic MRI findings) were collected on patients for registry purposes. Criteria for entry into the IDR were: presence of at least 2 of the 3 components of the triad; or, presence of 1 component plus typical callosal lesions on MRI.

Results: A total of 88 patients, from 22 different countries, have been registered in the IDR. Sixty (68.2%) are female, 28 (31.8%) male. The mean age at time of diagnosis was 31.1 years (range 9-57 years). At the time of diagnosis, 8 were <17 years old, 5 were 17-19, 31 were 20-29, 26 were 30-39, 11 were 40-49, and 7 were 50-59. Seventy three patients (83%) are from northern countries--- 34 from USA, 6 from Canada, 13 from Germany, and 20 from other countries in Europe. Fifty seven (64.8%) had experienced all three components of the clinical triad, and 100% of these patients had typical corpus callosal lesions on MRI. Twenty eight (31.8%) had experienced only 2 of the 3 components: 15 with retinal and inner ear disease, but no encephalopathy; 10 with encephalopathy and retinal disease, but no inner ear disease; 3 with encephalopathy and inner ear disease, but no retinal disease. Three patients had only one component, but had callosal lesions.

Conclusions: The above data suggest a spectrum of Susac's syndrome: extending from the typical "encephalopathic form" with the complete triad and corpus callosal lesions---to partial forms, most notably, the "retinal/inner ear subset." The IDR and ICS will serve as rich sources of data on Susac's syndrome.

45. Readability and Suitability Assessment of Patient Education Materials in Rheumatic Diseases

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Objective: Patient education materials are frequently used by providers to inform patients about their rheumatic disease, but little attention has been given to the readability and appropriateness of patient materials. The objective of this study was to examine the readability and suitability of commonly used patient education materials for osteoarthritis (OA), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and vasculitis.

Methods: Several popular patient resources were chosen: American College of Rheumatology (ACR), Arthritis Foundation (AF), Mayo Clinic Health Information, National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), UpToDate Basics, UpToDate Beyond the Basics, Vasculitis Clinical Research Consortium (VCRC), and Vasculitis Foundation. Readability was measured using the Flesch-Kincaid Reading Grade Level and computed after removal of illustrations, tables, captions, disease and medication names, all forms of “rheumatology,” and defined terms. Suitability was determined by the Suitability Assessment of Materials (SAM), a score that considers characteristics such as content, graphics, and layout/topography. Three different reviewers rated the SAM score and means were used in the analysis. Scores were converted into a percentage of total possible score. A score of 0-39% was considered not suitable, 40-69% adequate, 70-100% superior.

Results: Twenty-three resources written on the four diseases were evaluated. The education material for all four diseases studied had readability above the 8th grade level and readability did not differ among the diseases. The mean grade level for each resource ranged from 4.8 to 12.5 with a median grade level of 9.6. UpToDate the Basics was the only resource that achieved a widely-recommended standard of a 6th grade reading level. Only 5 of the 23 resources received superior suitability scores. All four diseases received adequate suitability scores with OA have the highest mean suitability score.

Conclusions: Most patient education materials for rheumatic diseases are written at readability levels above the recommended sixth-grade reading level. Most resources have only adequate suitability. In general, reading grade level was lower for the material for OA compared with materials for the other three diseases, and OA had the highest mean suitability score suggesting that disease complexity contributes to poor readability and substandard suitability. Developing more appropriate educational resources for patients with rheumatic diseases may improve patient comprehension.

46. The Natural History of Giant Axonal Neuropathy

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Background: Giant Axonal Neuropathy (GAN) is a rare, pediatric neurodegenerative disease affecting the central and peripheral nervous systems. GAN is caused by an autosomal recessive mutation in the gigaxonin gene, producing swollen and degenerating axons due to neurofilament accumulation. Patients develop a progressive motor and sensory neuropathy that causes muscle weakness, severe gait impairment, visual tracking deficits, dysarthria, and possible dementia. Patients lose the ability to ambulate around age 10 and die from respiratory failure in their 20s. Around 40 cases of GAN have been reported worldwide, although the true incidence is unknown.

Objective: We will perform a comprehensive study of the natural history of GAN by describing the clinical, electrophysiologic, imaging, molecular, and cognitive characteristics of 12 subjects over 2 years.

Methods: 5 study visits will occur over a 2-year period. Study procedures include motor function testing using the Jebsen Taylor Timed Test (“Jebsen”), Friedreich Ataxia Rating Scale (FARS), Gross Motor Function Measure (GMFM), Charcot Marie Tooth Neuropathy Score (CMTNS), and Six-Minute Walk Test (6MWT); electrophysiologic studies; brain and spine MRI; cerebrospinal fluid collection, serum collection, and skin biopsy; verbal and non-verbal cognitive assessments; and standardized quality of life indices.

Results: The baseline visit has been performed for 10 subjects. All subjects have genetically diagnosed or biopsy confirmed GAN. The CMTNS and 6MWT appear to have limited applicability in non-ambulatory subjects. Subjects with hand contractures or impaired fine movements have been unable to perform the Jebsen. The GMFM, FARS, and other study procedures have been performed without issue. CSF, serum, and skin biopsy samples are being held at a repository for future research. We expect to exceed our planned enrollment of 12.

Conclusions: We aim to characterize the neuromuscular deficits that occur in GAN. We will use established clinical function measures and secondary outcomes to define the expected rate of disease progression. This data will be used to measure therapeutic effect in an upcoming clinical trial of a genetic therapy for GAN. We also aim to clarify the nature of cognitive impairment in GAN. Reports of cognitive impairment in the literature are inconsistent and may reflect dysarthria (rather than cognition *per se*) or be the product of other genetic anomalies in patients with consanguineous parents.

47. **Public reporting of metrics may improve outcomes in rare diseases.**
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Objective: To review current approaches for obtaining patient data in the rare disease, Duchenne Muscular Dystrophy (DMD), and consider how monitoring and comparing outcome measures between DMD clinics could improve patient care. The Cystic Fibrosis Foundation (CFF) has stratified patient data by CF center since 1999, and made it publically available in 2006. Reporting outcomes to the center and more recently to the public has challenged the CF community to improve outcome measures and clinical care. This method has the potential to be used in other rare diseases, including Duchenne Muscular Dystrophy, which has few evidence-based quality care indicators to standardize outcomes across specialized DMD clinics. Alternative ways to improve care, such as comparing practice patterns and outcomes across centers, should be considered for such diseases.

Methods: We reviewed standardized data obtained annually from CF clinics; published guidelines and consensus statements on the care of DMD; compared current approaches to obtain DMD patient data and outcome measures; and hypothesized on the best method to implement public reporting of outcome measures to drive improvements in health care delivery.

Results: Current methods to monitor DMD patient information (MD STARnet, DuchenneConnect, and TREAT-NMD) do not yet provide patients with comparative data on outcomes. Data obtained through the CF patient registry allows for reporting of standard outcomes across clinics and has been associated with improved CF outcomes. A similar patient registry is under development for the Muscular Dystrophy Association (MDA) clinic network. Suggested metrics for quality care include: molecular diagnosis, ambulatory status and age at loss of ambulation, age requiring ventilator support, and survival.

Conclusions: CF longevity has increased by almost 33% from 1986 to 2010, in part due to a CF patient registry that has been stratified by individual care centers and publically available. Implementation of outcome reporting for MDA clinics might similarly benefit patients with DMD.

48. **Process modeling of a translational research program for undiagnosed and rare diseases**
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Objective: The Undiagnosed Diseases Program (UDP) at the National Institutes of Health (NIH) pursues two goals: 1. To provide answers to patients with conditions that have eluded diagnosis, 2) To advance medical knowledge about rare and common diseases [1]. Each UDP case is a unique entity necessitating a unique research process. Clinical evaluation, tests, consultations and subsequent research may significantly differ between UDP cases. Depending on the results of the clinical and molecular testing, each case may end up in a very different downstream scientific investigation design to elucidate the pathophysiology behind the disease. With thousands of applications and hundreds of cases admitted to the program UDP has done research on many rare and undiagnosed cases using methods such as whole genome SNP chip analysis and next generation sequencing. UDP is currently being expanded to include external sites that would work in coordination with the current intramural program. Our goal is to support this expansion by creating a process model that would lend to improvement of the current processes and help design the next phase of our translational research program.

Method: We modeled the UDP process using Business Process Model and Notation (BPMN) to better understand the commonalities and differences that can be used to design an information system that can improve the translational research process in the intramural UDP and help facilitate the coordination and collaboration of the external UDP sites that are expected to join the UDP in the near future.

Result: The process modeling revealed a complicated process flow with many stakeholders and static, dynamic components that are represented in three distinct lanes of processes (i.e. Clinical, In-silico and molecular investigation). The modeling reveals clear bottlenecks that need more attention from the scientific management. Modeling of the dynamic aspects (e.g time based alerts and escalation rules) helps us to design information systems that support the management of each UDP case as a unique mini scientific project.

Conclusion: We believe the outcomes of our work can be used to establish a robust and tested model of translational research program in rare and undiagnosed diseases.

1 - <http://rarediseases.info.nih.gov/Resources.aspx?PageID=31>

49. Lysine restricted diet for pyridoxine dependant epilepsy: first evidence & future trials

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Objective: To evaluate the efficacy and safety of dietary lysine restriction as an adjunct to pyridoxine therapy on biochemical parameters, seizure control, and developmental/cognitive outcomes in children with pyridoxine-dependent epilepsy (PDE) caused by antiquitin (ATQ) deficiency.

Methods: In this observational study, seven children with confirmed ATQ deficiency were started on dietary lysine restriction with regular nutritional monitoring. Biochemical outcomes were evaluated using pipercolic acid and α -amino adipic semialdehyde (AASA) levels in body fluids; developmental/cognitive outcomes were evaluated using age-appropriate tests and parental observations.

Results: Lysine restriction was well tolerated with good compliance; no adverse events were reported. Reduction of mean biomarker levels ranged from 26 to 82% for plasma pipercolic acid, 51 to 87% for urinary AASA, 42% for plasma AASA. For the 1 patient in whom data was available, cerebrospinal fluid levels decreased by 86% for pipercolic acid and 81% for AASA. Improvement in age-appropriate skills and seizure control were observed in all children.

Conclusions: This observational study provides Level IV evidence that lysine restriction is well tolerated with significant decrease of potentially neurotoxic biomarkers in different body compartments, and improves developmental outcomes in children with PDE caused by ATQ deficiency. To generate a strong level of evidence before this potentially burdensome dietary therapy becomes the mainstay treatment, we have established: an international PDE consortium to conduct future studies with an all-inclusive integrated study design; a website containing up-to-date information on PDE; a methodological toolbox; and an online registry to facilitate the participation of interested physicians, scientists, and families in PDE research.

50.**Lipid Profile in Rett syndrome**

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Objective Rett syndrome (RTT) is a severe neurodevelopmental disorder of females with co-morbidities affecting nearly every body system, including gastrointestinal dysmotility, cardiac conduction abnormalities, and metabolic dysregulation leading to growth failure. Dyslipidemia has not been recognized as a component of the syndrome, and we aimed to examine the lipid profile in RTT.

Methods Participants were derived from the RTT IGF-1 clinical trial. Laboratory values were obtained as part of the trial, including total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL). Values were converted to Z-scores using a non-fasting, gender and age-specific reference. Data on genotype, diet and normal activity level were obtained, including stereotypies (type and frequency), sitting ability, ambulatory status, muscle mass, limb strength, and hand use. Diet and activity data were compared to lipid levels using linear regression and Pearson's correlation. Since lipid values were obtained to monitor for adverse effects of IGF-1, baseline lipid values were compared to those during IGF-1 administration using ANOVA.

Results Twelve participants were seen 1 to 3 times for a total of 32 observations. At baseline, 4 of 12 participants had total cholesterol levels above the 85th percentile, and 2 of these were above the 95th percentile for age. Triglycerides were elevated in 2/12 at baseline (1 above 95th percentile), and LDL was elevated in 7/12 (3 above 95th percentile). The average total cholesterol for the cohort was 0.54 standard deviations (SD) and LDL was 0.30 SD above the normative mean. Average triglycerides and HDL were close to the normative mean, while VLDL was 0.58 SD below the normative mean. The most severe lipid profile was in a participant with R255X (total cholesterol 246, LDL 169). No significant associations between lipid levels and activity level were found. A trend of increased LDL with higher calorie diet was found ($p=0.09$, $r=.51$). IGF-1 administration did not affect average lipid values.

Conclusions Dyslipidemia was prevalent in our RTT cohort, with greater than 50% demonstrating baseline LDL elevation. This finding was only weakly associated with diet, and was not associated with activity level, however sample size was small. If lipid values are concerning, repeat testing on a normal diet could be obtained. Dyslipidemia could contribute to the lifelong morbidity of girls with RTT, and lipid screening should be considered in all patients with RTT.

51. **Measuring Autism Symptoms and Diagnosis in Select Rare Genetic Diseases**

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Objective: This study examined autism spectrum disorder (ASD) in selected rare genetic disorders associated with intellectual disability and physical disabilities. Previous literature has indicated that ASD is common in a variety of rare genetic disorders, including a rate of 24% in Wilms Tumor Aniridia Genitourinary anomalies, and cognitive impairment (WAGR Syndrome) and rates between 53-71 % in Smith Lemli-Opitz syndrome (SLOS). Variable methodology has been used in prior studies to assess ASD. It is hypothesized that thorough research diagnostic evaluations utilizing parent interview and direct observation of autism symptoms will result in lower rates of ASD, compared to use of surveys or medical records, when clinical judgment classifications consider cognitive functioning, adaptive behavior, physical disabilities and sensory deficits.

Methods: Thirty individuals with WAGR Syndrome (age 2-28) and 19 individuals with SLOS (age 3-22 years) were evaluated through an autism screening protocol, which included administration of the Autism Diagnostic Interview-Revised, and the Autism Diagnostic Observation Schedule, tests of cognitive functioning and the Vineland Adaptive Behavior Scales, Second Edition. Assessments were altered for specific sensory and other deficits related to these syndromes, including use of a modified DSM-IV autism checklist for individuals with significant visual impairments.

Results: In participants with SLOS, 4 individuals were not mobile (21%), and 4 individuals had hearing impairments (21%), and in the WAGR group, 28 of 30 (93%) individuals had visual impairments. Cognitive functioning in the WAGR and SLOS groups was impaired at rates consistent to prior studies, which included 77% and 95% with estimated nonverbal IQ ≤ 70 in each group, respectively. In WAGR, 10% (n=3) were classified with an ASD diagnosis, and in SLOS 21% (n=4) were classified with ASD, rates lower than previously reported in these populations. In both groups, there were a significant number of individuals classified as non-spectrum that had been diagnosed with an ASD in the community.

Conclusions: In individuals with selected rare genetic disorders that include intellectual disability and various sensory deficits, measurement of ASD symptoms and diagnostic classification requires comprehensive diagnostic evaluation and specific considerations.

 52. **Novel Transporters for MPSI and MPSIIIa Enzyme Replacement Therapy**

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Objectives: Mucopolysaccharidoses (MPS) are inherited metabolic diseases caused by a deficiency in degradative enzymes that reside in lysosomes. A few of these disorders can be treated by enzyme replacement therapy. However, significant challenges remain because key organs and tissues remain inaccessible to intravenously injected enzymes. We are exploring the utility of guanidinoglycosides as carriers in cell culture and in vivo in the MPSI and MPSIIIa mice.

Methods: The approach is based on the ability of guanidinylated neomycin (GNeo) to deliver high molecular weight proteins across cell membranes and into lysosomes via cell surface heparan sulfate proteoglycans. Our studies suggest that GNeo is likely to enter the cell complexed to heparan sulfate chains and to localize in organelles where glycosaminoglycans are stored and metabolized making them ideal agents for enzyme replacement therapy. All cells highly express heparan sulfate proteoglycans, suggesting the potential utility of GNeo-conjugates for delivery to multiple tissues.

Results: Murine sulfamidase was expressed in CHO cells with good yield (0.5 mg to 1 mg/L) with low levels of mannose-6-phosphate determined by hydrolysis and chromatography. The high uptake form of iduronidase (Aldurazyme) was kindly provided by Genzyme. Conditions were obtained to remove mannose-6-phosphate recognition using alkaline phosphatase before conjugation to GNeo. Conjugation conditions were optimized with an N-hydroxysuccinimide derivative of guanidinylated neomycin containing different linkers. The conversion rate was ~90% leading to the overall yield of 60 to 80% based on enzyme activity. The ability of the conjugated enzymes to restore enzyme activity and their capacity to degrade glycosaminoglycans in human dermal fibroblasts from MPSIIIa and MPSI was established. Single dose studies demonstrate favorable biodistribution of sulfamidase in MPSIIIa mice and clearance of stored material in the liver and other organs.

Conclusions: Optimization of conjugation furnished conjugated enzymes without compromising enzyme activity, as validated by biochemical and cell assays. Adequate amounts of modified enzymes have been obtained for *in vivo* studies. Dose escalation studies are underway to determine the efficacy of conjugated enzyme delivery to tissues.

53. Etanercept Treatment Ameliorates the Autoinflammation in SH3BP2 Cherubism Mice

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Objective: Cherubism is a genetic disorder caused by a gain-of-function mutation in SH3BP2, which involves jaw bone destruction. We had previously created a mouse model for cherubism and demonstrated that homozygous mutant mice show TNF-dependent, but T/B cell-independent systemic macrophage inflammation leading to bone erosion and joint destruction. The critical role of TNF in this autoinflammation was confirmed by creating TNF-deficient homozygous cherubism mutants, in which systemic inflammation and bone destruction was dramatically rescued. Therefore, we hypothesized that postnatal administration of anti-TNF drug might also ameliorate the disease progression in cherubism mice.

Methods: Homozygous cherubism mice were injected with anti-TNF drug, Etanercept, which is currently used for the treatment of inflammatory disorders such as rheumatoid arthritis. Etanercept was subcutaneously injected twice a week into homozygous mutant and wild-type control mice starting from 6-11 days of age for 6.5 weeks. Homozygous mice were separated into 3 groups: low (0.5mg/kg) and high (25mg/kg) dose Etanercept and a PBS vehicle control group for comparison with wild-type mice. Histological analysis by H&E staining was performed on liver, lung, and stomach tissues to evaluate the anti-inflammatory effect of Etanercept treatment. Quantitative PCR analysis for TNF expression in the liver was performed. For the evaluation of inflammatory joint destruction, microCT was used.

Results: Homozygotes treated with the high dose Etanercept showed greatly decreased systemic inflammation and joint destruction, while no significant effect was observed in the low dose and vehicle treated groups. Expression level of TNF mRNA in liver tissue of homozygotes was decreased in the high dose (7.3 ± 1.1 -fold increase to wild-type, $n=3$) compared with low dose (25.8 ± 16.2 , $n=3$) and vehicle treatment (28.5 ± 12.4 , $n=4$) groups. Consistent with this observation, the high dose treatment group showed a significant decrease in liver inflammatory area ($0.1 \pm 0.1\%$, $n=7$), while the low dose ($9.9 \pm 7.2\%$, $n=6$) was comparable to the vehicle control group ($10.6 \pm 5.0\%$, $n=6$). Bone and joint destruction in the high dose group was rescued to the level of the wild-type control group. Decreased inflammation was also observed in other tissues including facial skin and lung from the high dose group.

Conclusions: Postnatal administration of high dose Etanercept significantly ameliorated the progression of inflammation and bone destruction in homozygous cherubism mice. These results provide evidence that human cherubism patients might benefit from anti-TNF therapeutics.

54. Lysosomal Gangliosidosis Longitudinal Study, and Pilot Studies of Combination Therapy with FDA-approved, Oral Medications

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Background: Ganglioside accumulation has been implicated in the mechanisms of central nervous system (CNS) neurodegeneration in several lysosomal diseases including Tay-Sachs disease, GM1-gangliosidosis and even the mucopolysaccharidoses. Miglustat, an inhibitor of glucosylceramide synthase in the glycosphingolipid pathway, has yielded inadequate results as a monotherapy in the gangliosidoses, with no benefit observed in most cases. Microglial activation with release of inflammatory mediators, is increasingly recognized as an important aspect of ganglioside-associated CNS damage in these conditions.

Hypothesis: The AIM protocol, a combination therapy for the gangliosidoses that targets glycosphingolipid precursor accumulation while simultaneously targeting microglial activation and downstream inflammatory processes, may provide a more comprehensive treatment strategy intended to slow and/or stabilizing disease progression of the gangliosidoses.

Materials and methods: The AIM protocol includes miglustat and when appropriate, pyrimethamine to reduce ganglioside accumulation, and minocycline, ibuprofen, and N-acetylcysteine to reduce CNS inflammatory processes. The agents are administered orally. Outcome monitoring at baseline, 3 months, 6 months, 9 months, 12 months, and then at least annually thereafter, includes volumetric MRI, neurocognitive testing, neurological exam, measurement of serum and CSF inflammatory markers, leukocyte enzyme activity, and sedated and awake ophthalmology exams. A retrospective and prospective study is assessing the safety and efficacy of this approach.

Results: To date, 7 patients have started AIM therapy: After one year of AIM treatment, a patient with juvenile Tay-Sachs disease showed overall gains in language, motor skills and adaptive behavior skills. The other 6 patients being treated with the AIM protocol are in the process of completing titration to the full dose on the AIM medications (titration takes approximately 6-12 months). Potential drug toxicities have been monitored closely, and prevented or abated with appropriate interventions. Available AIM results, including serum and CSF inflammatory markers will be reported.

Conclusions: The AIM protocol has demonstrated improvement in juvenile Tay-Sachs disease and holds promise as a multi-targeted oral therapy for the gangliosidoses and related conditions.

55. **Diagnosis of Fragile X Syndrome Via Analysis of Family History**
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Objective: Fragile X syndrome (FXS) is the most common inherited form of intellectual disability with a prevalence of 1 in 4,000 males and 1 in 8,000 females. As an inherited condition, the fragile X mutation has critical consequences to the proband and family members at all levels in the generations as they may be premutation carriers and at risk for fragile X associated disorders (e.g., primary ovarian insufficiency or fragile X-associated tremor ataxia syndrome). We reviewed the fragile X pedigrees from families at our Center to determine the average number of family members diagnosed as having full mutations or premutations following the diagnosis of a proband.

Methods: The Fragile X pedigree review found 176 probands, including 108 males (61%) and 68 females (39%). The probands had a total of 785 diagnosed family members, including 278 who were hemizygote (35%) and 507 who were heterozygote (65%). 227 (29%) of the family members had full mutation FXS and 558 (71%) were premutation carriers.

Results: After the diagnosis of a proband with FXS, there are, on average, at least 5 family members who are diagnosed with the condition. While males tend to be diagnosed in large numbers initially, more females are identified during subsequent analysis of the family history and pedigrees.

Conclusions: These findings emphasize the need for all health care professionals to ensure that a detailed family history is constructed for each patient affected by FXS and to encourage testing and evaluation of all at-risk family members. Identification of a proband's affected family members brings affected individuals to early treatment and decreases the diagnostic odyssey of symptomatic relatives who are being evaluated without the benefit of family history. One of the study's limitations is the possible refusal of affected family members to be evaluated. Therefore, our finding of 5 affected relatives per family represents a minimum number of individuals expected to be found in a pedigree analysis.

56. **Profiling Human Urinary mRNA.**

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Objective: Depletion of podocytes drives the progression process in animal model systems and human glomerular diseases. In animal models, increased urine podocyte specific product measurements, such as podocin and nephrin, are associated with both acute glomerular injury and progression. The long term objective of the proposed research is to determine whether or not the urine mRNA biomarker methodology can be of potential use in patients with glomerular diseases to both understand the biology of glomerular diseases as well as to non-invasively identify and monitor podocyte stress, loss and response to therapy. Initially, the proposed methodology to be evaluated on the cohort of patients established at the University of Michigan. Then the urinary markers will be validated on well-phenotyped Nephrotic Syndrome Study Network (NEPTUNE) cohort. Incorporation of large NEPTUNE cohort will allow validation of the proposed methodology and improve statistical power to provide the information necessary to determine how the urine mRNA biomarker panel could be optimally deployed for clinical decision-making.

Methods: Using insights developed from the model systems we compared 682 urine samples *from adult and pediatric nephrology clinics* to 110 *control samples*. Spot urine samples were collected, processed for mRNA purification, reverse transcribed and assayed using the TaqMan system for podocin, nephrin, aquaporin2 and TGFb1 mRNA, protein and creatinine concentrations. A two sample comparison was used to analyze urine podocin:creatinine ratio and urine protein:creatinine ratio of clinic samples vs controls.

Results: Compared to controls, clinical samples had elevated urine protein:creatinine ratio ($P=3 \times 10^{-21}$) and elevated urine podocin:creatinine ratio ($P=3.6 \times 10^{-33}$). Podocin:nephrin mRNA ratio was increased in clinic samples compared to control samples ($P<0.001$). Urine podocin:creatinine ratio did not correlate highly with the urine protein:creatinine ratio ($r^2=0.12$), thereby demonstrating that urine podocin mRNA provides additional information.

Conclusion: Urine mRNA biomarkers can provide clinical information complimentary to proteinuria. Longitudinal analysis of phenotypically well-characterized patients will be required to establish the potential utility of urine mRNA biomarkers for clinical decision-making within defined settings.