



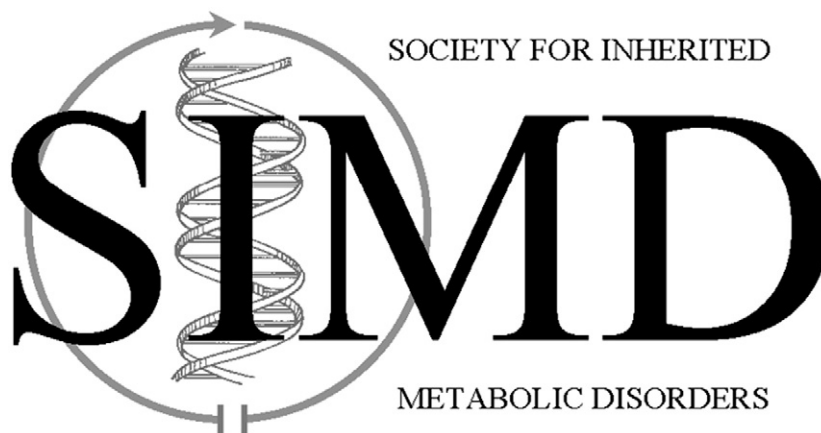
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Abstracts



**Society for Inherited Metabolic Disorders
39th Annual Meeting**

**Sunday, April 3, 2016–Wednesday, April 6, 2016
Sawgrass Marriott Golf Resort and Spa
Ponte Vedra Beach, Florida**

Program and Abstracts

ABSTRACTS

Invited Speakers

Name

Georgianne Arnold, M.D.
 Penelope Bonnen, Ph.D.
 Kim Chapman, M.D., Ph.D.
 Marni Falk, M.D.*
 Juan Francisco Cabello, M.D.
 Cary Harding, M.D.

 Sean Hofherr, Ph.D., FACMG*

 Alex Kemper, M.D., M.P.H., M.S.

 Priya Kishnani, M.D.
 Irini Manoli, M.D., Ph.D.*
 Mike Milburn, Ph.D.
 William Rizzo, M.D.*
 Wendy Smith, M.D.
 Peter Stacpoole, Ph.D., M.D.
 Kevin Strauss, M.D.
 Marshall Summar, M.D.

 Mendel Tuchman, M.D.*
 Gerard Vockley, M.D., Ph.D.*
 Melissa Wasserstein
 Saskia Wortmann, M.D., Ph.D.

*Abstract submitted

Title of abstract

Eminence vs Evidence Based Medicine
 Metabolomics in molecular diagnostics, matched metabolomics-exome data
 Human hepatocyte biochemistry in a differentiated stable system
 Nutrition, dietary supplements and medical food therapy in mitochondrial diseases
 Building infrastructure for IEM in Latin
 First presentation of pivotal PRISM 302 data: A phase 3, randomized, double-blind clinical trial evaluating efficacy and safety of pegvaliase for the treatment of adults with PKU
 Considerations in next-generation sequencing applications in newborn screening: supporting, supplementing, and expanding current approaches
 Making Decisions: The Role of Condition Review for the Advisory Committee on Heritable Disorders in Newborns and Children
 Diet therapy in GSD
 Rethinking the role of medical food in MMA
 METABOLON, Inc., Research Triangle Park, NC
 What's new in the STAIR Consortium
 Evidence Based Protocols in practice, the PKU experience
 Congenital and acquired disorders of the pyruvate dehydrogenase complex
 Medical food and the brain
 The SIMD and Newborn Screening for LSD
 and
 Consensus based Protocols as Evolving Tools, The Urea Cycle Disorders Experience
 Hyperammonemia: new surprises from research into an old problem
 INFORM – The Linkage Between Fatty Acid Oxidation, Mitochondrial Dysfunction and Disease
 Clinical Outcomes of Infants with Abnormal Newborn Screens for Krabbe Disease in New York State
 Causes of 3-methylglutaconic aciduria

ABSTRACTS:

Nutrition, Dietary Supplements, and Medical Food Therapy in Mitochondrial Diseases

Marni J. Falk¹

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Mitochondrial diseases are a collectively common but notoriously heterogeneous group of inherited metabolic disorders at the genetic, biochemical, and clinical levels. The central problem in respiratory chain disorders is impaired energy production, with secondary adaptations occurring throughout the cell that substantially alter diverse aspects of nutrient processing and intermediary metabolism. Limited knowledge, with no therapeutic guidelines, currently exists regarding the use of dietary modification to improve the health of patients with mitochondrial respiratory chain disease. Furthermore, the clinical implementation of either macromolecular nutrient modification or dietary supplements has been highly variable and poorly studied. Understanding how to provide proper nutrition and effectively use nutritional supplements in mitochondrial disease is an area of active regulatory interest and community activity. Indeed, these were central topics discussed in the Critical Path Innovation Meeting on Mitochondrial Disease Clinical Trial Development that was held at the Food and Drug Administration in October 2015. We will review the basic alterations in macronutrient processing and nutrient-sensing signaling pathways that occur in mitochondrial disease. Animal model data from *C. elegans*, zebrafish, and mouse models of primary respiratory chain disease will be discussed that highlight the potential therapeutic value in mitochondrial disease of low-fat, high glucose diets. Further, animal model data suggests that significant health benefit in these disorders may result from use of several dietary supplements, such as specific chocolate-derived compounds, vitamin co-factors, and fatty acid molecules that are now being developed. Thus, while primary mitochondrial disease predictably disrupts key aspects of basic cellular metabolism, it is highly likely that systematic study of nutritional therapies that are tailored to restore cellular physiology will enable the identification of therapeutic regimens that improve health in patients with mitochondrial respiratory chain disease.

CONSIDERATIONS IN NEXT-GENERATION SEQUENCING APPLICATIONS IN NEWBORN SCREENING: SUPPORTING, SUPPLEMENTING, AND EXPANDING CURRENT APPROACHES

Sean Hofherr¹, Sarah Viall², Mary Beth Seprish¹, and Kristina Cusmano-Ozog^{1,2}

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Background: Newborn screening (NBS) in the United States started in the mid-1960s in an effort led by Robert Guthrie (Guthrie and Susi, 1963). Originally, the test was for a single disease, phenylketonuria (PKU), and it utilized a bacterial inhibition assay (BIA). The BIA test was fast, cheap, and could be performed on a large scale using filter paper dried blood spots. Over the years, through the advent of clinical mass spectrometry many

additional disorders have been screened for in the US. In 2008, congress passed the Newborn Screening Saves Lives Act, which mandated all states to screen for a minimum of 29 conditions, and recommended the screening of an additional 20 secondary conditions (Green et al., 2007). Since that point, there have been improvements to newborn screening, including second tier testing (Matern et al., 2007; Turgeon et al., 2008), and some “big data” analysis (Hall et al., 2014; McHugh et al., 2011), both of which have increased the positive predictive value, but overall NBS is still using the same technology. In this work we explore the potentials and pitfalls of leveraging next-generation sequencing (NGS) applications in NBS to support, supplement, and expand current biochemical approaches.

Methods: There are many different approaches to leverage next-generation sequencing applications in newborn screening, and it is essential that these approaches be evaluated in the context of public health screening, benefit to patient, cost efficacy, and technical capabilities. As a quaternary pediatric hospital, Children’s National Medical Center has a large population of patients regularly followed up for an abnormal NBS in DC, Maryland, and Virginia with NGS in our diagnostic laboratory. Each patient with an abnormal NBS is sequenced for a medical exome (4813 clinically relevant genes), and using bioinformatic filters we analyze variants only in clinically relevant genes. It is through this experience that we have studied the potential use of this technology in newborn screening to support, supplement, and expand current biochemical approaches.

Results: We have used our clinical NGS program and its clinical application in sequencing all patients with abnormal newborn screening that are referred to our institution to evaluate the benefits of each approach of sequencing in NBS.

Conclusions: It is essential to study and evaluate the potential of using next-generation sequencing in newborn screening. Using our clinical NGS program data and our large abnormal NBS patient population we explore the different approaches to utilize NGS to support, supplement, and expand current newborn screening.

RETHINKING THE ROLE OF MEDICAL FOODS IN METHYLMALONIC ACIDEMIA

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Organic Acid Research Section, Medical Genomics and Metabolic Genetics Branch, NHGRI, NIH, Bethesda, MD

Background: Unlike medical foods for phenylketonuria, where multiple studies support their efficacy in preventing long-term complications of the disorder, formulations developed for methylmalonic and propionic acidemias have been based on limited and contradicting data. These products are deficient in a number of amino acids, yet contain an arbitrarily high amount of leucine. Leucine administration is known to cause depletion of plasma valine and isoleucine concentrations, competition in the uptake and tissue distribution of large neutral amino acids through their respective transport systems, and a multitude of other metabolic effects that have not been specifically examined in developing dietary guidelines for this patient population. Moreover, there is no theoretical rationale or experimental support for the practice of administering methionine-depleted medical foods to patients with *cbIc* deficiency.

Methods: A large and diverse cohort of 61 patients with isolated MMA (46 *mut*, 9 *cbIa* and 6 *cbIb*; age range 2.5 to 35 years) and 28 early onset *cbIc* patients (2–27y of age) were studied under a natural history protocol (clinicaltrials.gov ID: NCT00078078). Branched chain amino acid intakes from complete and incomplete protein sources (medical foods) were correlated with biochemical parameters, anthropometric measurements and body composition.

Results: Patients with isolated MMA had poor growth outcomes, with significantly low height and head circumference Z-scores and a high incidence of obesity. The majority received medical foods, with total protein intake reaching twice the RDA for protein and leucine administration reaching 5 times the daily-recommended intake. A number of patients required supplementation of propiogenic amino acids (valine and isoleucine) despite adequate complete protein intake. Plasma valine and isoleucine concentrations were strongly correlated with the consumption of medical foods. In *cbIc* patients protein restriction or use of medical foods was associated with lower plasma concentrations and predicted brain influx of methionine and were correlated with worse height and head circumference z-scores.

Conclusions: Distorted branched chain amino acid composition of MMA diets results in iatrogenic plasma amino acid deficiencies, complicating the management of MMA patients and possibly contributing to poor growth outcomes. Methionine restriction and the risk of leucine competition for methionine uptake to the brain render MMA/PA formulas unsuitable for *cbIc* patients. Our observations raise important concerns that should help design better medical foods for the dietary management of this group of disorders.

What’s New in the STAIR Consortium?

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The Sterol and Isoprenoid Research (STAIR) Consortium is a member of the Rare Diseases Clinical Research Network (RDCRN) and is funded by the National Center for Advancing Translational Science and Eunice Kennedy Shriver National Institute of Child Health & Human Development. STAIR provides a framework for conducting clinical studies on diseases of sterol and isoprenoid metabolism and training new investigators in rare disease research. STAIR consists of research sites located around the country and abroad. A centralized Data Management Coordinating Center collects clinical data and provides statistical support, along with managing a Patient Contact Registry and website (<http://www.rarediseasesnetwork.org/cms/stair>). The close involvement of patient advocacy groups is critical for recruiting subjects, assisting with study design and supporting STAIR efforts to advance knowledge of these diseases. Clinical studies include longitudinal natural history investigations and shorter pilot studies on disease characterization, therapy and biomarker discovery. Most STAIR diseases are considered ultra rare and few centers follow enough STAIR patients to conduct

clinical studies on their own. Current or past investigations have focused on Smith-Lemli-Opitz syndrome, Sjögren-Larsson syndrome, sitosterolemia, and mevalonate kinase deficiency/hyper-IgD syndrome. Additional studies on disorders of dolichol metabolism and methylsterol oxidase deficiency are expected to begin later this year. To encourage the next generation of researchers, STAIR supports post-doctoral trainees and/or junior faculty to become proficient in clinical research on these inborn errors of metabolism. New efforts of STAIR include formation of a central IRB to lessen the administrative burden of studies, recruit new clinical sites, sponsor patient/investigator conferences, and develop informal open discussion groups on STAIR diseases. The STAIR Consortium, and others within the RDCRN, is a critical

HYPERAMMONEMIA: NEW SURPRISES FROM RESEARCH INTO AN OLD PROBLEM

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Background: Hyperammonemia that results from inherited metabolic disorders, liver failure, extreme catabolic stress, complication of major surgeries or chemical toxicity, remains a major medical challenge. However, continuous progress is being made in diagnosis, better understanding and treatment. I will highlight new discoveries related to hyperammonemia both from basic, translational and clinical research by our and other groups.

Methods: The findings presented in this talk include those derived from catalytic, structural and thermal studies of NAGS and CPS1, characterization of a new animal models for hyperammonemia, molecular and stable isotope investigations of unique patients with hyperammonemia and clinical trials and natural history studies.

Results: The following finding will be discussed: [¹³C]urea labeling studies can predict response to drugs such as N-carbamylglutamate or other drugs affecting ammonia load. N-carbamylglutamate has been identified as a protein chaperon in a patient with CPS1 deficiency. Zebrafish and the rescued NAGS knockout mouse are useful hyperammonemia models suitable for neuroprotection drug screening. A new identified inborn error, carbonic anhydrase 5A deficiency can result in a urea cycle defect and hyperammonemia. A substantial proportion of patients with urea cycle disorders have mutations in regulatory regions which are not included in clinical DNA testing. Blood ammonia level seems to be a better marker of metabolic stability than glutamine level.

Conclusions: Since some patients with recurrent hyperammonemia remain undiagnosed in spite of exhaustive metabolic and molecular studies, additional inherited disorders causing hyperammonemia may remain to be discovered. Alternatively, some of these patients may have mutations in the regulatory regions of urea cycle genes. The availability of Zebrafish for testing of hyperammonemia in combination with a reliable hyperammonemia mouse model allows for neuroprotection high-throughput drug screening. Stable isotope dilution in vivo testing can help assess the effect of drugs that augment ureagenesis and can help to select effective treatment in individual patients.

INFORM: THE LINKAGE BETWEEN FATTY ACID OXIDATION, MITOCHONDRIAL DYSFUNCTION, AND DISEASE

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Three major energy pathways intersect in mitochondria: fatty acid β -oxidation, the tricarboxylic acid (TCA) cycle, and oxidative phosphorylation. The first two pathways provide reducing equivalents to drive ATP production from the first, as well as key metabolic intermediates for other cellular processes. Inborn errors of mitochondrial fatty acid oxidation are among the most frequent now identified through expanded newborn screening by tandem mass spectrometry. INFORM (the International Network for Fatty Acid Oxidation Research and Management) provides a collaborative framework for clinicians and basic scientists to exchange information on disorders of fatty acid oxidation and their global effect on metabolism. The network has met at SIMD each of the past 4 years and has sponsored an international symposium for the past 2 years. This presentation will review current INFORM projects, detail upcoming clinical trials, and discuss novel aspects of the pathophysiology of fatty acid oxidation disorders.

Abstracts of Short Oral Presentations Travel Award Recipients and SIMD Hyperion Fellow

ORAL PRESENTATIONS

Oscar Aubi
Jirair K. Bedoyan, M.D., Ph.D.

Sean Froese, Ph.D.
Young Joon (Fred) Kwon
Lisa Pan, M.D.
Zarazuela Zolkipli Cunningham,
M.B., Ch.B., MRCP
Didem Demirbas, Ph.D.
SIMD Hyperion Fellow

TRAVEL AWARD WINNERS

Phenylalanine hydroxylase screening. Pharmacological chaperones for phenylketonuria treatment
Diagnosis of genetically unresolved subjects with functional pyruvate dehydrogenase complex deficiencies using advanced genomic technologies with functional confirmation
Structural insights into the MMACHC–MMADHC protein complex involved in vitamin B12 trafficking
Flunarizine rescues short lifespan of CLN3 knockout *Caenorhabditis elegans* model of Batten Disease
Neurometabolic disorders: potentially treatable abnormalities in patients with treatment refractory depression and suicidal behavior
The diagnostic utility of aerobic exercise testing in Mitochondrial Myopathies
iPS cell-based modeling of galactosemia

PHENYLALANINE HYDROXYLASE SCREENING. PHARMACOLOGICAL CHAPERONES FOR PHENYLKETONURIA TREATMENT

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Background and Objectives: Phenylketonuria (PKU) is the most common inborn error of metabolism, caused by mutations in the gene encoding phenylalanine hydroxylase (PAH), resulting in increased phenylalanine levels in blood and toxic levels in brain. Owing to the fact that the two current treatments of PKU, namely a strict phenylalanine diet and the administration of Kuvan® present some pitfalls (neurodevelopmental or psychosocial problems and low responsiveness, respectively), new therapeutic strategies are accordingly needed [1]. The use of small molecular weight compounds that selectively bind to misfolded proteins and recover a native structure and function (at least partially) has proven to be successful in different diseases (e.g. [2]). For the particular case of PKU, the majority of the mutations in the enzyme PAH lead to misfolding. Hence, pharmacological chaperones -acquired name for these molecules- could potentially be applied as therapeutic agents to correct this defect.

We thus initiated a screening searching for stabilizers of PAH that could be developed into pharmacological chaperones for PKU treatment.

Materials and Methods: The screening consisted of a sequential multi-staged workflow comprising an initial high-throughput screening (HTS) of a commercial 10,000-compounds library by differential scanning fluorimetry assay, a validation of the binding through surface plasmon resonance and an efficacy assessment in cultured cells constitutively expressing PAH, both in terms of specific activity and amount of protein. Subsequently, a further characterization of the mode and thermodynamics of the binding was performed.

Results and Conclusions: We obtained a total of 109 positive compounds in the HTS after removal of promiscuous binders which represent the 1.09% of the entire library. In the aforementioned following stages, the number was reduced down to 2 candidates and the characterization phase resulted in the selection of our final hit compound. This compound displayed good affinity ($K_D \sim 10 \mu\text{M}$), enthalpically driven binding and excellent scores in cultured cells with an increase of $70.3 \pm 9.7\%$ in PAH activity and $12.9 \pm 1.5\%$ in PAH amount. Therefore, it is envisioned to be used as a pharmacological chaperone for PKU after future derivatization.

1. Blau, N. and N. Longo, Alternative therapies to address the unmet medical needs of patients with phenylketonuria. *Expert Opin Pharmacother*, 2015. 16(6): p. 791–800.
2. Wang, Y., et al., Specific rescue of cystic fibrosis transmembrane conductance regulator processing mutants using pharmacological chaperones. *Mol Pharmacol*, 2006. 70(1): p. 297–302.

DIAGNOSIS OF GENETICALLY UNRESOLVED SUBJECTS WITH FUNCTIONAL PYRUVATE DEHYDROGENASE COMPLEX DEFICIENCIES USING ADVANCED GENOMIC TECHNOLOGIES WITH FUNCTIONAL CONFIRMATION

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Background: Pyruvate dehydrogenase complex (PDC) deficiencies are a major subgroup of mitochondrial disorders associated with a high incidence of major neurological morbidity and mortality. The genetic etiology for about a third of PDC-deficient subjects remains unknown after primary genetic testing in several experienced diagnostic referral laboratories. These subjects may have different causes, inheritance mechanisms and outcomes, and benefit from different diagnostic tests, counseling, and treatment options. We used advanced genomic technologies including a 23-gene next generation sequencing (NGS) panel and whole exome sequencing (WES) along with functional confirmatory strategies to assign genetic causality for some of our genetically unresolved PDC-deficient subjects.

Methods: We employed a clinically validated 23-gene NGS panel for pyruvate-metabolism disorders that covers the coding sequences of PDHA1, PDHB, DLAT, DLD, PDHX, PDP1, PDP2, PDK1, PDK2, PDK3, PDK4, SLC19A2, SLC19A3, SLC25A19, TPK1, LIAS, LIPT1, LIPT2, BOLA3, NFU1, PCB, PCK1, and PCK2, and a WES pipeline that employs HiSeq 2500, CLC Genomics Workbench and Illumina Variant Studio along with an additional arm to the pipeline that includes Omicia VAAST and Phevor, to identify candidate variants and ranked candidate genes for subsequent functional confirmation. Our strategies for functional confirmation of novel variants included identifying metabolites/biomarkers in metabolic pathways being perturbed, Western analyses, enzyme assays and kinetics analysis, and using a lentivirus-based expression vector to rescue the mutant functional phenotype as well as CRISPR/Cas9 to generate targeted candidate gene-specific mutation(s) in a cell line with subsequent assay for functional PDC deficiency thus implicating the pathogenicity of the candidate variant(s).

Results: Our approach identified novel variants in genes not previously known to cause secondary PDC deficiencies such as succinyl-CoA lyase A2 (SUCLA2) and mitochondrial phosphoenolpyruvate carboxykinase (PCK2), as well as known and novel exonic and intronic PDHA1 pathogenic variants. Subjects with the SUCLA2 and PCK2 mutations shared some clinical/metabolic phenotypes with subjects with known catalytic or regulatory genes encoding PDC, but also exhibited some unique clinical/metabolic characteristics.

Conclusions: We describe a multifaceted strategy using advanced genomic technologies and generation of targeted mutation(s) in genes by CRISPR/Cas9 to demonstrate the utility of our approach to retrospectively and prospectively identify the genetic etiology of PDC-deficient subjects not yet molecularly resolved. Our discovery of new genes associated with PDC deficiency has expanded the known genetic and metabolic etiologies and clinical manifestations of these disorders. Our work will help with the design and development of future clinical trials and in the selection of relevant subgroups of PDC-deficient subjects for specific therapeutic interventions.

STRUCTURAL INSIGHTS INTO THE MMACHC–MMADHC PROTEIN COMPLEX INVOLVED IN VITAMIN B12 TRAFFICKING

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Conversion of vitamin B12 (cobalamin, Cbl) into the cofactor forms methyl-Cbl (MeCbl) and adenosyl-Cbl (AdoCbl) is required for the function of two crucial enzymes, mitochondrial methylmalonyl-CoA mutase and cytosolic methionine synthase, respectively. The intracellular proteins MMACHC and MMADHC play important roles in processing and targeting the Cbl cofactor to its destination enzymes, and recent evidence suggests that they may interact while performing these essential trafficking functions. To better understand the molecular basis of this interaction, we have mapped crucial protein regions required, indicate that Cbl is likely processed by MMACHC prior to interaction with MMADHC, and identify patient mutations on both proteins that interfere with complex formation, via different mechanisms. We further report the crystal structure of MMADHC C-terminal region at 2.2 Å resolution, revealing a modified nitroreductase fold with surprising homology to MMACHC despite their poor sequence conservation. Since MMADHC demonstrates no known enzymatic activity, we propose it as the first protein known to repurpose the nitroreductase fold solely for protein–protein interaction. Using small angle X-ray scattering we reveal the MMACHC–MMADHC complex as a 1:1 heterodimer and provide a structural model of this interaction, where the interaction region overlaps with the MMACHC-Cbl binding site. Together, our findings provide novel structural evidence and mechanistic insight into an essential biological process, whereby an intracellular ‘trafficking chaperone’ highly specific for a trace element cofactor functions via protein–protein interaction, which is disrupted by inherited disease mutations.

FLUNARIZINE RESCUES SHORT LIFESPAN OF CLN3 KNOCKOUT CAENORHABDITIS ELEGANS MODEL OF BATTEN DISEASE

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Background: Juvenile neuronal ceroid lipofuscinoses (JNCL, also known as Spielmeier–Vogt–Sjogren–Batten) is a devastating pediatric neurodegenerative disorder caused by mutations in CLN3 for which there currently is no effective treatment. Progressive neuronal death occurs, which may be triggered by abnormal intracellular calcium levels leading to neuronal apoptosis. Batten disease also involves the lysosomal accumulation of subunit c of mitochondrial ATP synthase and other proteolipids. Previously, our research using calcium channel antagonists has demonstrated the reversal of calcium effect in a neuroblastoma cell line and primary rat neurons. We hypothesized that this treatment could improve survival in an animal model of CLN3.

Methods: C. elegans CLN3-KO worms (XT7 strain, harboring deletion mutations in three genes that function as homologues to CLN3) were studied relative to wild-type controls (N2 Bristol). Lifespan studies were conducted to test the most effective calcium channel antagonists identified in cellular studies (amlodipine, flunarizine, nifedipine, nimodipine; all at 1 μM) and identify the most effective dose range (flunarizine at

10 μ M, 1 μ M, 0.5 μ M, 0.1 μ M). In vivo mitochondrial physiology was studied by fluorescence-based relative quantitation of mitochondrial mass (MitoTracker Green FM), membrane potential (TMRE), and matrix superoxide burden (MitoSOX).

Results: XT7 was significantly short-lived at 20 °C relative to wild-type worms (median lifespan 8.4 vs 12.6 days, $p < 0.01$). XT7 worms also had significantly altered in vivo mitochondrial physiology, with 10.1% increased mitochondrial mass ($p < 0.05$) and 8.7% increased matrix superoxide levels ($p < 0.05$); their mitochondrial membrane potential was not significantly changed. XT7 worms treated weekly with 1 μ M flunarizine had a significantly increased lifespan (median lifespan 8.4 vs 10.4 days, $p < 0.05$). Evaluation of drug effects on the altered mitochondrial physiology of XT7 worms is underway.

Conclusion: *C. elegans* CLN3-KO worms (XT7 strain) show increased mitochondrial mass and reduced lifespan. Flunarizine treatment rescued their shortened lifespan at similar concentrations as the effective dose identified in neuronal cells. These data suggest that the XT7 strain is a valuable model organism in which to further investigate the pathophysiology and novel therapeutic interventions for Batten disease.

NEUROMETABOLIC DISORDERS: POTENTIALLY TREATABLE ABNORMALITIES IN PATIENTS WITH TREATMENT REFRACTORY DEPRESSION AND SUICIDAL BEHAVIOR

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Background: Treatment refractory depression (TR-MDD) is a devastating condition with significant morbidity, mortality, and societal cost. At least 15% of major depressive disorder cases remain refractory to currently available treatments. We identified a young adult with TR-MDD and multiple suicide attempts with an associated severe deficiency of cerebrospinal fluid (CSF) tetrahydrobiopterin, a critical cofactor for monoamine neurotransmitter synthesis. Treatment with the tetrahydrobiopterin analogue sapropterin led to dramatic and long lasting remission of depression. This sentinel case led us to hypothesize that the incidence of metabolic abnormalities contributing to TRMDD is under-recognized.

Methods: We conducted a case-controlled, targeted, metabolomic evaluation of 31 adolescent and young adult patients with well-characterized history of depression refractory to at least three maximum-dose, adequate-duration medication treatments, and 17 healthy controls. Plasma, urine, and CSF metabolic profiling were performed by coupled gas chromatography/mass spectrometry, and high performance liquid chromatography, electrospray ionization, tandem mass spectrometry (LC-ESIMS/MS).

Results: Cerebral spinal fluid (CSF) metabolite abnormalities were identified in 19 of 31 TR-MDD participants. Cerebral folate deficiency ($n = 14$) was most common, with normal serum folate and low 5-methyltetrahydrofolate (5MTHF) in CSF. All patients with cerebral folate deficiency, including one patient with low 5MTHF and low tetrahydrobiopterin intermediates in CSF, showed improvement in depression symptom inventories after treatment with folinic acid and sapropterin, respectively. No healthy controls were found to have a metabolite abnormality.

Conclusions: Examination of the role of metabolic disorders in severe, TR-MDD identified an unexpectedly large proportion of patients with potentially treatable abnormalities. These disorders appear to be under-diagnosed, and identification with appropriate intervention could result in life-changing and life-saving treatment. The etiology of these abnormalities remains to be determined.

THE DIAGNOSTIC UTILITY OF AEROBIC EXERCISE TESTING IN MITOCHONDRIAL MYOPATHIES

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Background: Exercise intolerance (EI) is one of the most common symptoms in patients with mitochondrial myopathies (MM). EI results from impaired muscle oxidative phosphorylation (oxphos) capacity, where an exaggerated circulatory and ventilatory response to exercise exceeds the capacity of skeletal muscle to utilize the increased oxygen delivered (Haller and Bertocci, 1994). Exercise testing provides a useful adjunct to quantify EI in the clinical evaluation of MM. Specifically, impaired oxphos capacity manifests as decreased maximal oxygen consumption (VO₂max), increased delivery of oxygen to the muscle relative to its extraction, and disproportionately increased ventilation and carbon dioxide production for a given workload (Tanopolysky, 2004; Taivassalo et al., 2003). Other factors that could contribute to EI in MM should be excluded, such as cardiomyopathy or arrhythmia. Here, we describe results of exercise testing from MM patients with genetically-confirmed and suspected mitochondrial diseases and symptoms of EI as compared to patients with cardiomyopathy from other etiologies (CM) and healthy controls.

Methods: Aerobic exercise evaluation was conducted using cycle ergometry at The Children's Hospital of Philadelphia. Male and female patients with subjectively reported EI having genetically-confirmed MM ($n = 2$, BCORL1 and m.3288A>G), 'probable' or 'unlikely' mitochondrial disease based on clinical phenotype alone ($n = 2$ each, mean age 20.5 ± 10.6 years), and patients with cardiomyopathy ($n = 14$, mean age 14.4 ± 3.5) were compared with healthy control data.

Results: Heart rate, oxygen saturation, and blood pressure were normal in all subjects. Peak work in patients with definite (1.05 ± 0.2 W/kg) or probable (1.6 ± 0.6 W/kg) mitochondrial disease was significantly lower compared to subjects with unlikely mitochondrial disease (2.6 ± 0.15 W/kg), cardiomyopathy (2.4 ± 0.19 W/kg) and controls (3.0 ± 0.5 W/kg, mean \pm SD).

Conclusion: Reduced VO₂max is one of the most common findings in MM patients with EI. However, other causes for a low VO₂max include cardiovascular and respiratory disease. Our results indicate that the VO₂ max and peak work capacity in patients with definite or probable mitochondrial disease are significantly lower than those with cardiomyopathy. We conclude that exercise testing offers a reliable way to quantify EI in MM, with results used to both aid in diagnosis as well as monitoring over time and in response to treatment interventions.

IPS CELL-BASED MODELING OF CLASSIC GALACTOSEMIA

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Background: Classic galactosemia is an inborn error of carbohydrate metabolism that is caused by absent or barely detectable activity of the galactose-1-phosphate uridylyltransferase (GALT) enzyme. Long-term complications of classic galactosemia include cognitive deficits, language delays, speech defects, tremors, ataxia, dystonia and premature ovarian insufficiency. Despite years of research, the reasons behind the pathophysiology of galactosemia in central nervous system have still remained mainly unexplored. We aim to model classic galactosemia by generating induced pluripotent stem (iPS) cells from patients and differentiating them and their CRISPR corrected control lines into neurons to study the molecular mechanisms of galactose-1-phosphate uridylyltransferase (GALT) deficiency.

Methods: Cells from three classic galactosemia patients with Q188R/Q188R GALT genotype (two fibroblast cell lines and CD34⁺ cells) along with one fibroblast line with Q188R/Q188P genotype were reprogrammed into pluripotent stem cells using episomal vectors. iPS cells derived from normal fibroblasts as well as a pathological fibroblast line with phospholipase C beta deletion were used as controls. These iPS cells were differentiated into early stage neurons using monolayer based dual SMAD inhibition. Galactose metabolites in iPS cells and neurons were quantified with LS-MS/MS after methanol extraction.

Results: Galactosemic iPS cells accumulated large quantities of galactose metabolites but not UDPgalactose upon galactose exposure. Additionally, differentiated galactosemic neurons, which were grown in neuronal media containing B27 supplement and approximately 20 μ M of galactose, had lower UDPgalactose levels compared to wild-type counterparts. Studies to investigate the effect of this UDPsugar imbalance on the N- and O-linked glycosylation patterns of control and galactosemic iPS cells and neurons are currently underway.

Conclusions: iPS cell-based modeling of galactosemia may serve as a valuable tool to help us better understand the mechanisms of brain disease observed in classic galactosemia patients.

Oral presentations — Selected Abstracts of Broad Interest

To be presented Wednesday, April 6, 2016

These five abstracts were selected for oral presentation by the program committee from all abstracts submitted for poster presentation

Curtis Coughlin, MS, MBE
Matthew Olson, Ph.D.
Parthasarathy Sonaimuthu, Ph.D.
Ilona Tkachyova, M.Sc.
Yudong Wang, Ph.D.

The genotypic spectrum of classic nonketotic hyperglycinemia due to mutations in GLDC and AMT
Quantitation of acyl-coenzyme A and acyl-carnitine in primary human hepatocytes from propionic acidemia patients
Functional characterization of NAGS protein using gene therapy
Novel approach for the treatment of six mucopolysaccharidoses by targeting NDST1
Characterizing the molecular architecture of mitochondrial energy metabolism apparatus and its importance to clinical pathophysiology

THE GENOTYPIC SPECTRUM OF CLASSIC NONKETOTIC HYPERGLYCINEMIA DUE TO MUTATIONS IN GLDC AND AMT

Curtis Coughlin II¹, Michael Swanson¹, Kathryn Kronquist^{1,2}, Cecile Acquaviva³, Tim Hutchin⁴, Pilar Rodriguez-Pombo⁵, Shigeo Kure⁶, Marja-Leena Vaisanen⁷, Elaine Spector^{1,2}, GERALYN Creadon-Swindell^{1,2}, Ana Bras-Goldberg⁴, Elisa Rahikkala⁸, Jukka Moilanen⁸, Vincent Mahieu⁹, Gert Matthijs⁹, Irene Bravo-Alonso⁵, Celia Perez-Cerda⁵, Magdalena Ugarte⁵, Christine Vinaey-Saban³, Gunter Scharer^{1,10}, Johan Van Hove¹

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Classic nonketotic hyperglycinemia (NKH) is an autosomal recessive epileptic encephalopathy caused by mutations in either GLDC or AMT, encoding proteins of the glycine cleavage enzyme system. The phenotype is heterogeneous ranging from severe with profound developmental delay and intractable seizures, to attenuated with developmental delay, hyperactivity, behavioral problems, and chorea. We present a retrospective review from five international centers consisting of 578 unique families suspected of NKH. This comprehensive review reports 407 unique mutations including 243 novel mutations. 80% of subjects had mutations in GLDC, and missense mutations were noted in 52% of all GLDC disease causing alleles, the majority private. A missense mutation of an amino acid in the carboxy-terminal half was 1.5 times as likely to be pathogenic than in the amino-terminal half of the gene. Intragenic copy number variations (CNVs) in GLDC were noted in 140 subjects. Biallelic CNVs were present in 39 subjects including 4 subjects with non-overlapping CNVs. CNVs contributed 20% of all disease causing alleles in GLDC, with breakpoint hotspots prior to exon 1, and introns 2, 8, and 15 which correlates with intron size and number of repeat elements. In AMT, missense mutations were also the most common type of mutation with 67% of all disease causing alleles, the majority recurring. CNVs were not present in subjects with AMT mutations although four variants were noted in the 5 prime untranslated region between c.1-55 to c.1-66 suggesting the identification of a regulatory region for AMT. Four missense mutations had an ethnic enrichment. Sequencing and CNV analysis identified biallelic pathogenic mutations in 99% of subjects who completed testing. This data suggested a minimum incidence of NKH at 1:76,000 live births and that, based upon genotype, approximately 15% of individuals would have an attenuated phenotype. Following this comprehensive review, 484 unique mutations are now reported in GLDC and AMT.

QUANTITATION OF ACYL-COENZYME A AND ACYL-CARNITINE IN PRIMARY HUMAN HEPATOCYTES FROM PROPIONIC ACIDEMIA PATIENTS

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Propionic acidemia (PA) is an autosomal recessive disease impacting branched-chain amino acid catabolism. The genetic drivers for PA are missense or nonsense mutations in the PCCA and PCCB genes that encode the heterododecameric propionyl-CoA carboxylase (PCC) enzyme complex. The functional consequence of these mutations is the inability to catabolize propionyl-CoA to methylmalonyl-CoA, and a deficit in the subsequent generation of succinyl-CoA that enters the Krebs cycle for energy production. This metabolic “choke-point” in PA results in the accumulation of propionyl-CoA and other metabolic intermediates, such as propionylcarnitine, 3-hydroxypropionic acid and methylcitrate, which are routinely used as diagnostic biomarkers in the clinic. We previously described a novel rare disease platform that recapitulates the pathobiology of PA, including hepatocyte morphology, organization and function, and metabolic defects associated with PA, using an organotypic system that deploys primary hepatocytes from patients with PA. Here, we further validate our PA disease model by measuring clinically relevant biomarkers, such as acyl-CoAs and acyl-carnitines. Coupling metabolite enrichment with solid phase extraction mass spectroscopy, we show the ability to monitor these critical metabolites at single- to sub-nanomolar concentrations, over a 3-log dynamic range and with low coefficients of variation. These same metabolites are monitored in both cellular lysates and cell-conditioned media from normal and PA patient-derived primary hepatocytes. This work provides

direct biochemical validation for the first in vitro disease model of PA with the potential to facilitate drug-discovery and accelerate therapeutic development.

FUNCTIONAL CHARACTERIZATION OF NAGS PROTEIN USING GENE THERAPY

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N-acetylglutamate synthase (NAGS) is a urea cycle enzyme that catalyzes formation of N-acetylglutamate (NAG), an allosteric activator of carbamylphosphate synthetase I (CPS1). Defects in NAGS result in reduced or absent CPS1 activity and block of ureagenesis that cause neurological damage and can lead to death. We have created a mouse model of NAGS deficiency; the NAGS knockout (NAGSko) mouse survives and reproduces when treated with N-carbamylglutamate and citrulline (NCG + Cit) initially via injections and then in the drinking water. Upon NCG + Cit withdrawal, the NAGSko mice die of hyperammonemia within 24–48 h. We are now using gene transfer in the NAGSko mice to study function of the NAGS enzyme in vivo. The adeno associated virus type 2/8 (AAV2/8) combined with the liver-specific thyroxine binding globulin (TBG) promoter were used to achieve liver-directed gene transfer and expression of the mouse NAGS (mNAGS). The dose of AAV2/8TBG.mNAGS needed to rescue NAGSko mice was determined first. A single dose of 1×10^9 (Group I), 1×10^{10} (Group II) or 1×10^{11} (Group III) viral particles was delivered intravenously to the NAGSko mice; the NAGSko mice injected with the vector lacking mNAGS coding sequence were used as a control (Group IV). The efficacy of the mNAGS delivery and expression were determined by measuring the duration of activity of the injected mice on the voluntary wheel following withdrawal of (NCG + Cit) from the drinking water. Mice in groups II and III remained active for at least 48 h. After removal of (NCG + Cit) from the drinking water the average duration of activity of mice in Groups I and IV were 29.1 ± 11.1 and 21.3 ± 1.6 h, respectively. The expression of mNAGS mRNA and protein were more variable in Group II than in Group III. Therefore, we tested persistence of mNAGS expression in the NAGSko mice injected with 1×10^{11} viral particles. Real-time PCR and Western blotting were used to measure abundance of mNAGS mRNA and protein 1, 5, 7, 14 and 28 days after injection. Wild-type mice from the same colony were used as control. Both mRNA and mNAGS protein were detectable on the first day after injection and their expression persisted for at least 28 days after injection. On day 5 after injection, the mNAGS mRNA expression was 4-fold higher in the injected NAGSko mice than in the wild type mice and slowly decreased thereafter. The NAGS protein peaked on day 14 after injection and was 9 times higher in the injected mice compared to the wild-type animals. We are now testing whether replacement of the TBG promoter with the mNAGS natural promoter will allow mNAGS expression closer to levels observed in wild-type mice. This methodology will allow in vivo study of structure-function relationships in NAGS and evaluation of effects of the mutations found in patients on NAGS function.

Keywords: NAGS deficiency, Adeno-associated viruses (AAV), liver gene therapy, NAGS knockout mice

NOVEL APPROACH FOR THE TREATMENT OF SIX MUCOPOLYSACCHARIDOSES BY TARGETING NDST1

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Background: Accumulation of un-degraded heparan sulfate (HS) results from deficiencies in any of eight HS degrading lysosomal enzymes. Stored HS eventually affects the function of the lysosomes, which directly impacts many organs including the central nervous system (CNS). Currently used therapies lack efficiency in lysosomal storage disorders (LSDs) with neurological complications since access to the CNS is precluded by the blood brain barrier. We set out to identify small molecules that can be used in substrate reduction therapy (SRT) to lower the O-sulfation and/or epimerization of the HS sugar moieties. Our aim is to down-regulate the transcription of the first modifying enzyme in HS biosynthesis, N-deacetylase/N-sulfotransferase 1 (NDST1).

Methods: We developed a high-throughput assay to screen for small molecules that lower the HS biosynthesis through down-regulation of NDST1. We used HeLa cells stably expressing human NDST1 promoter-firefly luciferase in a high-throughput screen assay for transcriptional inhibitors of NDST1. The effectiveness of our best candidates was validated by several different assays, including western blots, enzyme activity, qPCR, and 35S pulse/chase analysis.

Results: Screening of one of the drug libraries resulted in 35 potential NDST1 transcription inhibitors. Further evaluation of dose response curves resulted in 22 bona-fide hits. One of the best compounds, SAHA (histone deacetylase inhibitor), significantly reduced the activity and protein levels of endogenous NDST1. Also, SAHA decreased HS synthesis in wild type fibroblasts and significantly reduced HS storage in fibroblasts of a patient with mucopolysaccharidosis type IIIC.

Conclusion: Substrate reduction therapy by targeting NDST1 represents a novel approach to developing a therapy for multiple lysosomal storage disorder since one single drug inhibiting substrate production can be used to ameliorate the clinical phenotypes produced by several different deficient lysosomal enzymes.

CHARACTERIZING THE MOLECULAR ARCHITECTURE OF MITOCHONDRIAL ENERGY METABOLISM APPARATUS AND ITS IMPORTANCE TO CLINICAL PATHOPHYSIOLOGY

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Mitochondrial energy metabolism is comprised of three major biochemical pathways: oxidative phosphorylation (OXPHOS), fatty acid oxidation (FAO), and the tricarboxylic acid cycle. OXPHOS is organized into functional enzymatic complexes that, in turn, assume higher order structures called super complexes. We have previously demonstrated functional and physical interactions between OXPHOS and FAO, forming a mitochondrial energy super-structure that promotes metabolic channeling, potentially allowing multiple enzymatic reactions to occur without release of intermediates, and thus optimizing metabolic efficiency. We now report the use of two-dimension blue native/SDS-PAGE western blots, mass spectrometry proteomic techniques, and immune-electron microscopy studies to characterize the physical interaction of FAO proteins with each other and with OXPHOS super complexes. We find that the mitochondrial trifunctional protein, an NADH⁺-generating enzyme, directly interacts with complex I NADH⁺-binding domain, as well as with very long-chain acyl-CoA dehydrogenase of FAO. Electron transfer flavoprotein dehydrogenase (ETF_{ADH}), which funnels reducing equivalents from acyl-CoA dehydrogenases in FAO to OXPHOS, interacts with the co-enzyme Q reduction site of complex III and complex I, which is consistent with the known interaction of these two enzymes in super complexes. Two-dimension blue native/SDS-PAGE also identifies short-chain 3-hydroxyacyl-CoA dehydrogenase, pyruvate dehydrogenase, α -ketoglutarate dehydrogenase, and malate dehydrogenase extensively interacting with OXPHOS complex I and the super complexes.

Patients with deficiencies of either FAO or OXPHOS often show clinical and/or biochemical findings indicative of a disorder of the other pathway. In a series of explanted hearts from transplant patients with a variety of disorders, interactions between ETF_{ADH} and mitochondrial trifunctional protein and OXPHOS super complex function were disrupted, implicating their importance for normal heart bioenergetics. These results provide an expanding view of the molecular architecture of mitochondrial energy metabolism apparatus and its potential impact on disease.

POSTER PRESENTATION ABSTRACTS

ABSTRACTS ARE LISTED IN ALPHABETICAL ORDER BY FIRST AUTHOR
THE ABSTRACT NUMBER IS THE POSTER BOARD NUMBER

1) DNA SEQUENCING OF OTC 5' REGULATORY REGION REVEALS NOVEL MUTATIONS IN SYMPTOMATIC PATIENTS WITH BIOCHEMICAL DIAGNOSIS OF OTC DEFICIENCY

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Background: Ornithine transcarbamylase deficiency (OTCD) is the most common urea cycle disorder (UCD). In approximately 10–20% of biochemically suggestive OTCD cases with a combination of low citrulline, elevated glutamine, and elevated urinary orotic acid or reduced hepatic OTC enzyme activity, no mutation is detected via conventional DNA sequencing of exons and splice junctions and deletion/duplication analysis. In such cases, deleterious mutations may be located in non-coding regions of the OTC gene, such as the regulatory domain. Mutations within the regulatory regions of genes currently represent a very small proportion of known disease-causing mutations and are likely underdiagnosed. We thus hypothesized that mutations in regulatory regions may account for many OTCD cases in which no mutation was identified via conventional DNA analysis.

Methods: We retrieved and de-identified 24 DNA samples sent to our laboratory for OTC analysis in which no mutation was identified. Additionally, we enrolled and obtained DNA samples from 13 subjects with biochemically suggestive of proximal urea cycle disorders in which DNA sequencing revealed no mutation in the N-acetylglutamate synthetase, carbamylphosphate synthetase I and ornithine transcarbamylase genes. In all samples, we then sequenced 1 kb upstream (OTC promoter) and the 900 bp liver-specific enhancer, 9 kb upstream of the OTC start codon.

Results: We identified 9 families with 7 novel variants in conserved OTC 5' upstream regulatory region. Six variants were in the OTC promoter and 1 in a liver-specific enhancer. None of these variants were listed in the 1000 Genomes and dbSNP databases. All symptomatic individuals were hemizygous males, with presentation ranging from neonatal hyperammonemia to absence of documented hyperammonemia, but with neurological symptoms and brain MRI findings consistent with a urea cycle disorder.

Conclusions: Regulatory mutations likely contribute a significant proportion of the biochemically confirmed OTC deficiency in which no mutation is identified via conventional sequencing. These mutations likely diminish, but do not abrogate OTC expression, resulting in an attenuated phenotype. We are currently performing functional studies of these mutations to characterize the impact of these mutations on OTC expression.

2) RISING RATE OF EXCLUSIVE BREASTFEEDING IS ASSOCIATED WITH INCREASED MORBIDITY AND MORTALITY AMONG NEONATES WITH MEDIUM CHAIN ACYL-CoA DEHYDROGENASE (MCAD) DEFICIENCY

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Background: Since widespread implementation of universal newborn screening (NBS) using tandem mass spectrometry, morbidity and mortality from MCAD has decreased drastically. However, it is known that some infants become ill prior to NBS results being available. Previous research reported that among MCAD patients born in the early 2000s, 4–5% were symptomatic before their NBS resulted. Among this population, exclusive breastfeeding was associated with symptomatic presentations and higher octanoylcarnitine levels. Since this time rates of exclusive breastfeeding have increased nationwide. In this study we set out to quantify the current risk of early clinical decompensation in neonates with MCAD. We also worked to identify factors associated with poor outcomes prior to return of NBS results.

Methods: This is a retrospective analysis of all neonates referred to our center and confirmed to have MCAD after an abnormal newborn screen between January 1st 2010 and August 1st 2015.

Results: 46 infants were diagnosed with MCAD during the study period. 11 of 46 (23.9%) were symptomatic prior to or at the time of the abnormal NBS report. Of these patients 4 presented with death or cardiac arrest, and 7 had hypoglycemia and/or lethargy. 100% of symptomatic patients were exclusively breastfed, while only 40.6% of asymptomatic patients were exclusively breastfed ($p = 0.0008$). During the study period, there was an upward trend in breastfeeding rates among patients without a known family history of MCAD. In 2010–2011 45.5% of patients were exclusively breastfed; this increased to 64.7% in 2012–2013, and 87.5% in 2014–15. Over this same time period rates of decompensation prior to NBS results significantly increased from 9.09% in 2010–2011, to 23.5% in 2012–2013, and 75% in 2014–2015 ($p = 0.003$).

Conclusions: Neonates with MCAD can become symptomatic before they are identified on NBS. Among these patients, exclusive breastfeeding is associated with an increased risk of morbidity and mortality. Closer monitoring and management of feeding difficulties in exclusively breastfed infants prior to the return of the NBS may help minimize adverse outcomes in this population.

3) WHOLE EXOME SEQUENCING DIAGNOSIS OF INBORN ERRORS OF METABOLISM AND OTHER DISORDERS IN THE UNITED ARAB EMIRATES

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Advantages and limitations of implementing whole exome sequencing (WES) in the clinic practice have yet to be fully illustrated. This study reports on the use of this technique to diagnose children with inborn errors of metabolism (IEM) and other disorders in the United Arab Emirates (UAE). From January 2012 to December 2014, 85 patients were evaluated using WES as a diagnostic tool. These patients were seen by our metabolic service at Tawam Hospital (Al Ain, Abu Dhabi) and the test was requested since a definitive diagnosis was unable to reach by conventional methods. In 6 (7%) patients, WES did not yield clinically relevant information. In 14 (17%) patients, WES confirmed IEM disorders (five mitochondrial disease, three lysosomal storage diseases, and six other entities). In 18 (21%) patients, WES confirmed genetic disorders (15 neurological diseases and three non-neurological diseases). In the remaining 47 (55%) patients, WES findings were of uncertain significance and required further studies. Of these, eight (9%) patients had confirmed 'pathologic variants' based on consistent phenotype, biochemical findings, family studies or previously suggested pathogenicity. Nineteen (22%) patients had 'likely pathologic variants' based on consistent phenotype or previously suggested pathogenicity. Twenty (24%) patients had 'non-pathologic variants' based on inconsistent phenotype and biochemical or familial findings. Thus, WES diagnosis was particularly helpful in 59 (69%) patients. Developing proper WES guidelines and policies (including training staff competent in obtaining consent and providing counseling) are essential prior to incorporating this technology in the routine clinical practice.

4) HEPATOSPLENOMEGALY AND FEVER FROM EBV INFECTION LEADING TO DIAGNOSIS OF CHOLESTEROL ESTER STORAGE DISEASE

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Hepatosplenomegaly in children frequently initiates a diagnostic workup for hematologic and infectious etiologies. Much rarer causes of hepatosplenomegaly include storage disorders for which diagnosis is often reached after more common causes have been excluded. However, mild storage disorders, such as cholesterol ester storage disease, are considered to be under diagnosed in the general population and may first be identified during another illness. We describe the case of a 18-month old boy who presented with hepatosplenomegaly and fever and found to have an acute EBV infection as well as cholesterol ester storage disease. We review the literature and discuss EBV infection as a frequent precipitating event leading to the diagnosis of CESD in otherwise mildly affected childhood.

5) PHENOTYPIC AND GENOTYPIC SPECTRUM OF N-LINKED CONGENITAL DISORDERS OF GLYCOSYLATION

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Background: Congenital disorders of glycosylation (CDGs) are a heterogeneous group of inherited metabolic disorders. Over 50 different types of N-linked CDG have been identified so far. We performed a retrospective cohort study to determine phenotype, genotype and prevalence of subtypes of N-linked CDG in our center.

Methods: Electronic patient charts were reviewed for clinical features, neuroimaging, biochemical investigations, genetic studies and long-term outcome.

Results: Eleven patients (age 5 months–19 years) from 10 unrelated families were included. In one family, there were 2 affected children with PMM2-CDG. All patients had global developmental delay. Seizures were present in 28.5% of the PMM2-CDG patients, whereas 75% of the patients with other subtypes of N-linked CDG. Neuroimaging showed cerebellar hypoplasia in all patients independent of subtype. Additionally, cortical dysplasia was present in a PMM2-CDG patient. Transferrin isoelectric focusing was suggestive of N-linked CDG pattern in all patients. PMM2-CDG was the most common subtype in 64% (7/11) of patients. Four patients had one of the other subtypes including ALG3-CDG, ALG9-CDG, AGL11-CDG and MPDU1-CDG. There were 4 novel mutations in the PMM2 (c.422G>A; c.91T>C; c.61T>C; c.430T>C).

The follow-up time ranged from 1.5–18 years following diagnosis.

Conclusions: We report 11 new patients with N-linked CDG. PMM2-CDG was the most common subtype in our patient cohort. For the other N-linked CDG subtypes ALG3-, ALG9-, AGL11- and MPDU1-CDG, there are less than 10 patients reported in the literature and our new cases add to the phenotypic spectrum of these ultra rare disorders. Due to multisystem involvement, surveillance is important to identify and treat disease related complications symptomatically; despite there is no disease specific treatment.

6) PHENOTYPIC SPECTRUM OF PATIENTS WITH PYRIDOXINE DEPENDENT EPILEPSY CAUSED BY MUTATIONS IN THE ALDH7A1 IN A SINGLE CENTER

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Background: Pyridoxine dependent epilepsy (PDE) is an autosomal recessively inherited disorder of lysine catabolism caused by mutations in the ALDH7A1 (PDE-ALDH7A1). We report phenotypic spectrum of patients with PDE-ALDH7A1.

Methods: The electronic patient charts were reviewed for clinical, biochemical and molecular genetic studies, neuroimaging and long-term outcome.

Results: Eleven patients from 9 unrelated families were included (six males, five females). In two non-consanguine families, there were two affected children. The seizure onset was neonatal in 63% and early infantile (up to 3 months of age) in 37% of the patients. Pyridoxine was started in the first month of life in four and early infantile (up to 12 months of age) in seven patients. Generalized tonic-clonic seizures were the main seizure type in all patients. Brain MRI showed corpus callosum hypoplasia, increased white matter signals and cerebral atrophy in single patients. Urinary alpha-aminoadipic semialdehyde levels ranged from 2 to 39.3 (reference 0–0.5 mmol/mol creatinine). There were fourteen different mutations (3 novel) in 22 alleles: four homozygous from consanguine families. The current average age was 9.1 ± 5.9 years (6 months to 18 years). Three patients (aged 3.5–18 years) had normal neurocognitive functions and two patients (6 months of age) had normal development. Five patients had mild to moderate global developmental delay or cognitive dysfunction. One patient had profound global developmental delay. Two patients had obsessive-compulsive and anxiety disorder. Eight patients had no seizures after pyridoxine was initiated (current dose 200 mg/day). Three patients had occasional seizures requiring pyridoxine dose up to 1000 mg/day. Only two patients were on anti-epileptic medications. In addition to pyridoxine therapy, four patients were treated with lysine-restricted diet and four patients with arginine supplementation.

Conclusion: We report eleven patients with PDE-ALDH7A1: eight new patients and three novel mutations. Delayed diagnosis was associated with unfavourable neurodevelopmental outcome.

7) COMBINATORIAL APPLICATION OF FUNCTIONAL OMICS TESTING UNCOVERS MITOCHONDRIAL DEFICIENCY IN SMITH-MAGENIS SYNDROME

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Mitochondrial diseases are a clinically, biochemically, and genetically heterogeneous group of disorders with a variable age onset and rate of disease progression. The involvement of both the mitochondrial and nuclear genome confers a significant challenge in discerning pathogenicity in an individual, often delaying diagnosis. Mitochondria dysfunction has increasingly been reported across a wide range of genetically distinct neurodevelopmental disorders, but the cellular defects that contribute to the pathogenesis of mitochondrial disease in this context are largely unknown. Smith–Magenis syndrome (SMS) is a neurodevelopmental disorder caused by reduced gene dosage of retinoic acid induced 1 (RAI1). In addition to intellectual disability, sleep disturbance, and significant behavioral problems, individuals with SMS experience hypotonia, seizures, progressive hearing loss, vision impairment, constipation, and also fatigue easily, all features typically reported in mitochondrial disease. In order to explore potential contributing factors to these features in SMS, we devised an innovative combinatorial functional omics approach to survey mitochondrial function. We employed transcriptional profiling of neuronal cells with targeted knockdown of RAI1 in conjunction with an untargeted small molecule metabolomics screen of plasma samples from individuals with SMS. Overrepresentation analysis of differentially expressed transcripts within RAI1 haploinsufficient neuronal cells identified a significant enrichment of 60 mitochondria-associated genes. The metabolomics screen revealed significantly increased mitochondria-associated metabolites including pyruvate and lactate, both of which have also been shown to be altered in individuals with autism spectrum disorder and other neurodevelopmental disorders. The convergence of both transcriptomic and functional metabolomic profiles prompted the diagnostic evaluation of mitochondrial function in SMS patient fibroblast cell lines. A battery of mitochondrial assessments performed on these patient-derived cell lines identified significantly diminished mitochondrial membrane potential indicative of compromised mitochondrial function. SMS patient cells also demonstrated abnormal respiratory chain activity, elevated oxygen consumption rates, and elevated mitochondrial DNA content, as well as elevated citrate synthase protein levels, reflecting abnormal mitochondrial proliferation, likely in response to mitochondrial dysfunction. Additionally, fluorescence microscopy revealed an unusual perinuclear distribution of mitochondria in SMS fibroblast lines. Taken together, our results demonstrate that mitochondrial function and integrity are compromised in SMS patient-derived cells. The mitochondrial deficiencies observed in this study resemble those reported in other neurodevelopmental disorders, including Down syndrome, autism, fragile X syndrome, and Rett syndrome. This finding suggests a model in which mitochondrial dysfunction elicits or contributes to a similar pattern of clinical manifestations including intellectual disability, developmental delays, hypotonia, seizures, and gastrointestinal symptoms across several genetically distinct disorders. Overall, our integrative functional omics approach has pinpointed cellular defects in SMS similar to those observed in other neurodevelopmental disorders and highlights the need for additional diagnostic mitochondrial screening in SMS patients.

8) STOP, CRITICIZE, AND RETHINK BEFORE THE INTENDED BENEFIT BECOMES A HARM: LESSONS LEARNED FROM A COBALAMIN C DEFICIENCY PATIENT

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Cobalamin C deficiency is the most common inborn error of intracellular cobalamin metabolism. This complex disease causes methylmalonic acidemia, homocystinuria, and hypomethioninemia. Despite early diagnosis via newborn screening and treatment, the outcomes remain unfavorable with neurological and ocular impairment almost always present. The disease pathophysiology is poorly understood and no well-defined treatment guidelines or protocols are currently in existence. For years several patients have been treated with low-protein diet and medical foods designed for isolated methylmalonic acidemia leading to iatrogenic methionine deficiency and potentially additional neurological damage. Most recently experts have been raising concerns about the safety of these medical foods and urging the need for better treatment approaches.

A five-year-old Amish boy with a known diagnosis of Cobalamin C deficiency presented to our clinic in October 2013 to establish long-term care. He had been on a methionine-free formula since the first week of life. His parents reported a very slow, almost static, progress in his development especially in language, communication, and social skills. His behaviors were consistent with a diagnosis of autism spectrum disorder. A detailed review of his previous records revealed good metabolic control but his methionine levels ranged between 3 and 10 $\mu\text{mol/L}$. Knowing the role of methionine in brain growth and development, he was started on a trial of methionine supplementation. Within 3 months parents reported major improvements in speech and social interactions. They also noted that he was overall a happier child. This favorable response to methionine supplementation triggered a long pause to rethink the treatment strategy. In May 2014 the patient was weaned off the medical formula and transitioned to a vegetarian diet. His medications and supplements were adjusted to keep a good metabolic control. Since there were no standardized protocols outlining hydroxocobalamin (OHCbl) and betaine dose adaptations and long-term management, the adjustments were made based on his homocysteine and methylmalonic acid levels as well as his overall performance. Our patient is now 7 years old. He is currently on 2.0 mg intramuscular OHCbl, 500 mg/kg/day betaine, and 5 mg/day folinic acid. He remains on levocarnitine due to low plasma carnitine levels. His growth parameters, including head circumference, are all within 50–75%ile for age. He has made a remarkable progress in his communication and social skills. He interacts well with peers, maintains eye contact, and is able to make friends. He is more focused and advancing in school. His nystagmus has improved while his macular atrophy has remained stable over the past 2 years. Overall, this revised treatment approach has been a turning point towards an improved quality of life and a better future for our patient. These observations should encourage others to reconsider the use of medical foods without being cautious and mindful.

Similar case studies combined with a better understanding of the pathophysiology of this disease would make the development of successful treatment protocols possible.

9) INTEGRATION OF MULTI-SCALE DATATYPES IN MOUSE GENETIC REFERENCE POPULATIONS TO REVEAL CANDIDATE MODIFYING BIOLOGY IN INBORN ERRORS OF METABOLISM

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Background: Current approaches to delineate the pathophysiology responsible for the clinical heterogeneity of inborn errors of metabolism (IEMs) have relied heavily on investigation of knockout (KO) mouse models. However, studies of KO models are on their own insufficient as these models are often congenic and thus designed to display minimal phenotypic variability, which is in contrast to the human situation. We view our data-driven multi-scale approaches in genetic reference populations of mice as complimentary.

Methods: We characterized the influence of genetics and diet on plasma metabolite abundances in the BXD mouse genetic reference population and related these data to numerous clinical traits scored in the same population. Approximately 75 metabolites including acylcarnitines, amino acids and (glyco)sphingolipids were measured in plasma from 40 BXD strains fed either a chow or high fat diet. Archived molecular, genetic and clinical data were integrated with the current metabolite data through expression QTL mapping as well as coexpression network analysis.

Results: Integrative genomics analysis revealed several known and novel genes contributing to plasma metabolite levels. First we highlight that natural variation in *Mlycd* expression controls plasma levels of C3DC-carnitine and is a physiologic regulator of hepatic ketogenesis. We also identify and validate using CRISPR/Cas9 technology two novel enzymatic regulators, a mitochondrial ornithine decarboxylase controlling plasma ornithine and an E3 ubiquitin ligase affecting plasma phenylalanine levels. Integration of molecular and metabolite data also revealed that plasma levels of long-chain acylcarnitines and amino acids were correlated to fasting-induced weight loss with liver lipid metabolism and muscle protein degradation as the underlying molecular pathways.

Conclusion: Novel pathways underlying IEM pathophysiology and potential therapeutic targets can be derived by including IEM-relevant metabolites in multi-scale biology approaches in mouse genetic reference populations.

10) THREE-DAY TRIAL OF N-CARBAMYLGLUTAMATE IMPROVES NITROGEN METABOLISM AND DECREASES AMMONIA IN INHERITED HYPERAMMONEMIAS

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Background: Hyperammonemia (HA) secondary to urea cycle defects causes lethargy, encephalopathy, coma and even death. Current therapy includes nitrogen scavengers, dietary protein restriction and supplementation with citrulline/arginine, but these treatments often fail to prevent recurrent hyperammonemic crises, underscoring the need for new therapies.

N-acetylglutamate (NAG) is the essential allosteric activator of carbamyl phosphate synthetase I (CPS1), which catalyzes the first step of the hepatic urea cycle. NAG is produced by NAG synthase (NAGS). Absence or insufficiency of NAG diminishes urea cycle flux and results in HA.

N-carbamylglutamate (NCG), a synthetic analogue of NAG, improves ureagenesis in patients with NAGS deficiency. We have studied the efficacy of NCG in other forms of HA.

Methods: We have enrolled 34 subjects with one of the following: NAGS deficiency, CPS1 deficiency, ornithine transcarbamylase (OTC) deficiency, methylmalonic acidemia (MMA), propionic acidemia (PA) and undiagnosed recurrent HA. Identical studies were performed immediately before and at the end of a 3-day trial of oral NCG (Carbaglu, Orphan Europe). Following an oral bolus of [^{13}C]sodium acetate, blood samples were collected at pre-determined intervals to measure the levels of [^{13}C]urea, ammonia, urea, and amino acids.

Results: In NAGS deficiency, NCG normalized blood ammonia, reduced glutamine, increased urea levels and restored ureagenesis to normal. In subjects with PA, MMA and in some subjects with CPS1 deficiency and undiagnosed recurrent HA, NCG administration was associated with decreased ammonia and increased ureagenesis. NCG had little effect in subjects with OTC deficiency.

Conclusion: NCG improves or restores ureagenesis and decreases or normalizes ammonia in stable patients with some forms of inherited HA. We are currently conducting a randomized, double blind, placebo-controlled trial of Carbaglu during acute HA in CPS1 and OTC deficiency, PA and MMA to collect data for potentially expanding the indication of this drug if the above results are confirmed.

11) SMALL FIBER NEUROPATHY IN A COHORT OF PATIENTS WITH GLYCOGEN STORAGE DISEASE TYPE III

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Background: Autopsy reports demonstrate the presence of glycogen in the Schwann cells in patients with glycogen storage disease type III (GSD III). Despite these findings, definitive clinical evidence of peripheral nerve involvement has not been reported.

Methods: We report the first confirmed cases of small-fiber neuropathy (SFN) in GSD III and present the results of an attempt at screening for SFN in this population. Fifteen patients enrolled in an IRB approved GSD III natural history study at Duke University between September 2013 and July 2015 were asked to complete the 21-item Small-Fiber Neuropathy Screening List (SFNSL), where a score of ≥ 11 is considered to be a positive screen. Additionally, 6 patients had diagnostic testing for SFN via a skin biopsy (calf, deltoid, thigh) examining sweat gland and epidermal nerve fiber density.

Results: Thirty percent of patients (5/15) had a positive SFNL screen ($n = 5$; median score 18, range 11–26; median age 15 years, range 8–52 years; and 4F:1M). Two patients with positive SFNL (2/5) had diagnostic testing for SFN and tested in the low normal range and abnormal range within the sweat gland nerve fiber (15 and 52 years, respectively). Four additional patients had a diagnostic skin biopsy with results indicating 1 patient with abnormal sweat and epidermal nerve fiber density (did not complete questionnaire), 1 patient with low normal epidermal nerve fiber density (scored “no SFN”) and 2 patients with normal sweat and epidermal nerve fiber density (both scored “no SFN”). No additional risk factors were identified as an underlying cause of the SFN.

Conclusions: We continue to learn about peripheral nerve involvement in GSD III as we build on previously reported abnormalities of the median motor response. Carefully conducted natural history studies allow for better characterization of rare disease.

12) DEVELOPMENT AND VALIDATION OF A NEAR-PATIENT TEST CASSETTE AND METER FOR BLOOD AMMONIA QUANTIFICATION

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Background: Hyperammonemia is a life-threatening condition which can result in developmental delay, coma and if untreated, death. Hyperammonemia is a component of several inherited metabolic disorders, including urea cycle disorders and organic acidemias. For the last 40 years, measurement of ammonia has necessitated a visit to a hospital equipped with enzyme assay instrumentation. This archaic and impractical system limits long-term management options, and frequently results in patients presenting to the hospital only after the onset of altered mental status. There is thus a need for point-of-care blood ammonia measurement.

Methods: We have developed a quantitative point-of-care blood ammonia sensor, engineered utilizing a specific, colorimetric ammonia reaction, in tandem with a cation exchange membrane, which selectively extracts ammonia from whole blood. The Clinical Laboratory Standards Institute guidelines were utilized to evaluate the sensor. The developed microfluidic test cassettes were evaluated for accuracy, sensitivity, stability, variability and were compared to the conventional clinical laboratory method.

Results: The test cassettes were shelf-stable for as long as 150 days. The calibration curve, collected using only 40 μL of whole blood per sample, was linear over the physiologic range of 20–500 $\mu\text{mol/L}$ ($R^2 = 0.998$). The limit of detection was 8 $\mu\text{mol/L}$ and limit of quantification was 27 $\mu\text{mol/L}$. The variability of the measurements was $\leq 5\%$. **Conclusions:** Our ammonia sensor prototype can reproducibly measure ammonia levels in whole blood. It can not only differentiate between normal and elevated blood ammonia levels, but can quantify the ammonia with sufficient precision to potentially permit more refined daily ammonia control. This would introduce a new treatment paradigm for the inherited hyperammonemias.

13) IMMUNOHISTOCHEMICAL ANALYSIS OF MANNOSE 6-PHOSPHATE/INSULIN-LIKE GROWTH FACTOR II RECEPTOR (M6PR/IGF2R) IN WILD TYPE AND MPS3B MUTANT CNS VASCULATURE AND IMPLICATIONS FOR TRANS-BLOOD BRAIN BARRIER (BBB) TRANSPORT

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Enzyme replacement therapy (ERT) for central nervous system (CNS) disorders is challenging due to the presence of the blood brain barrier (BBB). This necessitates either invasive direct application into the CNS, or promoting systemically administered therapeutic enzyme to cross the highly restrictive barrier. Currently a variety of strategies are being explored to selectively promote transport across the BBB including the use of specific receptors lining the vasculature of the CNS such as the mannose 6-phosphate/insulin-like growth factor II receptor (M6PR/IGF2R). In the non-clinical setting, there is experimental evidence to suggest that using the M6PR/IGF2R may be an effective strategy in newborn, but not adult mice. The reason for this lack of effectiveness in adults is currently unclear. To address this, we undertook a systematic immunohistochemical analysis of M6PR/IGF2R in the vasculature of developing and adult wild-type mice. We find strong staining on the luminal surface of most vessels in animals 1 week of age or younger. The number of vessels with positive staining decreases by 2 weeks and is largely absent by 3 weeks. A similar developmental analysis in normal human CNS vasculature is currently underway. Given that metabolic disorders may result in changes in protein bio-distribution, we also evaluated M6PR/IGF2R immunostaining in alpha-N-acetylglucosaminidase (Naglu) mutant mice, a model for Mucopolysaccharidosis IIIB. In Naglu mutant mice, staining is present in most vessels at birth, but largely absent by 2 weeks of age, potentially indicating earlier loss compared to wild-type mice. In both, wild-type and Naglu mutant mice, M6PR/IGF2R staining persists in neurons throughout adulthood demonstrating cell-type specific differential regulation of M6PR/IGF2R. Additionally, we are also evaluating perivascular astroglial scarring in Naglu mutant mice, which may pose a significant barrier to diffusion from vessels with damaged endothelial cells due to the resulting metabolic defect.

14) GALACTOSE-1 PHOSPHATE URIDYLTRANSFERASE (GALT) DEFICIENCY INDUCES PLEIOTROPIC DOWN-REGULATION OF PI3K/Akt SIGNALING IN MOUSE FIBROBLASTS

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Background: For years, the pathophysiology of the chronic complications associated with Classic Galactosemia has remained enigmatic. Recently, we constructed a new mouse model of GALT deficiency and showed that these mice have growth restriction and reduced fertility, phenotypes also seen in many patients. Using fibroblasts derived from the GALT-deficient mice, we took the first step to delineate the role of galactose metabolism in cell growth and development.

Methods: Primary skin fibroblasts from three normal (controls) and three GALT-deficient (tests) mice were propagated in regular DMEM medium supplemented with 15% FBS. We compared the followings between the two groups: (1) Growth rates; (2) Steady-state mRNA and protein expression levels of key members of the PI3K/Akt signaling pathway; and (3) The activation kinetics of Akt phosphorylation upon 100 ng/mL IGF1 stimulation.

Results:

- (1) GALT-deficient cells grow significantly slower.
- (2) Of the 24 genes interrogated using QIAGEN RT2 Profiler PCR Custom Array, Akt1 and Hsp90aa1 were down-regulated (2+/-fold) in mutant fibroblasts.
- (3) Immunoblots showed that levels of pAkt(Thr308), pAkt(Ser473), pan-Akt, pPdk1, Hsp90, and IGF1 receptor were reduced (p
- (4) Both serum-fasted control and GALT-deficient cells responded to IGF1-induced activation of Akt phosphorylation at + 15 min, but the mutant cells took significantly longer time to reach the maximum phosphorylation level.

Conclusions: Our results demonstrated for the first time that GALT-deficiency induces down-regulation of the PI3K/Akt signaling pathway in mouse fibroblasts at both transcriptional and protein levels. As the PI3K/Akt growth signaling pathway plays a crucial role in oocytes development and neuronal survival, we hypothesize that an attenuated PI3K/Akt signaling pathway could be a pathogenic mechanism for the complications seen in patients with Classic Galactosemia.

15) POLYMORPHISM K333Q REDUCES ACTIVITY AND STABILITY OF LONG-CHAIN ACYL-CoA DEHYDROGENASE

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Background: In humans, long-chain acyl-CoA dehydrogenase (LCAD) is expressed at high levels in alveolar type II (ATII) pneumocytes in the lung. No cases of human LCAD deficiency have been described. However, we previously linked a common polymorphism (K333Q) to reduced LCAD antigen in the lung of infants who died of SIDS. Here, we hypothesized that K333Q is a deleterious polymorphism associated with infant and childhood lung disease.

Methods: LCAD K333Q activity, kinetics, and stability were evaluated using a recombinant LCAD expression system. Primary adult human ATII cells were genotyped for K333Q and used for western blotting. The prevalence of K333Q was determined in neonates with respiratory distress syndrome (N = 202) and in children with pneumonia of varying severity (N = 775). Lung inflammation was evaluated in LCAD knockout mice (N = 4) seven days post-infection with influenza A/PR/8/34.

Results: Recombinant LCAD K333Q demonstrated two-fold less enzymatic activity than wild-type LCAD. Molecular modeling revealed that residue K333 is within interacting distance of the essential FAD cofactor. In keeping with a role for K333 in FAD binding, recombinant LCAD K333Q had significantly less enzyme-bound FAD. Exogenous FAD partially rescued the activity of LCAD K333Q but did not improve stability. Primary human ATII cells homozygous for K333Q had five-fold less LCAD antigen. However, the frequency of the K333Q allele was not increased among infants with respiratory distress or among children with pneumonia-induced lung injury. Finally, in contrast to our hypothesis, LCAD knockout mice were observed to have less severe lung injury following influenza infection. We speculate that reduced LCAD activity may suppress the inflammatory response during lung infection and studies are underway to test this hypothesis.

Conclusions: The K333Q polymorphism significantly reduces the activity and stability of human LCAD due to a reduced ability to bind the FAD cofactor. However, K333Q is not associated with respiratory distress or acute lung injury in children. Rather, animal studies suggest that loss of LCAD activity in the lung may actually confer protection against tissue damage in the context of lung infection.

16) PRECIPITOUS ENCEPHALOPATHY IN A PATIENT WITH MSUD WITH MODERATE LEUCINE ELEVATION

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Cerebral edema with subsequent herniation is a known fatal complication of patients with maple syrup urine disease (MSUD), associated with severely elevated leucine levels. A similar presentation of encephalopathy and seizures associated with cerebral edema can be caused by encephalitis or meningitis.

We present a 13-year-old male patient with MSUD with a three-day history of headache and once daily vomiting, who developed seizures and worsening cerebral edema despite hydration and amino acid restricted formula. At presentation his mental status was normal including normal ambulation and normal speech. His initial leucine level was 631 and he was treated with 10% dextrose containing fluids, isoleucine and valine supplements as well as Ketonex formula. The subsequent leucine value was 738, at which point he developed generalized tonic clonic seizures, followed by fixed pupils and became unresponsive. Despite efforts to initiate hemodialysis to reduce leucine values, we were unable to do so due to progressive cerebral edema and herniation resulting in brain death. Although elevated, his leucine levels were similar to values that he had when well. Moderate elevation of leucine levels can be associated with mental status changes, however the degree of morbidity in our patient is concerning for an additional etiology such as infection. At the family's request, an autopsy was not performed; thus, post-mortem viral testing was used to identify his systemic viral infection. Cerebral spinal fluid was not available for testing. We propose that the degree of encephalopathy and precipitous clinical decline in our patient was due to an acute complication of MSUD in addition to an underlying infectious etiology.

17) DEVELOPMENT OF THE US ENGLISH VERSION OF THE PHENYLKETONURIA - QUALITY OF LIFE (PKU-QoL) QUESTIONNAIRES

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Background: Phenylketonuria (PKU) is a serious, life-long disease characterized by a defect in the metabolism of phenylalanine (Phe) leading to elevated blood and brain Phe levels which leads to cognitive, emotional, and psychosocial problems. Quality of life (QoL) measures are a useful tool to determine the impact of an individual's current treatment strategy and better aim for optimal outcomes from a patient's perspective. However, QoL questionnaires developed for the general population may not adequately address the specific and often more subtle consequences caused by PKU. The phenylketonuria — quality of life (PKU-QOL) questionnaires were the first self-administered instruments developed to assess the impact of PKU on daily life (such as symptoms, behavioral and emotional issues, practical and social implications, financial and overall burdens) and its treatment (consisting of Phe-free medical food, a Phe-restricted diet, and any adjunct therapy) on health-related QoL. The four questionnaires (child, adolescent, adult and caregiver) share a similar structure, but reflect the specific realities of each of the populations. The questionnaires were simultaneously developed and validated in seven countries (i.e., France, Germany, Italy, The Netherlands, Spain, Turkey and the UK). The objectives of our study were to develop and validate the first PKU-QOL questionnaires designed for use by individuals with PKU and their caregivers in the United States.

Methods: The UK questionnaires served as a basis for the development of the US English PKU-QOL. The linguistic validation process consisted in 1) the adaptation of the UK versions into US English by a translator native of US English and living in the USA; 2) a clinician review; 3) cognitive interviews with patients (i.e., five children, five adolescents, and five adults) and five caregivers to test the appropriateness, understandability and clarity of the US translations; and 4) two proof-readings.

Results: The adaptation from UK to US English showed the usual differences between the two languages, such as differences in spelling [e.g., “dietician” (UK) vs. “dietitian” (US), or “mum” (UK) vs. “mom” (US)], or in words/expressions use [e.g., “please tick the box” (UK) vs. “please check the box” (US), “Following are” (UK) vs. “Below there are” (US), or “biscuits” (UK) vs. “crackers” (US)]. The major issue was cultural, and consisted in using a different terminology to describe PKU treatment throughout the questionnaires. The clinician, with the patients and the caregivers during the interviews, suggested to replace “supplement and amino-acid mixture” or “supplements” by “medical formula”.

Conclusions: The translation of the UK English PKU-QOL questionnaires into US English did not raise critical semantic and cultural issues. The evaluation of the psychometric properties of the US PKU-QOL questionnaires should provide more insight about their validity and reliability. The use of a validated tool to assess the impact of pharmacological treatments, such as sapropterin and potential new therapies, on clinical outcomes will be valuable for US healthcare providers in managing patients with PKU. The results of the linguistic validation study and the questionnaires will be presented.

18) FATAL CEREBRAL EDEMA IN A 21-MONTH OLD GIRL WITH PREVIOUSLY UNDIAGNOSED ORNITHINE TRANSCARBAMYLASE DEFICIENCY

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A 21-month old girl, previously healthy, presented to her local hospital with vomiting and diarrhea, and was diagnosed with tonsillitis and gastroenteritis. She received intravenous fluids and antibiotics as an outpatient and was discharged home. Later that evening, she had behavioral changes, followed the next morning by lethargy, ataxia, and vomiting. She was again seen in the local ED where her pupils were reactive, and there were mild elevations of hepatocellular enzymes in serum. A lumbar puncture and urine and serum drug screens were normal. She was admitted to the local ICU where she was intermittently hypertensive and apneic, and was intubated. A brain MRI at that time showed severe cerebral edema. She was given mannitol and air-transported to our hospital. On arrival, there was clinical evidence of brain stem herniation and brain death. Her blood ammonia at that time was 63 $\mu\text{mol/L}$, and plasma glutamine 1018 $\mu\text{mol/L}$. Urine orotic acid (168 mmol/mol Cr) and uracil (278 $\mu\text{mol/mol Cr}$) were markedly elevated. She died shortly after arrival.

A blood sample was obtained pre-mortem for DNA extraction. Sequencing showed that she was heterozygous for a known disease-causing missense mutation in the OTC gene (GCA to GAA) in codon 217, resulting in an Ala217Glu substitution. The mother did not carry this mutation.

While OTC deficiency can be associated with episodic hyperammonemia and cerebral edema in hemizygous males, this severe presentation without marked hyperammonemia in a previously well heterozygous female infant is unusual. The elevations of urine orotic acid and uracil are supportive of the diagnosis of OTC deficiency as the cause of her clinical crisis. The mechanism for her fatal cerebral edema is not clear.

19) GLOBAL DIFFERENCES IN PHENYLKETONURIA PHENOTYPES, GENOTYPES AND BH4 RESPONSIVENESS: A LESSON FROM BIOPKU AND PAHvdb DATABASES

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Background: Information from the BIOPKU database (www.biopku.org/biopku/), based on the genotype and phenotype data of over 9700 PAH-deficient patients from all over the world, suggests a heterogeneous global distribution of mild HPA, mild PKU and classic PKU. In addition, more than 950 different PAH gene variants are tabulated in the locus-specific database (www.biopku.org/pah/), and these result in almost 2000 different genotypes.

Methods: The BIOPKU database was linked with the PAHvdb and phenotypes, genotypes and BH4 responsiveness was calculated for Eastern Europe, Northern Europe, Middle Europe, Southern Europe, Middle East, Asia and the U.S. Allelic phenotype value (APV) was assigned to each PAH gene variant, where applicable.

Results: We found the global distribution of mild HPA, mild PKU and classic PKU to be approximately 17%, 27%, and 56%, respectively. There were significant differences in the frequency of severe classic PKU versus mild PKU and mild HPA between the eastern and southern European countries, with classic PKU being more common in the Eastern Europe and mild PKU and mild HPA in the Southern Europe. Reporting of mild HPA was lowest in Asia and the U.S. Accordingly, BH4 responsiveness was more frequent in regions with milder phenotypes. Both the phenotype and BH4 responsiveness correlated with the distribution of the genotypes.

Summary: Patients-based genotypes database, linked with the locus-specific database, is an invaluable tool to study both the PKU phenotypes distribution worldwide and the genotype-phenotype correlation and thus also potential BH4 responsiveness.

20) A QUANTITATIVE METHOD FOR THE MEASUREMENT OF DRIED BLOOD SPOT AMINO ACIDS USING ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY

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Background: Measurement of amino acids in dried blood spots has been extensively utilized for the detection of newborns with various inborn errors of amino acid metabolism including phenylketonuria (PKU) and maple syrup urine disease (MSUD). Whereas blood spot amino acid

measurement has been invaluable for initial diagnosis, the relative insensitivity of blood spot measurement has found limited use in lifelong monitoring of patients with these disorders. Most patients are currently monitored using plasma samples and measurement by ion-exchange chromatography, a process that can take up to two hours per sample. Many of the patients whom we monitor live several hours away from the hospital and find great inconvenience to travel this distance for routine monitoring purposes. We have previously found that ultra-performance liquid chromatography (UPLC) can provide a more rapid turnaround time for amino acid analysis and have routinely implemented this procedure in our laboratory for plasma amino acid analysis. We wanted to test if the assay was sufficiently sensitive to provide accurate monitoring of patients with amino acid disorders using blood spots. The work described here outlines our evaluation of blood spot amino acids using UPLC.

Methods: Amino acids from dried blood spots were derivatized with a proprietary reagent, AccQTag™. The derivatized amino acids were separated using UPLC and detected using fluorimetry. Plasma amino acids from dried blood spots were obtained from approximately 318 patient samples and compared to the corresponding plasma sample measured using the same UPLC.

Results: Intra- and inter-assay imprecision (mean CVs) for alioisoleucine, leucine, isoleucine, valine, phenylalanine, and tyrosine ranged from 0.1% to 4.3% and 4.2% to 20.2%, respectively. Recoveries were lower at high level, an observation that was not previously appreciated.

Conclusions: This UPLC based method can reliably measure significant amino acids in dried blood spots and finds the method to be sufficiently sensitive for accurate long-term monitoring of patients.

21) NOVEL SMALL MOLECULE THERAPY FOR MUCOPOLYSACCHARIDOSIS TYPE I

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Background: The mainstay of treatment for MPS I is enzyme replacement therapy with recombinant alpha L-iduronidase (rIDUA). rIDUA has limited efficiency on heart valves and the musculo-skeletal system and does not penetrate the BBB due to its molecular size and charge at the recommended dose. A number of pharmacologically active compounds were tested on skin fibroblasts from MPS I-HS patients. It was determined whether compound treatments result in an up-regulation of IDUA mRNA by utilizing Cells-to-CT technology (Life Technologies), which allowed for high throughput quantitative real-time PCR analysis in 384-well plates. A total of 7 different compound collections were screened. All compounds tested have known pharmacological activity and/or were from natural sources and were therefore deemed to be the most suitable for near term clinical utility.

Results: Two naturally occurring compounds (CTI-101 and CTI-102) were identified to increase IDUA activities in MPS I-HS fibroblasts to 2% of wild-type control fibroblasts in a dose dependent manner. CTI-102 showed the same 4-fold increase as CTI-101 but at a 10-fold lower potency. IDUA activities in wild-type mice were increased in plasma (10-fold) and in brain (+10%) following a 1 month administration of CTI-102 (50 mg/kg/d) compared to controls ($p < 0.05$).

Conclusions: Both compounds are brain penetrant and well tolerated in humans. CTI-101 and CTI-102 have the potential to address the currently unmet therapeutic needs for MPS I. A phase 2/3 clinical trial is in the planning stages.

22) ETHYLMALONIC ENCEPHALOPATHY: RESPONSE TO MEDICATIONS AND DIETARY TREATMENT

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Introduction: Ethylmalonic encephalopathy (EE) is a rare, neurometabolic disorder caused by mutations in the ETHE1 gene that encodes a mitochondrial dioxygenase crucial for hydrogen sulfide (H₂S) detoxification. Characteristic biochemical findings include elevated C4, C5 acylcarnitines, thio-sulfate, lactic and ethylmalonic acids in body fluids. The accumulation of toxic H₂S affects multiple systems resulting in a unique combination of GI, vascular, and CNS complications. Patients typically present in infancy with hypotonia, developmental delay, chronic diarrhea, orthostatic acrocyanosis and relapsing petechiae. The natural history of EE is characterized by a relentless, neurodegenerative course with death within the first decade.

Objective: To report the outcome of 2 newborn screen identified patients with EE treated with oral treatments aimed at lowering the production and promoting detoxification of H₂S and reducing the inhibitory effects of H₂S on COX activity.

Methods: Molecular testing for the ETHE1 gene was performed at the Division of Molecular Neurogenetics, IRCCS Foundation Neurological Institute "C.Besta", Milan, Italy. Biochemical testing was performed in CLIA/CAP certified laboratories by standard procedures. Baseline studies included brain MRI, EEG and biochemical testing. Patients were treated with N-acetylcysteine (105 mg/kg/d), Metronidazole (30 mg/kg/d) or alternating Neomycin (50 mg/kg/d) and Ascorbic acid (100 mg BID). Dietary treatment (methionine restriction 30–40 mg/kg/d) was added later.

Results: ETHE1 mutation analysis demonstrated a homozygous splice site mutation in patient 1 (c.505+1G>A) and compound heterozygous mutations in patient 2 (c.131_132delAG + c.566delG). Baseline studies demonstrated characteristic biochemical findings and normal EEGs. The baseline MRI of the brain was abnormal in patient 1 (small basal ganglia cystic lesions) and normal in patient 2. Patients have been on medical treatment and

closely followed for >10 months. Biochemical markers improved (44% reduction in ethylmalonic acid for both patients). Both patients maintained adequate growth and neither patient developed chronic diarrhea. Vascular abnormalities included mild orthostatic acrocyanosis for both patients and petechiae after tourniquet use in patient 1. CNS involvement was minimized but not prevented. Currently both patients have hypotonia and mild developmental delay and patient 2 developed seizures (infantile spasms) at 6 months of age.

Conclusion: The natural history of EE was amended with treatment; however, despite early diagnosis, there remain considerable neurologic abnormalities that are not fully corrected with the current treatment modalities.

23) PHENYLKETONURIA: A PROBLEM SOLVED? RESULTS OF A PATIENT SURVEY ON NEW TREATMENTS

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Phenylketonuria (PKU) is a rare metabolic disorder characterized by impaired conversion of phenylalanine (Phe) to tyrosine. If left untreated, the resultant accumulation of excess blood Phe can cause physiological, neurological, and intellectual disabilities. The National PKU Alliance (NPKUA) conducted a survey of its membership to assess current health status and interest in new treatments for PKU. Of the 625 survey respondents, less than half (46.7%) reported blood Phe within (120–360 µmol/L) – the range recommended by the American College of Medical Genetics and Genomics (ACMG). The survey results also showed that younger (= 18 years) subjects were about 3-times as successful in keeping their blood Phe concentrations within the recommended clinical range compared with adults. Blood Phe over 360 µmol/L was reported in one-quarter (25.5%) of = 18-year-old subjects and almost two-thirds (61.5%) of >18-year-old subjects. A little more than half (51.7%) of respondents reported having difficulty in managing their PKU, including the maintenance of a Phe-restricted diet. Subjects with PKU desire new treatments that would allow them to increase their intake of natural protein, discontinue or reduce their intake of Phe-free medical food and low-Phe foods, improve their mental health (including depression and anxiety), and reduce blood Phe concentrations. Respondents preferred oral administration of any newly developed therapies and, in general, disliked therapeutic injections. Injections at home were preferred over injections at a clinic. Payers, government agencies, clinicians, and industry partners should consider patient input when developing and approving new therapies and treatments for PKU.

24) HIGH PREVALENCE OF INHERITED METABOLIC DISORDERS IN PATIENTS WITH EPILEPTIC ENCEPHALOPATHY: A RETROSPECTIVE COHORT STUDY

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Background: Epilepsy is the most common childhood neurological disorder with a prevalence of 0.5–1.0% in children and adolescents. If it is associated with global developmental delay or cognitive dysfunction, it is defined as epileptic encephalopathy.

Objective: To determine the prevalence of inherited metabolic disorders (IMD) in childhood epileptic encephalopathy we performed a retrospective cohort study.

Methods: Electronic patient charts of the patients seen in the single Complex Epilepsy Genetics Clinic were reviewed for clinical features, neuroimaging, biochemical and genetic investigations.

Results: 120 patients between the age of 0–18 years were included. The prevalence of IMD was 8% (10/120 patients). The IMDs include PDE caused by ALDH7A1 mutations, Menkes disease, PNPO deficiency, cobalamin G deficiency, severe MTHFR deficiency, GLUT1 deficiency (2 patients), glycine encephalopathy, PDH complex deficiency and congenital disorders of glycosylation 1p caused by ALG11 mutations. 60% of these patients had a treatable IMD. The average age of seizure onset was 9.0 ± 16.5 SD months (1 h–4 years) and the average age of diagnosis was 48.9 ± 68.7 SD months (2–16.5 years). The diagnosis was delayed in two patients with GLUT1 deficiency for more than 10 years, despite atypical absence seizures started before the age of 5 years.

Conclusions: The prevalence of IMD was 8% in patients with epileptic encephalopathy. In 60% of patients there was one of the treatable IMD. Detailed metabolic investigations can lead to earlier identification and treatment of patients with epileptic encephalopathy.

25) N-CARBAMYLGLUTAMATE - A POTENTIAL LONG-TERM TREATMENT OPTION FOR PA AND MMA PATIENTS

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Introduction: Propionic aciduria (PA) and methylmalonic aciduria (MMA) are caused by a deficiency of propionyl-CoA carboxylase and methylmalonyl-CoA mutase and are characterized by elevated propionic acid and methylmalonic acid in the blood and urine. This results in the reduced N-acetylglutamate levels, an activator of the synthesis of carbamyl-phosphate. A frequent complication of these conditions is hyperammonemia. Treatments with ammonia scavengers including sodium benzoate and N-carbamylglutamate (NCG) are appropriate for use in acute severe decompensation. However, the effect of long-term NCG treatment on the rate and severity of decompensations due to propionic aciduria and methylmalonic aciduria is not known.

Methods: Data from patients with PA or MMA who experienced episodes of metabolic decompensation requiring hospitalization at the University of Padova in the last 2 years, failure to thrive and poor appetite, and received NCG were analyzed.

Results: Eight patients with PA ($n = 4$) or MMA ($n = 4$) aged 1.5–20 years were studied. Patients received a dose of NCG (50 mg/kg/day) for a treatment period of 6 to 16 months. In all patients, the number of episodes of metabolic decompensation decreased after initiation of NCG, with three of the patients having no episodes of metabolic decompensation after initiating treatment. After treatment, patients had an increase in natural protein intake of 20% to 50% and over this same treatment period patients gained 0–6.5 kg in weight. Parents reported improvements in attention, school activity, and appetite, and there was reduced need for gastrostomy in the patients enrolled.

Conclusions: This analysis demonstrates the long-term ability of NCG in stabilizing metabolic control in patients with severe PA or MMA, when administered as an adjunct to conventional management. Further studies in broader cohorts of patients are needed to confirm the results of this study, and to better define the correct drug dosage of NCG in this patient population.

26) NEW OBSERVATION OF SIALURIA PROMPTS DETECTION OF LIVER TUMOR IN PREVIOUSLY REPORTED PATIENT

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Sialuria, a rare inborn error of metabolism, was diagnosed in a healthy 12-year-old boy through whole exome sequencing. The patient had experienced mild delays of speech and motor development, as well as persistent hepatomegaly. Identification of the 8th individual with this disorder, prompted follow-up of the mother-son pair of patients diagnosed over 15 years ago. Hepatomegaly was confirmed in the now 19-year-old son, but in the 46-year-old mother a clinically silent liver tumor was detected by ultrasound and MRI. The tumor was characterized as an intrahepatic cholangiocarcinoma (IHCC) and DNA analysis of both tumor and normal liver tissue confirmed the original GNE mutation. As the maternal grandmother in the latter family died at age 49 years of a liver tumor, a retrospective study of the remaining pathology slides was conducted and confirmed it to have been an IHCC as well. The overall observation generated the hypothesis that sialuria may predispose to development of this form of liver cancer. As proof of sialuria in the grandmother could not be obtained, an alternate cause of IHCC cannot be ruled out. In a series of 102 patients with IHCC, not a single instance was found with the allosteric site mutation in the GNE gene. This confirms that sialuria is rare even in a selected group of patients, but does not invalidate the concern that sialuria may be a risk factor for IHCC.

27) A NOVEL KNOCK-IN MOUSE MODEL THAT RECAPITULATES PATHOBIOLOGY OF HUMAN PMM2-CDG DISEASE

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The most common Congenital Disorder of Glycosylation, PMM2-CDG, is caused by mutations in PMM2 that greatly reduce essential manose precursors for protein N-glycosylation. There is no therapy and no viable mouse model to test efficacy. To fill this gap, we generated hypomorphic mice containing compound heterozygous mutations, R137H/F115L, corresponding to the most prevalent human alleles, R141H/F119L. Pmm2 R137H/F115L mice showed embryonic and post-natal lethality while survivors had significantly stunted growth. Growth-related glycoproteins IGF-1, IGFBP-3, and acid-label subunit, along with antithrombin III, were all significantly deficient in Pmm2 R137H/F115L mutants, similar to PMM2-CDG patients. Their levels in Pmm2 R137H/WT and Pmm2 F115L/WT heterozygous were comparable to wild-type litter mates. Fibroblasts from Pmm2 R137H/F115L mice mimicked PMM2-CDG patient-derived fibroblasts. Both had reduced PMM2 activity, GDP-mannose, lipid-linked oligosaccharide precursor, total cellular protein mannosylation, and hypoglycosylation of a new endogenous glycoprotein biomarker, gp130. Over-expression of wild-type PMM2 in patient-derived fibroblasts rescued all these defects showing that restoration of mutant PMM2 activity is a viable therapeutic strategy. This functional mouse model of PMM2-CDG, in vitro assays, and the novel gp130 biomarker provide the essential tools to test potential therapeutics for this currently untreatable disease.

28) RECAPITULATION OF METABOLIC DEFECTS IN A MODEL OF PROPIONIC ACIDEMIA USING PATIENT-DERIVED PRIMARY HEPATOCYTES

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Background: Propionic acidemia (PA) is a disorder of intermediary metabolism with defects in the alpha or beta subunits of propionyl CoA carboxylase (PCCA and PCCB respectively) enzyme. We previously described a liver culture system that uses liver-derived hemodynamic blood flow and transport parameters to restore and maintain primary human hepatocyte biology and metabolism utilizing physiologically relevant milieu concentrations.

Methods: In this study, primary hepatocytes isolated from the explanted liver of a 8-year-old PA patient were cultured in the liver system for 10 days and evaluated for retention of differentiated polarized morphology. The expression of PCCA and PCCB was assessed at a gene and protein level relative to healthy donor controls. Ammonia and urea levels were measured in the presence and absence of amino acid supplements to assess the metabolic consequences of branched-chain amino acid metabolism in this disease.

Results: Primary hepatocytes from the PA patient maintained a differentiated polarized morphology (peripheral actin staining) over 10 days of culture in the system. We noted lower levels of PCCA and PCCB relative to normal healthy controls at the mRNA and protein level. Supplementation of branched-chain amino acids, isoleucine (5 mM) and valine (5 mM) in the medium, resulted in increased ammonia and decreased urea in the PA patient hepatocyte system, but no such response was seen in healthy hepatocytes or patient-derived fibroblasts.

Conclusions: We demonstrate for the first time the successful culture of PA patient-derived primary hepatocytes in a differentiated state that stably retain the PCCA and PCCB enzyme defects at a gene and protein level. Phenotypic response of the system to an increased load of branched-chain amino acids, not possible with fibroblasts, underscores the utility of this system in better understanding the molecular pathophysiology of PA and examining the effectiveness of potential therapeutic agents in the most relevant tissue.

29) PHENOTYPIC VARIABILITY IN *UMPS* HETEROZYGOES WITH OROTIC ACIDURIA

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Background: Orotic aciduria is clinically associated with disorders of the urea cycle and pyrimidine metabolism. Hereditary orotic aciduria (MIM #258900) is a disorder caused by a deficiency of uridine monophosphate (UMP) synthase. This bifunctional enzyme catalyzes the last two steps in the de novo pyrimidine biosynthetic pathway: orotate phosphoribosyltransferase (OPRT) converts orotic acid to orotidine-5'-monophosphate (OMP), and orotidine-5'-monophosphate decarboxylase (ODC) decarboxylates OMP to uridine monophosphate (UMP). Mutations within the *UMPS* gene (MIM *613891) are the only known cause of hereditary orotic aciduria. This autosomal recessive condition is extremely rare, with approximately twenty reported cases to date. Patients classically present with megaloblastic anemia in the first months of life. If untreated, this disorder can lead to neutropenia, failure to thrive, growth retardation, sparse hair and nail growth, developmental delay, and intellectual disability.

Methods: We identified mild orotic aciduria through initial metabolic screening of nine unrelated individuals with diverse clinical signs and symptoms, some of which overlapped with classical presentation. Genetic investigations were also performed.

Results/Conclusions: All individuals were found to have at least one rare variant in the *UMPS* gene on chromosome 3q13. Four of these variants were predicted to be null alleles with complete loss of function, and, to our knowledge, these are the first reported *UMPS* variants of their kind. The remaining variants were analyzed using several bioinformatics tools, and all missense changes were predicted to be damaging to the normal encoded protein using at least two different prediction programs. Because the presence of heterozygous *UMPS* variants was also evident in eight healthy family members, we conclude that such variants can lead to mild and isolated orotic aciduria without clinical consequence. Partial UMP synthase

deficiency should be included in the differential for any individual who has persistent orotic aciduria, with or without clinical sequelae. The discovery of heterozygotes manifesting clinical symptoms such as hypotonia and developmental delay are likely due to ascertainment bias.

30) ATAXIA PHENOTYPE ASSESSMENT IN A NEW MOUSE MODEL OF GALACTOSE-1 PHOSPHATE URIDYLTRANSFERASE (GALT) DEFICIENCY

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Background: Despite adequate dietary management, patients with Classic Galactosemia continue to have increased risks of developmental delay, speech problems, premature ovarian insufficiency, and abnormal motor development. A recent study of a new GALT-deficient mouse model revealed reduced fertility and growth restriction, phenotypes that are commonly seen in human patients. In this study, we further assess the fidelity of this new mouse model by evaluating the mutant mice for the manifestation of a common neurological sequela in human patients, cerebellar ataxia.

Methods: The balance, grip strength and motor coordination of 12 *GalT*^{-/-}, 5 *GalT*^{+/-}, 10 *GalT*^{+/+} mice between 6–10 months of age was assessed by the rotarod performance tests at different speeds. Results of the rotarod performance tests were compared to composite phenotype scoring tests, which evaluate coordination (ledge test), neurological/motor impairment (hind limb clasping), gait, and degree of kyphosis. The higher the composite phenotype score (on a scale from 0 to 12), the more severe the neuromotor impairment. Histological studies of cerebella were performed on hematoxylin and eosin (H&E), as well as calbindin D28k antibody-stained sections.

Results: In the rotarod performance tests, 87.5% of *GalT*^{+/+} and *GalT*^{+/-} mice were able to attain the one-minute mark at 6 rpm compared to 50% for *GalT*^{-/-}. At 12 rpm, 50% *GalT*^{+/+} and *GalT*^{+/-} were able to achieve the one-minute mark compared to 8.33% for *GalT*^{-/-}. For the composite phenotype scoring tests, we showed only 6.25% of all wild-type/heterozygous mice have composite score greater than or equal to 6, compared to 41.67% of the homozygous *GalT*-null mice under the same testing conditions. Measurements of the granular and molecular layers showed a 12.5% decrease in thickness ($p < 0.005$) in the *GalT*^{-/-} mice.

Conclusion: Our findings suggest the presence of an ataxia phenotype in the GALT-deficient mouse model with varying severities. This is in agreement with the varying degree of ataxia seen in human patients. Therefore, these animals can be a valuable tool for the study of the pathophysiology of Classic Galactosemia.

31) GNE PROMOTER DELETION: A NOVEL MUTATION IN SIBLINGS WITH GNE MYOPATHY

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GNE myopathy is an autosomal recessive muscle disease that presents with moderately progressive muscle atrophy, weakness, and muscular degeneration. The disease is caused by biallelic mutations in the *GNE* gene that codes for the bifunctional enzyme, uridine diphosphate (UDP)-N-acetylglucosamine (GlcNAc) 2-epimerase/N-acetylmannosamine (ManNAc) kinase. Since GNE is the rate-limiting enzyme in sialic acid biosynthesis, the pathophysiology is likely due to hyposialylation of muscle glycans (glycoproteins and glycolipids). Treatment for GNE myopathy is not available, but sialylation-increasing therapies are currently being developed for use in patients. For clinical trials to proceed, there is a need for accurate genetic diagnosis, especially for rare metabolic diseases.

We describe two siblings evaluated under our natural history protocol (www.clinicaltrials.gov, NCT01417533) who presented with anterior tibialis weakness and displayed the typical progressive pattern of muscle involvement in GNE myopathy. Muscle histology also revealed abnormalities characteristic of GNE myopathy, including marked variation in fiber size, scattered atrophic fibers, and rimmed vacuoles with some intracellular inclusions. In both patients, a targeted sequencing revealed a missense mutation in the *GNE* gene, NM_001128227.2:c.2179G>A (p.Val727Met), a known pathogenic mutation. In addition, the siblings had a c.52-8175_51+9417del variant, a deletion of ~9.9 kb encompassing the promoter region of most of the known *GNE* transcripts. Gene expression analysis using isoform-specific primers revealed reduction in the amount of all well-characterized coding transcripts of *GNE*. Further analysis of immortalized B-lymphoblastoid cell lines from the patients showed reduction in the amount of protein. These results, together with the clinical phenotype, suggest that the novel promoter deletion is likely pathogenic. Our study suggests consideration of deep non-coding regions of *GNE* for screening, to identify new patients for clinical trials or to establish a molecular diagnosis in those who have a clinical diagnosis of GNE myopathy but with a mutation only in one allele.

32) NEUROPSYCHIATRIC CONCOMITANT MEDICATIONS AND CO-MORBIDITIES IN PATIENTS WITH PHENYLKETONURIA: FINDINGS FROM THE PKUDOS REGISTRY

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Phenylketonuria (PKU) is a life-long inherited metabolic disease characterized by a defect in the metabolism of phenylalanine (Phe) which may lead to Phe accumulation and neuropsychiatric dysfunction. Sapropterin dihydrochloride (KUVAN®) is indicated to reduce blood Phe levels in tetrahydrobiopterin-responsive individuals with hyperphenylalaninemia in conjunction with a Phe-restricted diet. The Phenylketonuria (PKU) Demographics Outcomes and Safety (PKUDOS) registry is designed to provide longitudinal safety and efficacy data on subjects with PKU who are (or have been) treated with sapropterin dihydrochloride. As of September 16, 2015, 1447 subjects have enrolled in the PKUDOS registry with a mean enrollment duration of 47.2 ± 22.9 months. Baseline demographic characteristics include gender (female, 52.5%), race (white, 90.1%), and age at first sapropterin dose (mean, 16.5 ± 12.8 years). Of 1447 subjects, 90.3% reported use of concomitant medications (nutraceutical included) and 25.7% reported current use of at least one psychotropic or central nervous system medication. Of the 763 subjects assessed by their clinic for anxiety, 193 (25.3%) reported symptoms of anxiety (60.6% mild, 35.8% moderate, and 3.6% severe), of which 47 (24.4%) reported taking anxiety medication at baseline or during their first year in the registry. Of the 695 subjects assessed by their clinic for attention-deficit/hyperactivity disorder (ADHD), 170 (24.5%) reported symptoms of ADHD (39.4% mild, 53.5% moderate, and 7.1% severe), of which 85 (50.0%) reported taking ADHD medication at baseline or during their first year in the registry. Of the 752 subjects assessed by their clinic for depression, 133 (17.7%) reported symptoms of depression (57.9% mild, 36.1% moderate, and 6.0% severe), of which 48 (36.0%) reported taking anti-depressants at baseline or during their first year in the registry.

Observational data in the PKUDOS registry suggest that subjects with PKU have neuropsychiatric co-morbidities often requiring adjuvant treatment, further supporting that routine assessment of neurocognitive and psychiatric status should be incorporated into the routine management of PKU.

33) LONG-TERM ECONOMIC COSTS OF ORTHOTOPIC LIVER TRANSPLANT VERSUS STANDARD MEDICAL THERAPY FOR INTOXICATION-TYPE METABOLIC DISORDERS

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Background: Orthotopic liver transplant is a viable long-term treatment for some intoxication-type metabolic diseases, including the organic acidemias and urea cycle disorders. Few long-term studies have focused on the long-term medical economic costs of transplanting vis-a-vis continued medical therapy. We identified 39 patients currently followed at Children's National with severe Methylmalonic Acidemia, Propionic Acidemia, Argininosuccinic Aciduria, and Citrullinemia. Of these, 10 received orthotopic liver transplantation and 29 still receive standard medical therapy. We measured the downstream medical costs of these two cohorts.

Methods: Quantitative cost data on each patient was collected through enterprise systems from Children's National Health System and MedStar Georgetown University Hospital, including all subsequent hospitalizations, treatments, and prescriptions that were attributed to either transplant or metabolic disease. All cohorts were separated into those with and without transplant. We selected these specific disorders because they are identifiable by newborn screening.

Results: Patients with liver transplants had less long-term medical costs than those following standard medical therapy.

Conclusions: Given these findings, it could be inferred that liver transplantation may be considered a viable medical procedure for qualifying metabolic patients regarding long-term medical costs. Further studies regarding long term health outcomes and medical costs from a larger patient sample should be pursued.

34) BIOTINIDASE DEFICIENCY: A GENOTYPE-PHENOTYPE CORRELATION

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Background: Biotinidase deficiency (BTD) is a rare autosomal recessive disorder of biotin metabolism, usually detected by newborn screening of biotinidase activity. As newborn screening is not systematic in Lebanon, BTD patients are still diagnosed late when symptomatic. Clinical manifestations include neurological and cutaneous signs of variable severity, mainly psychomotor delay, seizures, deafness and skin rash. Treatment with Biotin can improve neurological manifestations and sometimes reverse hearing loss. So far, more than 165 mutations on chromosome 3p25 causing profound BTD have been described. However, genotype-phenotype correlation is not well established.

Objectives: To study the genotype-phenotype correlation in BTD patients.

Methods: Retrospective review of patients diagnosed with BTD at the Inherited Metabolic Diseases Clinic at AUBMC, from 2000 to 2015.

Results: Six families with BTD were identified. However, only two families with profound BTD (null activity) were genetically studied. Two siblings diagnosed, respectively at 36 and 13 months of age, had a previously unreported homozygous mutation in exon 2 of BTD gene (c.203_206dupTCCT p.Ser70Profs*23) associated to intractable seizures, alopecia and hearing loss. Biotin therapy improved hearing loss and controlled seizures. Another previously unreported mutation in exon 4 of BTD gene (c.1420G>T p.Gly474*) was detected in the second family. Index patient was diagnosed at 20 months of age with developmental delay, intractable seizures, optic atrophy and deafness. Biotin therapy decreased seizures frequency with no effect on hearing loss.

Conclusion: Two novel null mutations associated with reversible hearing loss and/or seizures control are reported in a country where newborn screening for BTD is still not systematic. Genetic screening in correlation with enzyme activity may help determine prognosis and biotin responsiveness in symptomatic, late-diagnosed patients.

35) THE UREA CYCLE DISORDERS CONSORTIUM: HIGHLIGHTS

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In 2003, the Urea Cycle Disorders Consortium (UCDC) was established as one of the first 10 consortia of Rare Disease Clinical Research Network (RDCRN), funded by the Office of Rare Diseases Research and the Eunice Kennedy Shriver National Institute of Child Health and Human Development with 5 participating institutions. The goals of the UCDC are to 1) further understanding of the urea cycle disorders (UCD); 2) develop and conduct clinical trials of novel therapies that improve survival and outcome; 3) create resources with information on UCD for clinicians, researchers, and patients; and 4) train the next generation of UCD investigators. The UCDC has established partnerships with the National Urea Cycle Disorders Foundation (NUCDF), philanthropic foundations and industry.

Now in its third 5-year funding cycle, the RDCRN is comprised of 22 Consortia and the UCDC has grown to include 16 sites: 13 in the US, one in Canada, one in Switzerland and one in Germany. The UCDC has conducted 9 major studies and 1–2 pilot and trainee projects each year, the largest of which is a longitudinal, natural history study with 742 enrolled as of November 30, 2015. We have gained insight into the pathophysiology and outcome of UCD and partnerships with industry have enabled the development and FDA approval of 3 orphan drugs to treat these disorders. The UCDC illustrates that multi-institutional collaboration is critical for better understanding of rare genetic disorders and for the development of new safe and effective therapies.

36) DIAGNOSIS, TREATMENT AND CLINICAL OUTCOME OF PATIENTS WITH LONG-CHAIN 3-HYDROXYACYL-CoA DEHYDROGENASE (LCHAD) DEFICIENCY

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Long-chain 3-hydroxy acyl CoA dehydrogenase (LCHAD) deficiency is a disorder of long chain fatty acid oxidation, presenting with hypoketotic hypoglycemia after fasting or illness, and variably associated with cardiomyopathy, liver dysfunction, and rhabdomyolysis. Because LCHAD deficiency can be life-threatening if untreated, it is included in most expanded newborn screening (NBS) programs. However, the impact of early treatment on long-term clinical outcome is still unclear. Moreover, there is variability in clinical management, particularly regarding carnitine supplementation. We have reviewed clinical and biochemical data in five patients with LCHAD deficiency (three patients identified by NBS) followed regularly in the Metabolic Clinic from the time of diagnosis. All patients (three males, two females; current ages 2 to 22 years) had signs and symptoms related to LCHAD deficiency. Treatment was started shortly after diagnosis: a diet restricted in long-chain fat and supplemented with MCT, essential fatty acids and low-dose carnitine (25 mg/kg per day). All patients achieved normal growth and development, with generally good metabolic control. Nevertheless, all patients but the youngest (2 years old) developed pigmentary retinopathy. Long-chain hydroxylated acylcarnitines did not change significantly with age, but did correlate with creatine kinase increase. Carnitine deficiency was never present in our patients following low-dose supplementation. We did not observe a correlation between long-chain hydroxylated acylcarnitines and carnitine levels, or an increased toxicity due to carnitine supplementation. Patients with LCHAD deficiency can have normal growth and development with proper treatment, which includes low-dose carnitine supplementation.

37) TRENDS IN HEALTH-RELATED QUALITY OF LIFE IN FAMILIES WITH INBORN ERRORS OF METABOLISM

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Background: Children with inborn errors of metabolism (IEMs) are living longer, healthier lives due to early detection and implementation of medical therapies. However, families face many challenges in caring for children with IEMs, due to complexities of medical and dietary treatments. In addition, frequent clinic visits, and in some instances, increased numbers of hospitalizations add stress to family life. Health-related quality of life (HRQoL) research can improve our understanding of parent and child perceptions of living with chronic disease.

Aims: The aim of this study was to explore trends in HRQoL in parents and children with diverse IEMs.

Methods: The Pediatric Quality of Life Inventory (PedsQL™) was administered to nineteen parents and children during routine metabolic clinic visits. Categories of IEMs for the children included phenylketonuria (PKU), glutaric aciduria type 1 (GA-1), ornithine transcarbamylase deficiency (OTC), medium-chain acyl-CoA dehydrogenase deficiency (MCAD), galactosemia, and neuromuscular disorders (NMDs). PedsQL™ scores for functional domains of physical, emotional, social, and school performance were compared for each parent/child dyad and for the different categories of IEMs.

Results: Across PedsQL™ domains, parents reported greater perceived difficulties than their children in each functional domain. For all IEM groups combined, the most significant discordances between parent/child functional scores were in the areas of emotional and social domains. For families of children with NMDs, the most discordance was demonstrated within social functioning, however, parents perceived greater social challenges than reported by children. The opposite pattern was revealed for families in the protein disorders grouping (PKU, GA-1, OTC). Children perceived greater difficulties than parents in all domains except emotional functioning. The greatest discordance between parent and child was in the domain of school performance. The degree of discrepancies between parent and child responses within the protein disorders grouping was less overall than all domains in the NMD category.

Discussion: Recognizing perceptual gaps between parents and children is important for understanding potential sources of anxieties and concerns in daily living. Health-Related Quality of Life (HRQoL) measurements provide beneficial information for intervention efforts and for recommending services for preserving family functioning. For future work, studies can be conducted to examine how child and parent-proxy reports change over time as children age. Additionally, investigations are needed to examine the impact of having a parent with chronic illness on the PedsQL™.

38) L-ASPARTATE AS POTENTIAL TREATMENT FOR CITRULLINEMIA TYPE 1 PATIENTS

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Background: Citrullinemia type 1 is an autosomal recessive urea cycle disorder caused by mutations in the ASS1 gene. It is characterized by increased plasma citrulline and urinary orotic acid, and decreased plasma arginine. Of the >65 ASS1 missense mutations reported, 21 map in or near the site for substrates binding (aspartate or citrulline), and thus, are potential kinetic mutations whose decreased activities could be rescued by the administration of L-aspartate (according to a previous report by Berning C. et al, Hum Mutat, 2008).

Methods: We used an E. coli expression system to study all known potentially kinetic ASS1 mutations. All mutations plus the wild-type enzyme were nickel-affinity purified, their activity and kinetic parameters were measured using tandem mass spectrometry and their thermal stability using Differential Scanning Fluorimetry.

Results: All 21 mutants could be expressed and purified. Fifteen mutants were totally inactive while six exhibited decreased affinity for aspartate and citrulline. One of these kinetic mutations (A118T) exhibited in addition decreased thermal stability. The function of these six kinetic mutations (P96S, P96H, A118T, R272H, R272C, R272L) could be rescued to ~20–80% of the wild-type ASS activity with doses up to 5 mM of L-aspartate.

Conclusions: The activity of ASS1 kinetic mutations with some residual activity seems to be rescued in our in vitro system by L-aspartate administration. Although the six kinetic mutations here characterized may affect only some of the ASS deficient patients, treatment with L-aspartate could offer a novel and probably safe therapeutic alternative. Since L-aspartate is already available as a drug (as di-peptide L-ornithine-L-aspartate), such treatment could be directly implemented for further evaluation in citrullinemia type 1 patients.

39) EPIGENOMICS IN NEUROPATHOLOGY OF PAH DEFICIENT PKU IN THE PAHENU2 MODEL

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Introduction: Phenylalanine hydroxylase deficient phenylketonuria (PKU) is the paradigm of a treatable inborn error where improved outcomes are realized with reduced concentrations of circulating phenylalanine (PHE). While PHE is the intoxicating agent in PKU, the mechanism(s) whereby PHE toxicity leads to neurologic dysfunction remain elusive. Chemically diverse small molecule toxins are recognized to cause epigenome dysfunction leading to aberrant DNA methylation. Paralleling this, we hypothesized chronic hyperphenylalaninemia would impact the epigenome and contribute to PKU neuropathology.

Methods: Post-weaning, PAHenu2 animals were either chronically hyperphenylalaninemic or under continuous PHE restriction. Heterozygous litter mates served as controls. DNA was prepared from brain tissue, methylated DNA enriched by immunoprecipitation and utilized to create libraries assessed by paired-end sequencing. Informatic data reduction compared cases to controls to identify differential methylation. Expression microarray analysis assessed the impact of methylation on gene regulation.

Results: Differential methylation of gene coding regions was observed in hyperphenylalaninemic and PHE restricted animals; however, hyperphenylalaninemic animals showed increased methylation. Differential gene body methylation targeted microRNA (miRNA) genes including a cluster in the imprinted Dlk1-Dio3 locus. Upregulated expression of several miRNAs was observed with concurrent down-regulation of target genes.

Differential promoter hypermethylation of protein coding genes was restricted to hyperphenylalaninemic animals and down-regulated expression was observed.

Conclusions: Aberrant DNA methylation is prominent in the brain of hyperphenylalaninemic animals and attenuated in PHE-restricted animals. Several genes with synaptic involvement are significantly down-regulated in hyperphenylalaninemic animals while in PHE-restricted animals expression is equivalent to controls. Similarly, dysregulated expression of miRNAs is prominent in hyperphenylalaninemic animals while similar to controls in PHE-restricted animals. Loss of epigenome homeostasis may be contributing to the neuropathology of PKU.

40) UNTARGETED SMALL MOLECULE METABOLOMIC PROFILING IN MILD CASES OF ZELLWEGER SPECTRUM DISORDERS REVEALS NEW DIAGNOSTIC BIOMARKERS

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Peroxisomes are involved in a variety of metabolic functions. Patients with Zellweger spectrum disorders (ZSDs) have impaired peroxisome biogenesis and multiple metabolic derangements resulting in elevations of very long chain fatty acids, pristanic acid, phytanic acid, pipecolic acid, and bile acid intermediates; and reduced levels of plasmalogens. The ZSDs comprise a clinical spectrum consisting of most severe Zellweger syndrome, and progressively milder phenotypes of neonatal adrenoleukodystrophy, and infantile Refsum disease. The phenotype of ZSDs is heterogeneous, ranging from intractable seizures, neuronal migration defects, neonatal hypotonia and death within 1 yr of life, to mild developmental delay, adrenal insufficiency and hearing loss. We performed untargeted small molecule metabolomic profiling on plasma samples from a cohort of 12 ZSD patients with mutations in PEX1, primarily compound heterozygous genotypes with one G843D allele. Not surprisingly, results from our metabolomic profiling showed elevated pipecolic acid, very long chain fatty acids, and several bile acids and reduced plasmalogens. Along with perturbations in the expected metabolites, we also observed perturbations in multiple analytes suggesting a global metabolic phenotype in PBD. For example, a significant reduction in sphingomyelin in every patient sample was observed, which was not observed in over 300 other samples. Sphingomyelin is a genuine biomarker for ZSD with a significant pvalue of 5.39E – 09 and a false discovery rate of 0.00061. The observation of reduced sphingomyelin was one of the strongest effects observed in these samples and was facilitated by untargeted metabolomic profiling. This observation contrasts with reports of accumulations of sphingomyelins in red cell membranes and may reflect altered distribution of these lipids in plasma samples in PBD. Sphingomyelin can be used as a biomarker for clinical trials, response to therapies, and for prognostic monitoring in PBD. Results from metabolomic profiling are concordant with values generated by quantitative clinical assays. The perturbations in the metabolomic profiles of plasma from these patients are unique, specific, and not previously seen in over 300 other samples analyzed as normal controls or for other indications. All of the patients in this cohort fall into a mild (9) and milder (3) spectrum of ZSD, with a common presentation of developmental delay, hearing loss, liver disease, and microcephaly. For such undifferentiated phenotypes, the differential diagnosis is often very long and includes metabolic, neurometabolic, genomic, and other Mendelian disorders. Our untargeted metabolomic screening approach that identifies several biomarkers is the most effective way to detect these mild cases of ZSD and end the diagnostic odysseys for these patients.

41) WHEN TWO CONDITIONS WITH SIMILAR FEATURES MEET: A CASE OF ERDHEIM CHESTER DISEASE IN A PATIENT WITH A COMMON GENETIC DISORDER

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Background: Primary Hemochromatosis is the most common genetic disease in Caucasians causing iron overload. About 1:10 Caucasians carry one copy of C282Y in the HFE gene. Clinical manifestations such as fatigue, joint pain, weight loss, hypogonadism, diabetes insipidus, arrhythmia and liver disease may not manifest until age 50. Hemochromatosis has overlapping features with Erdheim–Chester Diseases (ECD) which can also manifest at age 50 with fatigue, hypogonadism, diabetes insipidus among other manifestations. Here we present a case of ECD that remained undiagnosed for 5 years since the initial manifestation was diabetes insipidus thought to be secondary to the patient's known diagnosis of hemochromatosis.

Methods: A 53-year-old Caucasian male with diagnosis of hemochromatosis and ECD was evaluated at the NIH Clinical Center as part of ECD Natural History study. Clinical evaluations and images such as brain/pituitary/orbital/abdominal/pelvic MRI, CT scan of the heart and chest, FDG-PET and Tc-99 bone scans were performed.

Results: Skin biopsy and imaging findings confirmed ECD. Molecular testing detected the BRAF V600E mutation. Excess iron was seen on brain MRI, but no other complications associated with hemochromatosis were seen. The presence of ECD was seen in the bones, kidneys, heart, skin and pituitary stalk. Mild cerebral atrophy was reported. Endocrine abnormalities included hypogonadism and diabetes insipidus. Patient was treated with interferon (IFN) alpha, but because of side effects, therapy was modified to Anakinra. Today he is on BRAF/MEK inhibitor therapy.

Conclusions: Having a common and a rare disorder that share clinical manifestations can also be rare, but not impossible. Having a diagnosis does not make one immune to other common or rare diagnoses. When manifestations sound similar, but there is something that doesn't add up, keep looking and keep looking. New treatments are becoming available for rare diseases so it is important not to miss now treatable conditions.

42) HEALTH SYSTEM IMPACT OF FALSE-POSITIVE NEWBORN SCREENING RESULTS FOR MEDIUM-CHAIN ACYL-CoA DEHYDROGENASE DEFICIENCY AND PHENYLKETONURIA IN ONTARIO, CANADA

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Objectives: Many newborn screening (NBS) programs across the world recently celebrated 50 years of important health benefits for babies affected with rare diseases. However, the impact of false-positive NBS on services use (HSU) remains poorly understood. Ontario is Canada's most populous province with 140,000 births per year and universal, publicly-funded health care. We describe the frequency of false-positive NBS results for medium-chain acyl-CoA dehydrogenase deficiency (MCADD) and phenylketonuria (PKU), and compare HSU in the first few years of life among infants with false positive results to those with screen-negative results.

Methods: Eligible infants were those who underwent NBS in Ontario between April 1, 2006–March 31, 2010 for MCADD, and; April 4, 2006–November 4, 2012 for PKU. All infants were identified as false-positive or screen-negative. Emergency department (ED) visits, inpatient hospitalizations and physician visits occurring from birth until end of follow-up were summed and rates of HSU were calculated using person-years. Rate ratios (RRs) of HSU compared all children who screened false-positive for MCADD or PKU with all eligible screen-negative children in the respective birth cohort. RRs were adjusted for age, sex, gestational age, birth weight, socioeconomic status and rural/urban residential status.

Results: In the MCADD cohort, 43/545,355 (0.007%) eligible infants screened false-positive. Adjusted RRs comparing children who screened false-positive for MCADD with screen-negative children were: 0.96 (95% CI: 0.67–1.40) for ED visits; 1.57 (95% CI: 0.82–3.01) for hospitalizations and; 1.22 (95% CI: 1.03–1.46) for physician visits.

In the PKU cohort, 57/877,299 (0.006%) screened false-positive. Adjusted RRs comparing children who screened false-positive with those who screened negative were: 1.18 (95% CI: 0.99–1.44) for ED visits; 5.09 (95% CI: 3.80–6.86) for inpatient hospitalizations, and; 2.97 (95% CI: 2.71–3.26) for physician visits.

Age-stratified analyses in both birth cohorts indicated that associations between false-positive NBS results and HSU were strongest in the first year of life: for MCADD, children with false-positive results no longer had significantly higher HSU after 1 year of age, while hospitalizations and physician visits for children with false-positive NBS for PKU remained higher past 1 year of age.

Conclusion: Infants in Ontario with false-positive results for MCADD and PKU had higher frequencies of hospitalizations and physician visits compared with screen-negative controls. In children with false-positive NBS results for MCADD, these findings may be explained by residual confounding related to illness, visits related to follow-up diagnostic evaluation, and/or a psychosocial effect on parents' perception of their child's health. In children with false-positive NBS results for PKU, findings may be residually confounded by prematurity and/or underlying illness that requires total parenteral nutrition (TPN), as newborns with NBS results suggestive of TPN in Ontario are referred for diagnostic evaluation. Overall, our findings underscore the importance of a health system that can effectively support children with false-positive NBS results and their families.

43) BEHAVIORAL PHENOTYPE IN A LIVER TRANSGENIC MOUSE MODEL (MUT^{-/-};TgINS-ALB-MUT) OF METHYLMALONIC ACIDEMIA

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Background and Objectives: Methylmalonic acidemia (MMA) causes multisystemic disease, characterized by both acute metabolic crisis and chronic, progressive end-organ damage, and requires lifelong therapy with protein-restricted diets. Recently, liver transplantation has been

employed to improve stability in metabolically labile patients, yet these patients remain at risk for significant metabolic neurological injury, or metabolic stroke. We therefore evaluated the baseline neurological phenotype of transgenic $Mut^{-/-}$ mice that express Mut in hepatocytes under the control of the mouse albumin promoter ($Mut^{-/-};TgINS-Alb-Mut$) as a model for the neurological manifestations of MMA. Although these mice are rescued from neonatal lethality by the low level expression of Mut , the animals display massive elevations of methymalonic acid and 2-methylcitrate in brain extracts and, under dietary stress, develop the renal disease of MMA.

Methods and Materials: $Mut^{-/-};TgINS-Alb-Mut$ mice ($N = 9$, 66.6% males) and their heterozygous littermates ($N = 25$, 64% male) were generated by timed breedings with dams maintained on high fat rodent chow for breeding animals (PicoLab Mouse Diet 20; 20% protein, 9% fat).

Results: Nine $Mut^{-/-};TgINS-Alb-Mut$ mice began the early developmental testing period, with 2 deaths between the second and third weeks of life (77.8% survival) with no subsequent deaths throughout the testing period; the two affected pups demonstrated marked neurological decompensation within 24–48 h of death, were not able to be tested during this time, and thus were excluded from analysis. Among survivors, there were no statistically significant differences between $Mut^{-/-};TgINS-Alb-Mut$ mice and their heterozygote littermates.

Conclusions: Hepatic express of Mut rescues the neonatal phenotype and appears to confer protection against severe neurological changes under a relatively normal diet. Whether dietary stress will influence the neurophenotype remains to be explored, but the results suggest that intrinsic neurological injury does not appear to be a feature of this mouse model, despite the markedly elevated metabolites in both the plasma and CSF. The $Mut^{-/-};TgINS-Alb-Mut$ mice were nutritionally supported throughout the testing period using the PicoLab Mouse Lab Diet 20, which contains at least twice the fat content of standard rodent chow and 3% less protein content than standard rodent chow. The additional caloric support provided by this chow may have contributed to the neurological status of the knockout mice. The data from these mice, which were not under significant nutritional or metabolic stress, serve as the benchmarks for further studies on neurological outcomes of $Mut^{-/-};TgINS-Alb-Mut$ mice under stress and using therapeutic interventions to prevent lasting injury.

44) FUNCTIONAL STUDIES REMAIN THE BEST STRATEGY TO CONFIRM THE DIAGNOSIS OF PRIMARY CARNITINE DEFICIENCY

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Background: Primary carnitine deficiency is caused by mutations in the SLC22A5 gene encoding the OCTN2 carnitine transporter. This condition can present in children with hypoketotic hypoglycemia or cardiomyopathy and with sudden death in children and adults. The lack of functional carnitine transporters results in low carnitine levels detectable at birth by newborn screening and biochemically supported by low plasma carnitine levels. A precise diagnosis is confirmed by measuring reduced carnitine transport in fibroblasts or by SLC22A5 gene sequencing. It is unclear if functional studies are always necessary for diagnostic confirmation and how well molecular studies predict pathogenicity of missense variants.

Methods: Carnitine transport was measured in skin fibroblasts of 382 patients suspected of having primary carnitine deficiency because of clinical presentation, abnormal newborn screening or family history. Each of the 10 exons of the SLC22A5 gene was sequenced in 95 patients whose fibroblasts had reduced carnitine transport. Missense mutations identified in the OCTN2 transporter were stably expressed in CHO cells to determine their effect on carnitine transport.

Results: Carnitine transport was reduced to 20% or less of normal in fibroblasts of 142/382 subjects referred for possible primary carnitine deficiency. SLC22A5 gene sequencing in 95 of the 142 affected subjects identified 159 possibly causative variants in 84% of the alleles. Expression of 89 missense variants (some obtained from additional patients) in CHO cells demonstrated that 76 of them reduced carnitine transport, while 13 failed to reproduce defective carnitine transport. Studies of fibroblasts from patients carrying 2 of these putative mutations demonstrated that the sequence change affected RNA splicing and reduced mature OCTN2 mRNA levels. Common prediction algorithms (SIFT, Polyphen-2) correctly predicted the functional effects of expressed missense mutations in about 70% of cases.

Conclusions: Primary carnitine deficiency is caused by heterogeneous variations in the SLC22A5 gene. Measurement of carnitine transport in fibroblasts remains the best strategy to confirm or exclude a diagnosis, since DNA analysis fails to identify causative variations in about 16% of the alleles and existing software can correctly predict the pathogenicity of missense variants in only 70% of cases.

45) SEVERE COBALAMIN DEFICIENCY IN THREE FAMILIES DUE TO DIFFERENT MECHANISMS

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Background: Cobalamin (Vitamin B12) deficiency may be due to dietary restrictions, abnormal absorption, or inactivation due to nitrous oxide (N_2O). Nitrous oxide is used as an anesthetic, an analgesic, an oxidizer in motor racing, and a recreational drug. We present three patients with severe cobalamin deficiency, due to different mechanisms

Results: 1: An 18-year-old male was evaluated for generalized weakness, difficulty walking, and numbness of fingers and feet. He had elevated plasma methylmalonic acid (MMA), elevated total homocysteine (tHcy) and reduced cobalamin. He reported regularly inhaling N₂O from tanks intended for motorcycle racing. His mother and brother reported having had similar episodes.

2: A 9-month-old entirely breast-fed infant presented with developmental delays, hypotonia, and worsening oral motor function. Routine lab testing revealed macrocytic anemia. Subsequent testing included elevated MMA, tHcy and extremely low Vitamin B12. Mother had asymptomatic pernicious anemia. The infant was likely affected both by transplacental antibodies and having been exclusively breast fed with vitamin B12 deficient milk. He improved dramatically with parenteral vitamin B12.

3: A home-born twenty-three month old male presented with failure-to-thrive, developmental delay and hand tremor. He was exclusively breastfed for 1 year, then was given a home-made vegan formula. He had elevated plasma MMA and tHcy level, very low vitamin B12. Elevated MMA and tHcy reversed with parenteral vitamin B12, but overall neurological status improved only slightly.

Conclusion and discussion: Three patients presented with severe cobalamin deficiency due to different interference mechanisms in the cobalamin pathway. These cases highlight the importance of considering cobalamin deficiency in the differential diagnosis of patients of various ages with a variety of neurological symptoms. Prompt recognition through the measurement of plasma MMA, tHcy and cobalamin level will allow prompt treatment.

46) SUCCESSFUL TREATMENT OF CDG SYMPTOMS BY GALACTOSE SUPPLEMENTATION IN PGM1 DEFICIENCY

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Phosphoglucomutase 1 deficiency is a novel type of congenital disorder of glycosylation with a multi-system phenotype. Normal PGM1 enzyme function is essential for glycosylation, and also for normal glucose and galactose metabolism. We report on the first prospective multicenter observational study in 9 patients with PGM1 deficiency receiving dietary galactose supplementation.

Participants in our pilot study received oral galactose supplementation over 18 weeks. D-Galactose intake started at 0.5 g/kg per day, increasing to 1.0 g/kg per day after 6 weeks and to 1.5 g/kg per day after 12 weeks. Maximal daily dose of galactose was 50.0 g, an amount that is within the recommended daily intake. Three of the patients remained on long-term supplements up to 24 months.

There was a significant improvement in transaminase values, coagulation, thrombolytic factors and in the endocrine status. Liver function and coagulation abnormalities restored within weeks on galactose supplementation but endocrine changes required longer treatment periods. No rhabdomyolytic episode and no progression in cardiac dysfunction occurred on therapy. Creatine kinase and glucose levels remained variable.

Our study confirmed safety and significant beneficial effects of galactose supplementation in patients with PGM1-CDG. Controlling hypoglycemia and muscle dysfunction remained challenging. A mean daily oral intake of 1 g/kg was sufficient to maintain 80–100% of normal glycosylation-related laboratory values within the first 4 months on supplementation, followed by 0.5 g/kg/day maintenance. Intra- and inter-individual compliance remained variable. One noncompliant patient deceased due to cardiomyopathy. One patient used higher doses against dietary advice, but without side effects. In summary galactose treatment was proved to be effective in improving CDG-related symptoms in PGM1 deficiency.

47) ASHKENAZI FOUNDER MUTATION IN USMG5, A REGULATOR OF ATP SYNTHASE, IS A NOVEL CAUSE OF AUTOSOMAL RECESSIVE MITOCHONDRIAL DISEASE

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Background: We report here 3 idiopathic Leigh syndrome patients from 2 unrelated Ashkenazi Jewish families in whom exome sequencing revealed a recurrent biallelic mutation in USMG5. Not previously implicated in human disease, USMG5 (upregulated in skeletal muscle 5) is known to regulate ATP synthase, or mitochondrial respiratory chain complex V. USMG5 has one transmembrane domain and is hypothesized to serve as a chaperone for assembly of the F₀ subunit of ATP synthase, although its exact function is unknown, and assembled ATP synthase no longer requires USMG5 for functionality. USMG5 knockdown in human cells dramatically decreases complex V levels and ATP synthesis.

Clinical Case Reports: Patient 1, the first child born to a non-consanguineous Ashkenazi Jewish couple had gross motor delay, first walking at 18 months. Acute ataxia and spasticity developed at 23 months following a fever. He was cognitively normal and had slowly progressive development until age 9 years, when he developed a progressive gait abnormality, abnormal ocular movement, lethargy and abnormal breathing that progressed to respiratory failure and ultimately death following prolonged sedation from anesthesia. Autopsy revealed dark brain discoloration and atrophy of the hypothalamus, midline thalamic nuclei, pons and periventricular grey matter, with atrophy and absent pigmentation of the

substantia nigra and locus coeruleus. Degeneration, spongiform changes, gliosis and swelling were noted in the thalamus, hypothalamus and brainstem, as well as moderate, patchy loss of cerebellar Purkinje cells. His pathologic diagnosis was Leigh encephalopathy.

Patient 2 was the younger brother of patient 1. He had neck hyperextension in infancy and developmental delay, first walking at 27 months with an ataxic gait. Acute regression occurred at 2.5 years, when he stopped walking. He was cognitively normal and had slowly progressive development but experienced motor regression and gait difficulties with each febrile illness. He died at age 6 years. Autopsy showed moderate brain edema, stenosis of the cerebral aqueduct, diffuse moderate gliosis, periaqueductal and periventricular congestion, as well as changes in the brain stem white matter. His pathologic diagnosis was Leigh encephalopathy.

Patient 3 was the first child born to consanguineous Ashkenazi Jewish parents who were 4th cousins, but unrelated to the first 2 patients. He came to medical attention at age 6 months for developmental delay and hypotonia. He had slowly progressive development, sitting at 10 months, crawling at 14 months, walking at 26 months, and first spoke at 18 months. Sudden ataxia and left leg limpness developed at 4 years, at which point he had 15 words, some 2-word sentences, little emotional expression, and textural sensitivity. Brain MRI showed multiple scattered foci of signal abnormality in the bilateral lentiform nuclei and periaqueductal grey matter. He has a clinical diagnosis of Leigh syndrome.

Whole exome sequencing on all three patients revealed a rare, homozygous splice variant in USMG5, c.87+1G>C. This mutation has been seen in 16/119,782 controls in the ExAC browser. Functional validation of USMG5 and complex V functions in fibroblasts from these patients is underway.

Conclusions: USMG5 is a critical regulator of mitochondrial complex V function. We report a homozygous recessive splice site USMG5 founder mutation in 2 unrelated Ashkenazi Jewish families as a novel cause of Leigh syndrome. Testing additional Ashkenazi Jewish Leigh syndrome patients may diagnose additional cases and inform the frequency of this newly recognized mitochondrial disease. These cases have the potential to further elucidate USMG5 function(s) in complex V regulation.

48) TARGETED METABOLOMIC PATHWAY ANALYSIS. A CASE STUDY IN CYSTINOSIS

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Background: Cystinosis (OMIM #219800) is diagnosed and monitored by assaying the accumulated intracellular cystine. We measured all the compounds involved in the cysteine, methionine and homocysteine metabolism and the γ -glutamyl cycle, as well as the mixed disulfides, in diagnostic specimens, and in subsequent samples after cysteamine treatment, to get further insight into the pathophysiology of the disease. The isopeptide N^{ϵ} -(γ -glutamyl)-lysine (iEK), a product of transglutaminase crosslinking, was also investigated, as a potential marker of fibrosis.

Methods: We have developed a targeted comprehensive-metabolite profiling liquid chromatography-mass spectrometry (LC-MS/MS) method, using a perfluorobenzylalkyl HPLC column able to measure simultaneously the 48 metabolites involved, using mixed leukocytes lysates (39 controls, 5 diagnostic specimens) with stable-isotope dilution (16 labeled standards) and diversion to remove sulfosalicylic acid (SSA), added to quench thiol exchange. iEK resolves from *L*-Lysyl-*L*-glutamate, and the other 2 possible isopeptides. SSA suppressed the ionization in negative mode.

Results: In the diagnostic samples, we did not detect any deficiency of reduced glutathione (GSH), though γ -glutamylcysteine and cysteinylglycine concentrations were reduced, perhaps reflecting a limitation of cysteine. Despite earlier reports of 5-oxoprolinuria in cystinosis, no difference was observed for 5-oxoprolinone. We were not able to measure adenosine triphosphate (ATP), since nucleotides are not stable at low pH. A moderate reduction of the sum of AMP and ADP was observed.

Endogenous cystamine and cysteine-cysteamine (the therapeutic intermediate) were reduced at baseline, but were elevated after cysteamine treatment, as previously reported for GSH. An increase of putrescine was also present. A difference from controls was observed for free N^{ϵ} -(γ -glutamyl)-lysine. A signal at *m/z* 231, tentatively thiocysteine-cysteamine, varied in proportion to cystine, very elevated at diagnosis and moderated after treatment.

Conclusions: Metabolomic pathway analysis in isolated cells provides metabolic and mechanistic insight. We are particularly interested in the role the persulfide thiocysteine, and its disulfides, as it is a source of hydrogen disulfide, and may play a role in cell signaling. γ -glutamyl-lysine may be a useful biomarker of disease activity in cystinosis.

49) ASPIRIN INCREASES MITOCHONDRIAL FATTY ACID OXIDATION DESPITE HIGH-LEVEL PROTEIN ACETYLATION

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More than 20,000 pounds of aspirin is consumed annually in the United States. Aspirin has been linked to Reye syndrome and metabolic dysfunction but the mechanisms are not understood. We hypothesized that aspirin would inhibit mitochondrial function in part by increasing lysine acetylation of metabolic enzymes. In cultured cells, aspirin induced high-level protein acetylation in a dose and time-dependent manner. Incubation of recombinant proteins with aspirin also caused lysine acetylation, indicating that the acetylation is due to direct transfer of the acetyl group from acetyl-salicylic acid to lysine. Overnight incubation of cells with aspirin increased acetylation of key fatty acid oxidation enzymes by several-fold. However, contrary to our hypothesis, mitochondrial function was increased by aspirin rather than decreased. Aspirin increased overall cellular oxygen consumption and mitochondrial palmitate oxidation, while at the same time reducing peroxisomal fatty acid oxidation by half. The effect of aspirin on mitochondrial fatty acid oxidation did not require SIRT3 deacetylation of mitochondrial target lysines, as SIRT3 knockout mouse embryonic fibroblasts responded similarly to aspirin. The aspirin-induced increase in fatty acid oxidation was largely abrogated when measured with palmitoylcarnitine as substrate, which bypasses carnitine palmitoyltransferase-1 (CPT-1). Together, our findings suggest that aspirin increases transport of fatty acids into mitochondria through CPT-1, and that despite the high level of protein acetylation inside the mitochondria, overall flux

through the pathway is increased. The aspirin-induced drive on mitochondrial fatty acid oxidation combined with an inhibition of peroxisomal fatty acid oxidation may have negative consequences for patients with genetic defects in mitochondrial energy metabolism.

50) PHARMACOLOGICAL CHAPERONES AS A NEW TREATMENT FOR CPS1 DEFICIENCY

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Background: Carbamoyl phosphate synthetase 1 (CPS1), a 1462-residue mitochondrial enzyme, catalyzes the entry of ammonia into the urea cycle, which converts ammonia, the neurotoxic waste product of protein catabolism, into non-toxic urea. CPS1 deficiency (CPS1D) is a rare autosomal recessive inborn error of the urea cycle, caused by mutations in the CPS1 gene (>200 mutations reported). Unless promptly treated, the hyperammonemia caused by CPS1D can lead to encephalopathy, coma and death or mental retardation. For patients with CPS1D, the only possible cure is liver transplantation. Current management is in many cases insufficient for achieving long-term metabolic stability and survival, highlighting the importance of searching for alternative therapeutic approaches.

Methods: We produced, purified and characterized recombinant human CPS1 in a baculovirus/insect cell expression system. We exploited this system to explore the disease-causing nature of 38 mutations identified in patients with CPS1D. We purified large amounts of purified wild-type CPS1 and performed a high-throughput screening with 11,000 compounds (including 1000 FDA approved drugs) to identify CPS1-stabilizing pharmacological chaperones. The hits obtained were subjected to detailed characterization of their functional and molecular effects.

Results: Our results proved that ~60% of the characterized CPS1 missense mutations trigger enzyme misfolding and/or destabilization. From the high-throughput screening, eight compounds bound and stabilized wild-type CPS1. Three of these did not substantially inhibit CPS1 activity.

Conclusions: Most mutations affecting CPS1 are proven to be destabilizing. Thus, we approach a potential future treatment of CPS1D, based on enzyme stabilization by ligands acting as pharmacological chaperones. Out of the 11,000 compounds screened, three bound, stabilized and did not inhibit CPS1 activity, and thus, are a promising novel therapy for misfolding CPS1 mutations. This novel treatment will have an enormous impact on the quality of life of CPS1D patients, since up to date the only curable treatment is liver transplantation.

51) PREDICTIVE MODELING OF ALTERED METABOLIC ACTIVITY IN PROPIONIC ACIDEMIA

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Inborn errors of metabolism give rise to global alterations in metabolic function. Many of these alterations cannot be predicted from enzyme function per se, due to the high degree of interconnectivity between metabolic reactions. However, these emergent changes in metabolism can be studied using genome-scale metabolic models (GEMs). Constraint-based and topological analysis of metabolic networks can yield insights into disease-related perturbations that occur in cellular metabolite uptake, production, and internal utilization. Thus, these models provide a platform for predicting potential biomarkers and therapies for inborn errors of metabolism, as well as a wide variety of other diseases affecting metabolic function. Furthermore, experimental data (e.g., transcriptomic data) can be integrated into the analysis of GEMs to improve the accuracy of the predictions that they generate. Here we present an analysis of a human hepatocyte GEM in the context of propionic acidemia (PA). Using a novel analysis methodology that integrates transcriptomic data from hepatocytes of healthy and diseased donors, we generated predictions of perturbations to metabolic function that are associated with PA. We show that many of our predictions are consistent with well-characterized aspects of the PA phenotype, including amino acid metabolism, energy production, and secreted metabolite production. In addition, we present novel hypotheses regarding altered metabolite usage in the disease state. To our knowledge, this is the first analysis of transcriptomic data from a PA patient utilizing a genome-scale metabolic reconstruction.

52) METABOLOMIC PROFILE SCREENING OF URINE FOR INBORN ERRORS OF METABOLISM

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Metabolomics is the study of the distinctive chemical fingerprint produced by specific cellular processes. Untargeted mass spectrometry-based metabolomic profiling of body fluids, such as urine, is an emerging technique used to produce and analyze this chemical fingerprint. This technology holds the promise of providing new insights into human disease states and serving as a primary diagnostic tool for novel and previously characterized inborn errors of metabolism (IEM). The purpose of this study was to determine the breadth of utility of using urine and an integrated metabolomic workflow for IEMs screening. To evaluate the feasibility of urine metabolomics for detecting IEMs, we analyzed 100 urine samples with our integrated metabolomic workflow comprised of four chromatographic techniques followed by mass spectrometry. The study cohort consisted of 35 samples from individuals previously diagnosed with an IEM but blinded to the definitive diagnosis, and 65 samples from individuals not diagnosed with an IEM. The resulting data was analyzed for biochemical signatures of IEMs to identify the specific diseases, and enriched biochemical signatures

of each disease using two different normalizations, osmolality or creatinine. The sample set consisted of 17 different IEMs representing disorders of aromatic amino acid metabolism, branch-chain amino acid metabolism, fatty acid catabolism, nucleotide degradation, nitrogen homeostasis, and neurotransmitter metabolism. Disorders normally detected using urine samples such as isovaleric acidemia and succinic semialdehyde dehydrogenase deficiency were identified with the metabolomic workflow. The workflow also was able to detect disorders and identify molecules not identified in plasma samples from subjects diagnosed with the same disease. These differences in biochemical signatures between the sample types could be explained by the role that the excretory system plays in maintaining the homeostatic balance of plasma by removing end products of metabolism and excess molecules from circulation. Further, it also supports the possibility of using urine more universally for screening, even for conditions typically assessed with other sample types. Finally, consistent disease assessments were achieved with either creatinine or osmolality-normalized data. This analysis combined with our previously reported study (Miller et al 2015), has identified biochemical signatures for over 30 IEMs, supporting the use of metabolomics as a tool to screen and identify disease signatures of IEMs in clinical samples. In addition to its rich biochemical profile, urine is a biological matrix that can be obtained through non-invasive means as compared to plasma, serum, and cerebrospinal fluid. Thus, metabolomics may offer a route to collapse dozens of assays and multiple sample types into a metabolomics screen of a single urine sample.

53) SIALYLATION-INCREASING THERAPIES FOR GNE MYOPATHY

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The adult onset muscular disorder GNE myopathy, also known as hereditary inclusion body myopathy (HIBM), is caused by mutations in GNE (identified in 2001), the key enzyme of sialic acid (SA) biosynthesis. Based on animal studies, the paucity of SA due to GNE deficiency likely causes hyposialylation of muscle glycans (glycoproteins and glycolipids), resulting in progressive muscle weakness and atrophy. Therefore, sialylation-increasing therapies are plausible approaches for GNE myopathy. In 2005, we tested intravenous immunoglobulin G (IVIG) injections in 4 patients, since the abundance of SA on IgG could potentially be utilized to sialylate other glycans. In 2010, we were involved in a single patient study of intramuscular or intravenous injections of GNE cDNA. Results of these early studies were encouraging but inconclusive. Additionally, they were either impractical (frequent IVIG injections) or with an unclear path for regulatory approval (gene therapy). Preclinical studies demonstrated that oral SA pathway supplementation (Neu5Ac, ManNAc, sialyllactose) ameliorated muscle decline and glycan hyposialylation in GNE myopathy mice, leading to the rationale for oral supplementation approaches to be evaluated clinically. Substrate supplementation with SA (Neu5Ac) is in a Phase 3 trial (Ultragenyx Pharmaceutical). In parallel, we evaluate twice-daily oral administration of the neutral SA precursor N-acetylmannosamine (ManNAc) in a Phase 2 trial (NIH-Escala Therapeutics).

Cellular and biosynthetic pathway properties as well as our recent pharmacokinetic (PK) results of ManNAc provide evidence for potential significant benefit. ManNAc is uncharged with an efficient cellular uptake (diffusion), while SA is negatively charged and acidic, which requires more complex cellular uptake (micropinocytosis). In fact, ManNAc is commonly added to cell cultures for full sialylation and/or growth of the cells and their intracellular compounds (such as recombinant proteins). Oral SA PK studies showed a significant first pass effect in rats and mice, necessitating design of alternative SA-delivery formulations for human trials. Our studies of oral ManNAc in GNE myopathy subjects demonstrated that twice-daily ManNAc administration was safe and well tolerated and led to sustained increases in plasma SA levels, confirming intracellular (where GNE is located) conversion of ManNAc to SA. These studies also suggested that twice daily oral dosing of ManNAc is sufficient to maintain increased sialic acid levels.

Ongoing clinical trials will assess efficacy of the sialylation-increasing therapies under investigation. The presented timelines of gene discovery and therapeutic investigations for GNE myopathy are illustrative for therapeutic development for many rare metabolic disorders, which is often a costly, time-consuming process.

54) COMBINED ANALYSIS OF DISEASE-SPECIFIC MARKERS FOR PATIENTS WITH SUSPECTED SPHINGOLIPIDOSIS

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Background: Several lysosomal storage disorders (LSDs), including Gaucher (GD) and Niemann–Pick (NPD) type A/B may present clinically with hepatosplenomegaly or isolated splenomegaly. Current biochemical analysis of these disorders requires enzymatic activity measurement. However, in GD it is not always possible to ascertain affected individual from a carrier based only on beta-glucosidase activity. In addition, the chitotriosidase expressed by lipid-laden macrophages is not GD specific and the number of false-negative results is significant due to the presence of a low activity polymorphic variants. Combined assay for detection of plasma markers in GD and NPD can significantly improve the diagnostic approach and testing algorithm of sphingolipidoses with common clinical phenotype.

Methods: Plasma samples from patients with abnormal beta-glucosidase activity (including confirmed GD (n = 5), confirmed heterozygous carriers (n = 3) and undetermined (n = 5) cases), NPD type A and B (n = 14) and healthy individuals (59 male, 60 female) were tested using a combined liquid chromatography/tandem mass-spectrometry (LC–MS/MS) method for detection of GD and NPD biomarkers, recently developed in our laboratory. The analytes include: glucosylsphingosine (GD), lyso-sphingomyelin, cholestane-3 β ,5 α ,6 β -triol and 7-ketocholesterol (NPD).

Results: Glucosylsphingosine proved to be highly reliable biomarker to discriminate GD patients (298–1230 nM) from carriers (<9 nM) and healthy individuals (<6 nM). In NPD type A or B, plasma lyso-sphingomyelin levels were significantly higher (319–5,220 nM) as compared to the healthy control group (<17 nM).

Conclusions: The biomarker analysis, a highly reliable quick-performance test, could change the current algorithm and may be used as first line screening in a larger population. We propose this combined biomarker assay as a first-line test for patients with splenomegaly/hepatosplenomegaly and sphingolipidosis. Further studies are necessary to verify its application to monitor the enzyme replacement therapy.

55) SUCCESSFUL IMPLEMENTATION OF THE NEW YORK STATE (NYS) X-LINKED ADRENOLEUKODYSTROPHY (X-ALD) NEWBORN SCREENING (NBS) MODEL. 20 MONTHS FOLLOW-UP OF TWO AFFECTED BROTHERS AND THEIR AFFECTED MOTHER

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On January 1st 2014, NYS started NBS for X-ALD. The rationale for screening was based on the accepted eligibility criteria for NBS and the premise that affected boys will develop symptom late allowing for effective therapeutic interventions. The use of bone marrow transplant (BMT)/hematopoietic stem cell transplant (HSCT) as a curative intervention was one of the strongest arguments for the screening. The model includes diagnostic, surveillance and therapeutic interventions as detailed elsewhere. The initial diagnosis, counseling, management, treatment and surveillance of a newly diagnosed newborn, his older brother and their mother are described. The index case, currently age 20 months, was born full term after uneventful pregnancy. Newborn period was unremarkable. On day 47 of life a second tier positive NBS for ALD was reported with elevation of C26:0. Results were discussed with the pediatrician and mother over the phone. Based on normal clinical assessment of the newborn an appointment was set for the following day. The 36-year-old mother, her 4-year-old older son and the proband were seen. A careful and very detailed discussion was held addressing all aspects related with X-ALD especially those linked with confirmatory diagnosis, molecular testing, surveillance and treatment. Biochemical and molecular testing confirmed the diagnosis of X-linked ALD (X-ALD) on the proband, his older brother and the mother. Accordingly consultations with neurology, endocrinology and BMT team were done in the following days. Based on the NYS model all affected members were included in a surveillance protocol. The older brother was found to have a normal brain MRI using X-ALD diagnostic criteria. He is on Lorenzo oil. On treatment his C26:0 levels are currently normal and his neurological and endocrinological assessments are normal. The proband, now age 20 months, is also asymptomatic and recently has his first brain MRI with normal results. The mother is also asymptomatic, has a normal neurological and endocrinological exam and has normal laboratory results. During the evaluation a sister of the proband was found not to be a carrier and more importantly being a match for BMT. Mutation analysis found a hemizygous missense mutation, p.Arg163His in the ABCD1 gene that was only reported once without genotype-phenotype correlation. Thus, the implementation of the NYS model showed its value in this case. Beyond the pure medical issues, this case demonstrated how intense and difficult, but doable was the process of communicating the initial results to the family considering the immediate, mediate and long term implications. Moreover, the implications for the family put the model to the test. In hindsight all the previous work done by the ALD NBS working group, of which we are active participants, was key for this success. Moreover, the active involvement of all sub-specialists at our institution as well as X-ALD experts, including neuroradiologists and neurologists in other USA centers of excellence have been an integral part of the management of this family. In summary, this family exemplifies the successful implementation of the NYS model for X-ALD NBS. Finally, it is worth mentioning that without the active and proactive involvement of the mother and her family this successful story probably would have not happened so smoothly. Thus, a team effort including the patient, family and health providers prove that NBS for X-ALD is doable and effective. Follow-up of further patient and families like this one will reinforce the value of this NBS initiative.

56) INCORPORATING ROUTINE METABOLIC GENE ANNOTATION INTO THE EXOME ANALYSIS PIPELINE FOR UNDIAGNOSED PATIENTS

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The NIH Undiagnosed Diseases Program (UDP) sees patients with illnesses that remain undiagnosed despite extensive medical investigations. Most participants suspected of having a metabolic component to their underlying disease have been evaluated using standard clinical metabolic testing before presentation to the UDP. We hypothesize that some of these patients have metabolic clues that were not recognized during standard testing. Several contributing factors might include: 1. levels of some metabolites that are elevated, but below the threshold for reporting; 2. combinations of mildly-elevated metabolites that may not rise to clinical significance, but might still point to the involvement of relevant pathways; and/or, 3. certain elevated metabolites that have not yet been associated with human disease. Furthermore, we hypothesize that some potentially revealing metabolic abnormalities may be identified using a combination of family-based controls, candidates from genomic studies and detailed patient phenotyping.

We present a strategy for the incorporation of metabolomics into a multifaceted process for identifying clues toward disease causation. Metabolic parameters will be included as metadata modeled after the annotations used for DNA sequence variants. A panel of genes, selected for known metabolic relevance, will be used to define genomic variants for directed study. In addition, we propose a strategy to optimize the collection of metabolic data for nuclear families, in which carrier-level metabolic abnormalities can be used to support the prioritization of candidate metabolite measurements.

Our pilot implementation of metabolic gene annotation for patients evaluated for an undiagnosed disease suggests that inclusion of metabolic gene annotation in the exome analysis pipeline efficiently highlights variants of potential metabolic interest while not overburdening the overall work flow.

In addition to identifying potentially causal Mendelian variants, exome analysis may yield metabolome-modifying variants that may secondarily contribute to pathogenesis, the evolution of phenotype, and the patient's associated metabolic profile. Routinely incorporating metabolic annotation into exome analyses establishes a baseline metabolic genotype that may assist in the interpretation of metabolic data sets.

57) NATIONAL STUDY TO ASSESS CURRENT PRACTICES OF THE MANAGEMENT OF PHENYLKETONURIA

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Introduction: In order to optimize neurocognitive and psychological outcomes, individuals with phenylketonuria (PKU) require lifelong treatment to maintain their blood phenylalanine (Phe) levels within a therapeutic range. PKU management differs between clinics, in particular the targeted blood Phe ranges. The American College of Medical Genetics (ACMG) published a management guideline in 2014, updating the previous National Institute of Health consensus statement published in 2000. The ACMG guideline recommends all individuals with PKU maintain blood Phe levels between 120–360 $\mu\text{Mol/L}$. This study was designed to examine current PKU management practices in clinics across the US, and to determine adherence rates to ACMG and clinic guidelines.

Methods: Dietitians and physicians with experience managing PKU developed a questionnaire that assessed US metabolic clinics' treatment guidelines and patient adherence to these guidelines. The primary objectives were to assess patient adherence to their blood Phe targets and clinic adherence to ACMG recommendations. This questionnaire was distributed by a consultant to 130 clinics following 20 or more PKU patients. Only one questionnaire was completed per clinic. Participants were strongly encouraged to leverage their clinic's PKU team and patient database to provide appropriate and accurate responses. The data analysis was performed by the same consultant.

Results: Forty-four out of 130 clinics completed the questionnaire (33.8% response rate). Most (80%) of the responders were from academic centers. Respondents reported on a total of 3772 PKU patients, representing approximately half of the patient population being followed in US metabolic clinics¹. On average, the clinics were actively following two-thirds (68%) of their PKU patient population. The remaining third were reported to be inactive as these individuals have not been seen in the past 3 years. Approximately 40% of patients were infants and children, 20% adolescents, and 40% adults, with 2% that were pregnant or planning to become pregnant. The mean lower and upper targeted blood Phe ranges for children and adolescents used in the clinics were reported to be consistent with the ACMG guidelines of 120–360 $\mu\text{Mol/L}$. For adults, the targeted ranges in the clinics were slightly higher than ACMG guidelines, with a mean upper limit of 445 $\mu\text{Mol/L}$. Only 31% of 18–29-year-olds and 22% of ≥ 30 -year-olds are reported with blood Phe levels consistent with the ACMG guidelines. The mean range recommended for pregnant women was between 115 to 300 $\mu\text{Mol/L}$. A higher percentage of infants and children (83% of 0–4 years and 68% of 5–12 years) had mean blood Phe levels within the clinic's targeted range, as compared to adolescents (46% of 13–17 years) and adults (36% of 18–29 years, and 27% of ≥ 30 years), except for women who were pregnant or planning to become pregnant (71%). The majority (89%) of clinics had recommendations on frequency of blood Phe testing with more frequent testing recommended for infants (< 1 year of age, 49 tests per year) and children (1–12 years of age, 20–26 tests per yr), than adolescents (13–17 years of age, 18 tests per year) and adults (≥ 18 years of age, 15 tests per year).

Conclusion: These results from a national survey distributed to clinics across the US represent approximately half of the PKU patient population actively followed in clinic. The clinics' targeted blood Phe range for infants and children was consistent with that of the ACMG guideline, though less than a third of clinics complied with these guidelines for adults. The percentage of patients achieving their target blood Phe levels progressively decreased with age, suggesting compromised metabolic control with increasing age. Improving patient blood Phe control provides an opportunity to address a challenge in PKU management, with the goal to improve outcomes for these patients.

¹Berry 2013, Genetics In Medicine, http://www.nature.com/gim/journal/v15/n8/fig_tab/gim201310t2.html

58) AROMATIC L-AMINO ACID DECARBOXYLASE (AADC) DEFICIENCY - A NOVEL MUTATION CLINICAL, BIOCHEMICAL FEATURES AND GENE THERAPY OUTCOMES

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Aromatic L-amino acid decarboxylase (AADC) deficiency (MIM#608643) is an autosomal recessive inborn error of neurotransmitter metabolism affecting both serotonin and catecholamine synthesis. Mutations in the DDC gene (OMIM#107930) encoding for the AADC enzyme is responsible for the deficiency and severe neurological presentation. Brun et al (2010) reported 24 different mutations including 8 novel mutations. We describe clinical and treatment outcomes in a biracial toddler of non-consanguineous parents with a novel DDC mutation - c.286G>A (p.Gly96Arg), not previously reported and a founder mutation - IVS6+4 A>T. The clinical presentation included normal age appropriate growth and development until 4 months of age, neurological deterioration following a single acute febrile illness resulting in severe global developmental delay, profound hypotonia, emotional lability, oculogyric crises, severe feeding difficulty requiring g-tube placement. Routine blood chemistries, neuroimaging and electroencephalographic findings were unremarkable. Usual biomarker seen in urine organic acids was not persistent. Further CSF neurotransmitter analysis noted negligible HIAA and HVA levels and markedly elevated 3-OMD, and plasma AADC activity was undetectable.

Treatment initiation with dopamine agonist and cofactor (pyridoxine) noted very limited improvement in motor development. But, PDMS-2 (Peabody Developmental Motor Scales), AIMS (Alberta Infant Motor Scale), and Bayley III raw scores noted marked improvement from 6, 1, and 28 at baseline and 28, 6, and 32 at 6 months, respectively, after gene transduction.

Thus, we conclude this new mutation is deleterious, assuming limited protein production by the known splice variant and that gene therapy results in significant improvement in developmental and health outcomes.

59) RECURRENT LACTIC AND KETO-ACIDOSIS, HYPO- AND HYPER-GLYCEMIA WITH COMPLEX III DEFICIENCY DUE TO MUTATION IN CYC1

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Background: Patients presenting with recurrent episodes of ketoacidosis, sometimes with lactic acidosis or hypoglycemia, are not uncommon in metabolic clinics. Commonly recognized causes of recurrent ketoacidosis include succinyl-CoA oxoacid transferase deficiency (SCOT), mitochondrial acetoacetyl thiotransferase (T2, sometimes referred to as β -ketothiolase) deficiency, and more recently, monocarboxylate transporter 1 deficiency. Adding lactic acidosis and hypoglycemia may suggest mitochondrial dysfunction, glycogenolysis or disorders of gluconeogenesis.

Findings: A young male born to first cousins once-removed had > 10 admissions for recurrent episodes of acidosis with marked ketosis and lactic acidemia (that over time became permanent). Episodes typically presented with hypoglycemia followed by marked hyperglycemia requiring insulin when glucose therapy was initiated. The acidosis did not resolve until insulin was infused. Ketosis was not present between episodes. Eventually, he developed diabetes and required daily insulin therapy. Extensive work-up ruled out defects of pyruvate and FAO metabolism, SCOT and T2 deficiencies, GSD type 0, and gluconeogenic defects.

Results: A commercially available SNP microarray revealed no significant copy number variants, but several regions of homozygosity were identified, including 2 regions > 5 Mb in length on chromosomes 7 and 8. Four candidate genes were identified based on the phenotype, and bi-directional Sanger sequencing revealed a homozygous variant, c.288G>T (p.Trp96Cys), predicted to be deleterious, in the CYC1 gene on chromosome 8. Cultured skin fibroblasts were assayed for respiratory chain function. ETC testing was normal, but high resolution oximetry using a method optimized in one of our laboratories (Anal Biochem 457:52 2013) revealed a defect in complex III integrated function, consistent with the expected defect in CYC1. Experiments are underway using CRISPR/Cas-9 to engineer the variant in a normal human cell line to confirm pathogenicity.

Discussion: We report here the third case of a newly described cause of episodic ketoacidosis with lactic acidosis and abnormal glucose metabolism due to a respiratory chain defect in the complex III component cytochrome c1 (CYC1 gene). This phenotype was recently independently described in 2 patients with a similar presentation, one of whom, of the same geographic origin as our patient, was also homozygous for the p.Trp96Cys variant (AJHG 93:384, 2013). Cytochrome c1, cytochrome b (mtDNA encoded) and the Rieske iron-sulfur protein form the functional cytochrome bc1 center of complex III. The protein is anchored in the phospholipid bilayer of the mitochondrial inner membrane, contains a heme center, and mediates the transfer of electrons from Co-Q10 to cytochrome c via the iron-sulfur protein component of complex III. This report confirms that CYC1 mutations are a cause of autosomal recessive complex III deficiency that appears to typically present in the 2nd year of life with lactic and ketoacidosis and acute fasting hypoglycemia that are associated with insulin resistance during recovery and eventually leads to insulin dependent diabetes.

60) ORGANIC CATION TRANSPORTER 1 (OCT1) EFFLUXES CARNITINE AND ACYLCARNITINES FROM THE LIVER TO BLOOD

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Background: Acylcarnitines are diagnostically important intermediate metabolites of fatty acid oxidation proposed to have independent effects on intermediary metabolism by other mechanisms. Despite the heightened interest in acylcarnitines, the metabolism of acylcarnitines themselves has not been closely examined. Recent genome-wide association studies for blood metabolomic markers revealed a strong association between SLC22A1, a gene encoding the organic cation transporter 1 (OCT1), and blood acylcarnitine levels. OCT1 is a hepatic membrane transporter that mediates transport of small molecules between the liver and blood. In this study, we sought to examine how OCT1, a hepatic transporter, could affect whole-body acylcarnitine metabolism.

Results: We generated OCT1 liver-specific knockout mice (LSKO) and used mass-spectroscopy to measure carnitine and acylcarnitine levels in their livers and compared to those of wildtype control mice. We found that the levels of both metabolites were significantly elevated in the OCT1 deficient livers, suggesting that OCT1 mediates the efflux of carnitine and acylcarnitines from the liver. To test this, we performed in vitro efflux assays using [3H]-carnitine in isolated primary hepatocytes, and found impaired efflux of [3H]-labeled compounds in the OCT1 deficient hepatocytes. Mass-spectroscopy confirmed that the impaired efflux impacted both free carnitine and acylcarnitines. As liver is the major organ for carnitine homeostasis, we tested whether OCT1 affects whole body carnitine metabolism. We gavaged [3H]-carnitine to control and LSKO mice, and measured the distribution of the [3H]-label to different tissues. We observed that in LSKO mice, [3H]-label is highly elevated in the liver, and is reduced in the blood and

extra-hepatic tissues including heart and muscles. This supports the hypothesis that OCT1 effluxes carnitine/acylcarnitines from the liver to the blood where they become available to extra-hepatic tissues.

Conclusions: We found that OCT1 mediates the efflux of carnitine/acylcarnitines from the liver to blood and thereby affects the whole body metabolism of these compounds.

61) INFUSION-ASSOCIATED REACTIONS AND IMMUNOGENICITY IN THE ADVANCE STUDY OF ALGLUCOSIDASE ALFA PRODUCED AT 4000 L SCALE IN PATIENTS WITH POMPE DISEASE

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Background: ADVANCE (NCT01526785), a phase 4 open-label prospective study, investigated safety and efficacy of alglucosidase alfa produced at 4000L scale (4000L rhGAA) in patients with Pompe disease who had previously received rhGAA produced at 160L scale. Presented here are infusion-associated reactions (IARs) by time and immunogenicity parameters.

Methods: Patients with Pompe disease, aged ≥ 1 year, received 4000L rhGAA IV for 52 weeks, at the same dose as their previous therapy, and could continue on 4000L rhGAA in an extension phase. Adverse events (AEs) were monitored continuously. IARs were defined as treatment-emergent AEs (TEAE) related/possibly related to 4000L rhGAA occurring intra-infusion or 0–24 h post-infusion; TEAEs ≥ 24 h post-infusion were considered IARs if judged to be delayed reactions. AEs and IARs were scored by time periods versus infusion.

Results: 113 patients were treated. Following the 52-week treatment phase, 100 patients continued into the extension, in which 45 patients received 4000L rhGAA for at least 18 months longer and 55 discontinued before 18 months (0 for AEs, 4 deaths, and 51 for other reasons). 270 intra-infusion and 0–72 h post-infusion TEAEs occurred (intra-infusion: 40 patients had 112 TEAEs; 0–2h post-infusion, 19 patients had 35 TEAEs; 2–24 h post-infusion, 28 patients had 62 TEAEs; 24–48 h post-infusion, 19 patients had 43 TEAEs; and 48–72 h post-infusion, 12 patients had 18 TEAEs). 35 patients had 149 IARs. Most TEAEs intra-infusion (78/112) and 0–2 h post-infusion (17/35) were IARs. In contrast, 2–24 h post-infusion a minority of TEAEs (8/62) were IARs, no IARs occurred 24–48 h post-infusion, and only 2 (diarrhea) occurred 48–72 h post-infusion. IARs affecting ≥ 2 patients intra-infusion were urticaria, pyrexia, flushing, nausea, vomiting, headache, throat irritation, tachycardia, increased blood pressure, and erythema. The only IARs affecting ≥ 2 patients 0–2 h post-infusion were pyrexia (4 patients/5 events) and at 2–24 h post-infusion, diarrhea (2 patients/3 events). Diarrhea was a frequent TEAE (unrelated to study drug in 51 [45.1%] patients and related in 4 [3.5%]) but was infrequent as an IAR (3 patients/5 events). The most frequent IARs affecting $\geq 3\%$ of patients were pyrexia, tachycardia, urticaria, flushing, and vomiting. Serious IARs affected 6 patients (15 events); those in ≥ 2 patients were pyrexia and chills. 7 of the 24 patients remaining anti-rhGAA IgG-seronegative throughout the study had 14 IARs. For the 89 seropositive patients (77 seropositive at baseline; 12 seroconverted on-study) by quartiles of peak IgG titer: Quartile 1 (titers 0–400), 5/24 patients had 9 IARs; Quartile 2 (titer 800, a narrow range, but including 13 patients), 2/13 patients had 17 IARs; Quartile 3 (titers 1600–3200), 10/33 patients had 34 IARs; and Quartile 4 (titers 6400–102,400), 11/19 patients had 75 IARs. 8 patients experienced IARs associated with the Day 1 4000L rhGAA infusion, at which time 3 patients were seronegative and the remaining 5 patients' titers ranged from 1600 to 25600 (1 seronegative and 1 seropositive patient had received immunomodulation). No patients with IARs tested positive for anti-rhGAA IgE.

Conclusions: 4000L rhGAA had a safety profile comparable with the collective experience of patients receiving 160L rhGAA. All IARs were mild-to-moderate in intensity; most were considered non-serious.

62) 2-ETHYLHEXANOIC ACID, FOUND IN COMMON PLASTICIZERS, LEADS TO ARTIFICIAL INCREASE IN C8 ACYLCARNITINE IN A NEONATE ON EXTRACORPOREAL MEMBRANE OXYGENATION (ECMO)

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Plasma acylcarnitine analysis may be performed in neonates as part of the metabolic evaluation for an abnormal newborn screen or in patients with clinical symptoms that suggest an inborn error of organic acid and/or fatty acid metabolism. Our laboratory received specimens for metabolic testing on a 6-day-old male infant who presented at birth with hypoglycemia, severe anemia, and hydrops who had received a blood transfusion and

was on extracorporeal membrane oxygenation (ECMO). Plasma acylcarnitine analysis revealed an increased level of C8 and low levels of C0 and C2 acylcarnitine species with increased C8/C2 and C8/C10 ratios. Although this profile suggested a diagnosis of medium-chain acyl-CoA dehydrogenase (MCAD) deficiency, the normal levels of C6 and C10:1 acylcarnitines were inconsistent with this diagnosis. As in most laboratories, we perform routine acylcarnitine screening by flow infusion tandem mass spectrometry, which does not separate isobaric compounds. When the sample was re-analyzed using a chromatographic LC–MS/MS method, we found that only a small fraction of the C8 acylcarnitine represented octanoylcarnitine, with the majority being an unidentified compound. Based on the absence of the C8 carboxylic acid, valproic acid, and the presence of very high levels of another C8 carboxylic acid, 2-ethylhexanoic acid, by urine organic acid analysis, we hypothesize that the unidentified C8 acylcarnitine species is 2-ethylhexanoylcarnitine. 2-Ethylhexanoic acid and phthalic acid, also markedly increased in the urine sample from this patient, are derived from plasticizers used in the manufacture of polyvinylchloride (PVC) and have been shown to be present in body fluids of neonates exposed to PVC products including those used in blood transfusions, intravenous nutrition, and ECMO. Repeat studies in samples collected from this patient at age 2 weeks showed a significantly lower urinary 2-ethylhexanoic acid level and a normal plasma level of C8 acylcarnitine.

63) THE USE OF PENTOSAN POLYSULPHAFATE AS ADJUNCT THERAPY IN MUCOPOLYSACCHARIDOSIS I

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Mucopolysaccharidosis I (MPS I) is a multi-system disease due to progressive accumulation of glycosaminoglycans (GAGs). This triggers inflammatory and apoptotic responses leading to the clinical phenotype, including involvement of the musculoskeletal system. Current therapeutic approaches, including hematopoietic stem cell therapy and enzyme replacement therapy (ERT), have shown limited efficacy in the treatment of joint and/or skeletal disease.

Pentosan polysulfate sodium (PPS or Elmiron) has recently been shown to treat the skeletal phenotype and complications in a rat model of a related disease, MPS VI, when early treatment was initiated. PPS, therefore, may be of value as an adjunct therapy in patients with MPS.

Here we report a series of attenuated MPS I patients who are being treated with oral PPS (4.2 mg/kg/d; compassionate use) as an adjunct therapy to ERT. A total of 4 patients with MPS I (2 females, 2 males, ages: 6 years–25 years) have been treated with PPS for 3–39 months. Initial dosing was at 1/3 of the final dose, and was titrated up by thirds until the final dose was reached. PPS was well tolerated in all four patients without any reported side-effects and/or laboratory abnormalities.

Near normalization of joint mobility was demonstrated in one 8-year-old female who was treated for more than 3 years. One adult male reported reduced joint and back pain after 9 months of therapy. Overall, there were no changes in urine GAG levels.

Our limited experience with PPS as an adjunct therapy in MPS I suggests that patients may benefit from treatment. Larger studies are needed to explore its clinical utility in a broader MPS patient population.

64) INFANTILE ONSET POMPE DISEASE: SIBLING CASES IN THE PRE-NEWBORN SCREENING ERA

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Introduction: With the introduction of Myozyme Enzyme Replacement Therapy (ERT) for the treatment of Infantile Onset Pompe Disease (IOPD), and the potential of early diagnosis through newborn screening, the natural history of this once universally fatal disorder is changing forever. Here we review three sets of siblings with IOPD to see how the prospects for the second child have changed.

Methods: The charts of 3 sibling pairs of patients with IOPD were reviewed to understand the change in natural history as a result of earlier diagnosis, use of ERT and changes in treatment protocols.

Results: The patients in this group were born between 1999 and 2014. The average age of diagnosis was 5 months for the first sibling. Two of the second siblings were diagnosed within the first month of life, while the third was diagnosed late at 5 months of age due to an erroneous prenatal diagnosis.

Sibling pair A: The oldest sibling was diagnosed in 1999 (prior to FDA approval of Myozyme in 2006); She succumbed at 9 months of age following multiple hospitalizations for respiratory failure. Her younger sibling was born in 2008, and became symptomatic at 4 months. He was started on ERT prior to a confirmed diagnosis because of the family history. Though diagnosed late he is doing well on biweekly ERT, and is able to attend school and walk independently.

Sibling pair B: The older sibling was born in 2006, and was CRIM negative. He was diagnosed at 4 months of age and started shortly thereafter on ERT without immunomodulation and succumbed at 11 months of age. His younger sibling was born in 2012 and received a course of immunomodulation with initiation of ERT. He made good progress and started walking at 14 months of age. However, he has required two subsequent courses of immunomodulation and did poorly following an RSV infection which eventually necessitated tracheostomy tube placement.

Sibling Pair C: The older sibling, born in 2008, was diagnosed at 6 months of age. He was started on ERT shortly thereafter, however due to the severe nature of his presentation he required tracheostomy tube placement and progressed poorly. He developed high antibody titers that resolved without immunomodulation, but during this time his muscle weakness worsened and he succumbed at 2 years of age. His younger sibling was born in 2014, and she was given a modified course of immunomodulation at the start of ERT. She has made good progress, meeting developmental milestones.

Discussion: Earlier diagnosis is the most clearly important determinant in outcome, though its combination with changing ERT protocols and immunomodulation are vital to their improved outcome. The diagnosis of the second sibling with IOPD is akin to newborn screening, as earlier diagnosis leads to optimized treatment outcomes. As is consistent with the Pompe Disease registry, the average delay in diagnosis is close to 6 months

for IOPD patients diagnosed on clinical grounds. These cases highlight the benefit of newborn screening for Pompe Disease that has already started in several states.

65) THE iNTD REGISTRY: THE CLINICAL DATABASE OF PATIENTS WITH INBORN NEUROTRANSMITTER RELATED DISORDERS

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Background: Inherited defects of biogenic amines, tetrahydrobiopterin (BH4), folate, serine and glycine metabolism lead to progressive neurological symptoms in early infancy. Immediate diagnosis and treatment may result in an improved outcome. Until today there is no standardized systemic evaluation of diagnostic processes, therapeutic approaches and long term outcome of affected patients.

Methods: The “International Working Group on Neurotransmitter Related Disorders” (iNTD) provides a platform for clinicians and scientists to exchange expertise and to foster international collaborations in research projects in the field of neurotransmitter related disorders. To date, it includes 27 metabolic centres from 18 countries worldwide. The web-based iNTD patient registry for inherited defects of neurotransmitter related disorders enables a standardized assessment of the epidemiology, genotype/phenotype correlation and outcome of these diseases, their impact on the quality of life of patients and current diagnostic and therapeutic strategies. The already existing registers (JAKEdb and BIODEFdb) will be unified with the iNTD registry. Based on the evaluation of the patient registry, the development of consensus care guidelines for the clinical and therapeutic management is in progress. Conclusion: The iNTD network is a growing international initiative to encourage scientific and clinical exchange on neurotransmitter related disorder. Together with the iNTD registry it aims to improve current research, basic knowledge and clinical management strategies considering the rare neurotransmitter related diseases.

66) THE YIELD OF BIOCHEMICAL SCREENS FOR CONGENITAL DISORDERS OF GLYCOSYLATION

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Background: Congenital disorders of glycosylation (CDGs) are a group of more than 100 genetic disorders due to defective glycan modification of biomolecules. Until 2000, laboratory diagnosis and screening was achieved by isoelectric focusing of transferrin. Recently, however, mass spectrometric methods have replaced isoelectric focusing as the standard method for screening for CDGs. We evaluated the frequency with which these tests show abnormalities in patients known to have a CDG.

Methods: Patients with biallelic pathogenic mutations in various genes known to cause CDGs were enrolled in the NHGRI study 14-HG-0071 (NCT02089789) and/or 76-HG-0238 (NCT00369421). Blood and urine were collected from these subjects and samples were analyzed by Mayo Medical Laboratories and Emory Genetics Laboratory. At Mayo, serum carbohydrate deficient transferrin and ApoCIII isoforms were analyzed. At Emory, plasma N-glycan profiles, plasma N-glycan structure, plasma O-glycan profiles, and urine oligosaccharide and glycan screen were performed.

Results: Fifteen individuals were included in this study: 1 with a dolichol synthesis disorder, 2 with a GPI anchor disorder, 9 with an N-linked glycosylation disorder, and 3 with an O-linked glycosylation disorder. These individuals underwent an average of 4.4 of the 6 possible biochemical screening tests for CDGs. Seven of 15 had at least one test return highly abnormal and suspicious for a congenital disorder of glycosylation. Of these seven, five had N-linked glycosylation defects. 3/15 returned borderline results on their most abnormal test. In 5/15 patients, all tests returned normal.

Discussion: In our study only 47% of patients with known CDGs showed clear abnormalities in biochemical tests currently used for CDG screening. The sensitivity of the screens may depend on the type of CDG, but even within N-linked CDGs, only 56% had results considered highly suspicious, so there is room to improve biochemical screens for CDGs. Moreover, a negative result in these tests does not definitively rule out a glycosylation defect. While recently most individuals with CDG have been identified through whole genome/exome sequencing, biochemical screening can be used to validate molecular findings and track clinical status.

67) IMPAIRMENT OF MITOCHONDRIAL BIOENERGETICS IN FIBROBLASTS FROM PATIENTS WITH COMPLEX I DEFICIENCY

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Background: Mitochondrial complex I deficiency is the most frequent cause of oxidative phosphorylation disorders presenting in childhood. It is clinically heterogeneous, but the predominant symptoms include hypotonia, basal ganglia dysfunction, psychomotor retardation, and failure to thrive. Complex I consists of 46 structural subunits including NDUFV1 and ND6, nuclear and mitochondrial DNA encoded

proteins, respectively. Diagnosis of mitochondrial diseases is difficult due to poor reproducibility of muscle and fibroblast respiratory chain activity measurement.

Objective: The impairment of complex I function on mitochondrial bioenergetics and oxidative status in fibroblasts from patients with NDUFV1 and ND6 deficiencies were investigated using whole cell techniques. **Methods:** Fibroblasts were cultured in media with or without glucose in order to force them to rely on oxidative phosphorylation for ATP production. We evaluated oxygen consumption rate, mitochondrial membrane potential and mass, and reactive oxygen species (ROS) production in patient fibroblasts using a Seahorse XFe96 flux analyzer, immunostaining and fluorimetry.

Results: The most prominent effects were observed in fibroblasts with ND6 deficiency. Cell oxygen consumption studies demonstrated that basal respiration and reserve capacity were decreased in ND6-deficient fibroblasts by 16% and 28%, respectively, whereas in NDUFV1-deficient cells only reserve capacity was decreased by 14%. Immunostaining has shown a decrease in membrane potential in NDUFV1- and ND6-deficient fibroblasts by 25% and 73%, respectively. Alterations in mitochondrial morphology in both cell lines were also observed, but a decrease of mitochondrial mass (30%) was found only in NDUFV1-deficient cells. Assays with DCF-DA and mitoSOX probes showed that ROS levels were normal in both cell lines.

Conclusion: Our findings show that impairment in bioenergetics and mitochondrial function, which contribute to the pathophysiology underlying the symptoms observed in complex I deficiency, can be detected in fibroblast cell lines with mutations in NDUFV1 and ND6 genes, providing options to better demonstrate dysfunction and evaluate the efficacy of potential therapeutic agents.

68) PRISM 301: AN OPEN-LABEL, RANDOMIZED, PHASE 3 CLINICAL TRIAL EVALUATING EFFICACY AND SAFETY OF PEGVALIAS FOR THE TREATMENT OF ADULTS WITH PHENYLKETONURIA

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Background: Phenylketonuria (PKU) is an inherited metabolic disease in which phenylalanine (Phe) cannot be metabolized to tyrosine due to deficiency of the enzyme phenylalanine hydroxylase. Phe accumulation can be toxic and may cause neurocognitive and neuropsychiatric dysfunction. Pegvalias (BMN-165), PEGylated recombinant *Anabaena variabilis* phenylalanine ammonia lyase, is a novel potential enzyme substitution therapy to reduce blood Phe levels in patients with PKU. Pegvalias converts Phe to ammonia and trans-cinnamic acid. The PRISM 301 study is an open-label, randomized, phase 3 clinical trial to assess the safety and efficacy of daily subcutaneous pegvalias treatment in adults with PKU.

Methods: This study enrolled pegvalias-naïve subjects with blood Phe concentration of ≥ 600 $\mu\text{mol/L}$ into an induction (Weeks 1–4), titration (Weeks 5–34), and maintenance (>2 weeks) dose regimen study of pegvalias. Subjects were randomized 1:1 to a maintenance target dose of 20 mg or 40 mg pegvalias daily for a minimum of 26 weeks and up to 36 weeks. During the induction period, all subjects received 2.5 mg pegvalias weekly. The titration period was of variable duration depending on pegvalias tolerability and subjects increased their dose until target dose was achieved. Subjects were required to maintain protein intake consistent with their intake at baseline. Blood Phe levels were evaluated at screening and every 4 weeks until the study completion or early termination visit. The inattentive domain of the Attention Deficit Hyperactivity Disorder Rating Scale (ADHD-RS) was used to assess inattentive symptoms (subscale score ranged 0–27 with higher scores indicating increased symptoms) at baseline and at study completion.

Results: At the interim analysis data cut (September 25, 2015), 257 subjects were enrolled in the study. Baseline demographic characteristics include gender (female, 50%), mean (range) age of 29.2 (16–55) years, body weight of 80.5 (41.5–139.2) kg, and body mass index of 28.5 (17.1–47.3). Baseline mean (SD; min, max) blood Phe level was 1237.9 $\mu\text{mol/L}$ (356.0; 567.5, 2337.5). Of 253 subjects (4 subjects did not have ADHD-RS baseline scores), the baseline mean ADHD-RS inattention score was 9.9 points and 47.0% had a baseline score > 9 points. For subjects with > 26 weeks of pegvalias exposure in this open-label study ($n = 131$), mean (SD; min, max) blood Phe decreased 34.7% (45.9%; –100%, 121.8%). The mean (SD) decrease from baseline in ADHD-RS inattention was –3.68 points (5.1; $n = 141$). A total of 96.5% of subjects had at least 1 adverse event (AE) and 9.7% had a serious AE. The most common adverse events (AEs) in subjects with ≥ 1 AE were arthralgia (61.5%), injection site reaction (51.4%), erythema (42.4%), headache (32.3%), and rash (24.9%).

Conclusions: Phase 3 interim results reported decreased blood Phe levels and decreased ADHD inattention symptoms in subjects treated with pegvalias. The safety profiles were similar between randomized dose groups and consistent with previous clinical studies of pegvalias. Pegvalias administered using an induction, titration and maintenance dosing regimen may be a potential therapeutic option for adult patients with PKU. The study is ongoing and updated efficacy and safety results will be presented.

69) ORTHOTOPIC LIVER TRANSPLANTATION IN CLASSICAL MSUD: A SINGLE CENTER EXPERIENCE

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Background: Maple syrup urine disease (MSUD) results from a deficiency of the branched chain keto-acid dehydrogenase (BCKD) complex, and results in elevations of the branched-chain amino acids, in particular leucine, which causes non-osmotic brain edema. Standard of care involves a strict low protein diet to reduce intakes of dietary leucine, and the supplementation of a branched chain amino acid-free formula. Adherence to dietary management is challenging. Even with appropriate dietary management, risk of metabolic decompensation remains high with any illness, injury or slight dietary indiscretion. Liver transplantation has been shown to improve long-term leucine control in MSUD patients.

Methods: We present 6 patients with classical MSUD, ages 10 months to 16 years, who underwent orthotopic liver transplantation with cadaveric grafts, 5 of whom participated in domino liver transplantation.

Results: Plasma leucine normalized in 4 of 6 patients within one week of transplantation. In the remaining 2 patients, plasma leucine was elevated, but within the treatment range for MSUD (less than 300 $\mu\text{mol/L}$). One patient had elevated plasma leucine for 2.5 years post-transplantation. The other remained slightly elevated eight months post-transplant. All patients post-transplant were on an unrestricted diet and did not require branched chain amino acid-free formula. Alloisoleucine was occasionally present in plasma samples post-transplant. The plasma amino acids of recipients of BCKD-deficient livers have remained normal.

Conclusions: Orthotopic liver transplantation is a viable option for long-term leucine control in patients with MSUD and domino liver transplantation is presented as a safe utility of BCKD-deficient livers.

70) IN VITRO FUNCTIONAL STUDIES IN PATIENT DERIVED DERMAL FIBROBLASTS REVEAL SALVAGE PATHWAYS THAT MIGHT RESCUE GLYCOSYLATION DEFECTS IN CLASSICAL GALACTOSAEMIA

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Background: The long-term outcomes of treatment of Classical Galactosaemia, (GALT deficiency: EC 2.7.7.12), are disappointing. Also, substantial variation in outcomes occurs in siblings and individuals homozygous for the Q188R mutation. We have identified significant variation in accessory glycosylation pathways in Galactosaemia using IgG galactose incorporation analysis and also significant variation in related glycan synthesis gene pathways (Stockmann et al., 2015, Maratha et al., 2015, in press).

Objectives: To develop an *in vitro* human GALT deficient model of galactose exposure to study potentially modifiable glycosylation salvage pathways in Galactosaemia; to study the glycosylation of the glycoprotein markers EGFR, IGF-1R and ICAM-1; and also to examine the effects of over-expression of two potential modifier genes, UDP-glucose pyrophosphorylase (UGP2) and Beta 1,4-Galactosyltransferase (B4GALT1).

Methods: Human dermal fibroblast (GHDFs) cells were cultured from three adult Galactosaemia subjects; (Q, M and U,) all homozygous for Q188R, and commercial control cell lines (NHDFs). Q and M have good neurological outcomes (FSIQ ≥ 80), U has a poor outcome (FSIQ < 80). GHDFs were cultured in galactose 'intoxicated' galactose media (0.1%) and at current treated/ambient galactose concentrations (0.01%). The NHDFs were treated with Tunicamycin (a glycosylation inhibitor), to study possible glycosylation salvage pathways with monitoring of cell viability using a MTT cell viability assay. We then studied the over-expression of UGP2 and B4GALT1, through viromer transfection reagent based transient transfections, using a modified luciferase assay (ER-LucT luciferase construct). Analysis of the N-glycan markers EGFR, IGF-1R and ICAM-1 was performed using Colorimetric Cell-Based ELISA assays.

Results: Initial Cell-Based ELISAs for EGFR, IGF-1R and ICAM-1 indicated dysregulated glycosylation patterns observed as decreased levels of total and phosphor forms of EGFR, IGF-1R and ICAM-1 at all intervention steps in the study. The cells for the poor outcome patient (U) showed significant abnormalities at all galactose concentrations. However, significant difference in expression of the three markers was only apparent for cell lines Q and M at intoxicated galactose concentrations (p.

Conclusions: Our data suggests that EGFR, IGF-1R and ICAM-1 can serve as *in vitro* markers of hypoglycosylation in Galactosaemia. Furthermore, we have observed significant differences in modifiable accessory glycosylation pathways in subjects, responsive to overexpression of modifier glycosylation genes. We believe that these studies validate our clinical observations of significant variation in glycosylation in Galactosaemia and a model to further develop effective treatment strategies.

71) COMPLEX I DEFICIENCY IN THE CORTEX OF HUNTINGTON'S DISEASE MICE

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Background: Huntington's disease (HD) is a disorder of the basal ganglia in the brain that affects a person's ability to think, talk, and move. The disease is caused by the hyper-repetition of a trinucleotide CAG in the HTT gene which codes for the protein huntingtin. While this gene, and its expansion, is known to be a causative agent in HD, little is known of the function of huntingtin. Additionally, the build-up of huntingtin has been reported to be the crux of disease pathogenesis, though the specific effects of this toxicity are still unknown.

Objective: This study examined the relationship between respiratory chain complex deficiencies and HD.

Methods: Various portions of the brain – the cerebellum, cortex, hippocampus, and striatum – were isolated from a mouse model of human Huntington disease. To analyze the extended effects of the disease at various ages, eight week, twenty week, and 80 + week old mice were grown with and without HD. Respiratory chain activity and structure was analyzed in mitochondrial extracts by blue native gel electrophoresis (BNGE) with in situ assay of complexes I, III, and IV.

Results: Eight week old HD and wild type mice had similar BNGE patterns of respiratory chain complexes in all brain sections. By 80 weeks, complex I in the cortex of HD mice showed a dramatic decrease in activity compared to controls. Respiratory chain supercomplexes were also disrupted. Other brain portions were similar between HD and wild type mice.

Conclusion: These results demonstrate a significant defect in the mitochondrial respiratory chain in cortex of aging HD mice compared to wild type animals. The mechanism of huntingtin protein aggregation in disrupting mitochondrial energy metabolism is unclear, and interruption of this process may provide new options for treatment of HD.

72) MULTIPLE VARIANT IDENTIFICATION IN THE DIAGNOSTIC WORK-UP FOR A MITOCHONDRIAL DISORDER

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Background: Our patient, a 10-month-old female, presented with new onset seizures, history of developmental delay and a 2–3 month history of developmental regression. She was born at term following an uncomplicated pregnancy. She failed her newborn hearing screen and was referred for further evaluation which revealed severe bilateral sensorineural hearing loss. Her California Newborn screening results were normal. Early milestones were mildly delayed: she lifted her head at 3 months and started rolling at 5 months. At 6 months of age, she lost the ability to roll and had declining head control. A Head MRI was normal. At 10 months of age, she was admitted with seizures to our hospital. Head MRI during her admission showed diffuse areas of diffusion restriction involving the cortical and subcortical white matter in bilateral cerebral hemispheres, most prominent posteriorly, as well as bilateral thalami. Genetics was consulted due to suspicion of an underlying inborn error of metabolism.

Methods: Urine organic acids, plasma amino acids, CSF neurotransmitter studies, glycosylated transferrin, urine oligosaccharides, and urine mucopolysaccharides were non-diagnostic. mtSEEK and nucSEEK from Courtagen Diagnostics Laboratory were ordered.

Results: nucSEEK identified 7 variants while mtSEEK identified one variant. nucSEEK revealed compound heterozygosity for variants of unknown clinical significance in the NARS2 gene. NARS2 encodes the mitochondrial asparaginyl-tRNA synthetase. Though NARS2 is not associated with any known syndrome, the variants are located in highly conserved regions and are predicted to be disease causing. There have been three previously reported cases with significant phenotypic variability. mtSEEK found a variant of uncertain clinical significance in ATP6, which codes for a mitochondrial encoded ATP synthase, which may be contributing to the patient's phenotype. Parental studies confirmed each parent to be a carrier for a NARS2 variant. Additional variants identified in our patient included FOXC1 (paternally inherited) and KCNE1 (paternally inherited). FOXC1 is an autosomal dominant glaucoma variant and KCNE1 is an autosomal dominant Long QT syndrome variant. The latter led to a comprehensive arrhythmia panel from GeneDx and the patient was found to have two more previously unreported cardiac arrhythmia variants.

Conclusions: Large gene panel testing identified the likely cause of this patient's symptoms. However, the identification of numerous variants led to additional testing on the patient and screening recommendations for the father.

73) GYNECOLOGIC AND REPRODUCTIVE HEALTH ISSUES IN PATIENTS WITH CHEDIAK-HIGASHI DISEASE

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Background: Chediak-Higashi disease (CHD; MIM #214500), due to mutations in *LYST*, is an ultra-rare autosomal recessive condition with partial oculocutaneous albinism, bleeding diathesis, immunodeficiency, and hemophagocytic lymphohistiocytosis. The *LYST* protein is thought to function in the formation and trafficking of lysosome-related organelles. Without bone marrow transplant (BMT), mortality is high within the first decade of life. Despite transplant, both classic patients treated with BMT, as well as CHD patients with an atypical, late-onset form develop slowly progressive neurological features by early adulthood. Prior to BMT, few children with recognized CHD survived into adulthood so there is little information available about ob/gyn issues in CHD despite risks associated with immunodeficiency and bleeding.

Objective: To evaluate the gynecologic and reproductive health issues in women with Chediak-Higashi disease.

Methods: Four adult females with confirmed CHD were evaluated at the National Institutes of Health Clinical Center. Evaluation included ob/gyn history, laboratory testing, review of outside records, physical examination and pelvic ultrasound if indicated.

Results: The patients ranged in age from 21 to 43 years; 2 patients were diagnosed with CHD in childhood and treated with BMT. Age at menarche was normal. Half of the patients reported monthly menses and half reported less frequent periods; 2 reported heavy menstrual periods, 1 improved with use of oral contraceptive pills. Although dysmenorrhea was reported in 2 patients, none report other pelvic pain. Likewise, no patients had recurrent vaginal or pelvic infections. The only sexually-transmitted infection reported was HPV in 1 patient who also had a history of an abnormal Pap smear that led to diagnosis of cervical dysplasia. Gynecologic procedures included bilateral tubal ligation in 1 patient and loop electrosurgical excision procedure (LEEP) in 1 patient with no abnormal bleeding. Two patients have been pregnant with a total of 3 pregnancies. Neither reported bleeding during pregnancy. One pregnancy was complicated by mild gestational hypertension leading to induction at term, vaginal delivery and retained placenta requiring manual removal; the others were uncomplicated and resulted in vaginal deliveries without excessive bleeding. One untransplanted patient had a history of infertility. Laboratory testing included normal estradiol, FSH, LH and TSH levels in all 4 patients. Prolactin and testosterone levels were normal in 3 of 4 patients; 1 patient with elevated levels normalized upon retesting.

Conclusions: Our results indicate a possible increased risk of menorrhagia. We did not, however, see an increased risk of bleeding during pregnancy or delivery. Though 1 patient had fertility issues, 2 others conceived without difficulty. Despite the known immunodeficiency associated with CHD, we did not see an increased risk of vaginal or pelvic infections. Gaining a better understanding of the range of ob/gyn problems in CHD helps to expand the phenotype and is the first step toward developing ob/gyn therapeutic strategies for this patient population.

74) HAS THE EVALUATION OF THE APPARENT LIFE-THREATENING EVENT FOR AN INBORN ERROR OF METABOLISM CHANGED?

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What is the proper evaluation for an Inborn Error of Metabolism (IEM) in a child presenting with a “concerning event” — often called an apparent life threatening event (ALTE) that is witnessed by a caregiver? A recent systematic review of ALTEs found only five studies addressing metabolic disorders and one report of vitamin D deficiency in infants with routine screening including serum chemistries, blood gases, glucose, calcium, lactate, ammonia, urine organic acids, and plasma amino acids. IEMs have only been reported in fewer than 5% of cases. The most common descriptions of ALTEs have reported urea cycle disorders and fatty acid oxidation disorders as well as examples of organic acidemias and lactic acidemias. Beyond this literature, there is only a limited number of studies systematically looking at ALTEs, often labeling them as “SIDS-like” or “near-SIDS” events, but these are not the same thing. Metabolic testing has been described in an inconsistent fashion. In reviewing these reports, risk factors for an increased likelihood of an IEM include a positive family history of SIDS and recurrent events. The diagnosis and treatment of IEMs has changed with the expansion of newborn screening to include diseases previously only diagnosed after an ALTE and changes necessary testing. Currently, literature only has only limited support for testing of lactate, serum bicarbonate, and glucose for the asymptomatic infant presenting with a brief episode. A tiered testing approach including additional metabolic screening may be warranted based upon the presence of specific historical and physical examination findings.

75) THE ISSUES WITH TANDEM MS PROFILES ARE RESOLVED WITH QUANTITATIVE ACYLCARNITINE ANALYSIS

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Background: Tandem MS “profiling” of acylcarnitines and amino acids was conceived of as a first-tier screening method, and its application to expanded newborn screening has been enormously successful. However, unlike amino acid screening (which uses amino acid analysis for second-tier validation of screening results), acylcarnitine “profiling” also assumed the role of second-tier validation. This was due to the lack of a generally accepted second-tier acylcarnitine determination method.

Methods: Tandem MS “profiles” are limited in both selectivity and quantitative accuracy. We instead used our validated (U)HPLC-MS/MS method for the accurate quantification of total carnitine, free carnitine, butyrobetaine, and acylcarnitines.

Results: We show results from proficiency test samples with known diagnoses: malonic acidemia (MA), 3-methylcrotonyl-CoA carboxylase deficiency (3-MCC), short-chain acyl-CoA dehydrogenase deficiency (SCADD), beta-ketothiolase deficiency (BKT), methylmalonic acidemia (MMA), and multiple acyl-CoA dehydrogenase deficiency (MADD). The ability to discriminate among constitutional isomers and diastereomeric acylcarnitines, along with a high level of accuracy and precision, are demonstrated. These features are unavailable when using tandem MS “profiles”.

We also show an example of research interest (cerebral spinal fluid), where separation of acylcarnitine species and accurate and precise acylcarnitine quantification is necessary.

Conclusions: The accuracy and precision of our validated (U)HPLC–MS/MS second-tier method for the quantification of total carnitine, free carnitine, butyrobetaine, and acylcarnitines should make it the method of choice for clinical and basic research projects. These include validation of newborn screening results, treatment protocol studies, acylcarnitine biomarker studies, and metabolite studies using tissue, urine, or other sample matrices.

76) RECURRENT INFECTION INDUCES APPARENT LIVER FAILURE IN A FEMALE WITH CITRULLINEMIA TYPE 1

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Citrullinemia type 1 (CTLN1) is a urea cycle disorder that usually presents in the neonatal period with hyperammonemia and concurrent neurological deterioration. We report a 40-month-old female with confirmed CTLN1 who presented with intercurrent liver failure apparently precipitated by an acute infection. The patient was diagnosed at 4 days of age when she presented with progressive lethargy secondary to hyperammonemia of 258 $\mu\text{mol/l}$, which peaked at 617 $\mu\text{mol/l}$ on the 5th day. She was started on standard treatment for Citrullinemia with the use of conjugating agents (sodium phenylbutyrate), L-arginine and protein restriction utilizing medical foods to provide essential amino acids and adequate calories. She was switched to glycerol phenylbutyrate at 28 months of age. Following her initial presentation, she has been in relatively stable metabolic control with just three short admissions in the first year of life. In the last year, there have been no hyperammonemic episodes. Her most persistent problem has been recurrent otitis media, which has necessitated the placement of ear tubes. However, she continues to have recurrent ear infections for which she has received multiple courses of antibiotics (oral & drops). Initially, during these episodes, mild elevations of AST/ALT (40/82 at 32 months of age) were noted which increased slowly in the next few months to levels in the 100–200 range. During her most recent episode of otitis media at 38 months of age, she presented with an area of purpura/bruising over the right lower quadrant of her abdomen. Her LFTs at that time were reported as AST/ALT of 698/1806 U/L (normal 4–35/6–55 U/L) with corresponding INR of 3.33 with PTT of 47.6 (normal 25–32 s) and PT of 36.5 s. Over a 2-week span, her INR rose to a peak of 4.38 with AST/ALT of 3390/4755 U/L with the appearance of several additional small areas of purpura which in one week's time, slowly improved with INR of 1.6 with PTT of 33.9 s and PT of 15.8 and corresponding reduction in AST/ALT down to 184/891 U/L. The patient has had persistent elevations of her liver enzymes throughout, but this most recent episode represents further deterioration in her condition. Despite these episodes, she has remained clinically/metabolically stable throughout with no evidence of hyperammonemia (glutamine of 871 $\mu\text{mol/l}$ with her highest AST/ALT). The parents have been advised about the potential need for liver transplantation. The ongoing consequences of these episodes are unclear.

77) A NOVEL CUTIS LAXA SYNDROME WITH CONGENITAL DISORDERS OF GLYCOSYLATION

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Metabolic cutis laxa syndromes form a heterogeneous group of inborn errors of metabolism eventually affecting the formation and function of extracellular matrix proteins leading to sagging, inelastic and wrinkled skin. Several genetic defects in different metabolic pathways have been described, including two disorders of glycosylation: COG7-CDG and ATP6V0A2-CDG.

We present two patients with a unique combination of features in association with cutis laxa and combined glycosylation abnormalities. Both patients presented at birth with severe wrinkled skin, dysmorphic and progeroid features, an abnormal fat distribution with almost absent subcutaneous fat. Growth parameters were normal after birth but started to deviate from the age of 2 years. Psychomotor development was severely delayed. Cutis laxa improved throughout the course of disease.

The first male patient additionally had bilateral cataract, and cardiomyopathy, which improved spontaneously over the years. Biochemical analysis showed hypercholesterolemia. Brain MRI revealed white matter abnormalities and widened ventricles.

Both patients had a mild glycosylation defect involving N-linked and O-linked glycosylation. Whole exome sequencing revealed a recessive mutation in a subunit of the V-ATPase proton pump, from which another subunit ($\alpha 2$) is deficient in ATP6V0A2-CDG, leading to abnormal Golgi trafficking, further bridging cutis laxa and CDG.

78) IDENTIFICATION OF MODIFIER GENES OF POMPE DISEASE PHENOTYPE BY VARIANT ANALYSIS OF WHOLE EXOME SEQUENCING DATA

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Background: Pompe disease is an autosomal recessive condition due to mutations in the GAA gene encoding acid alpha-glucosidase leading to lysosomal accumulation of glycogen. The disease phenotype shows a broad spectrum with various ages of onset and different degrees of systemic organ involvement including cardiac, skeletal and smooth muscles, as well as respiratory function due to diaphragmatic weakness. More recently, involvement of peripheral and central nervous system, bone, venous and arterial vessels, kidneys, gastrointestinal tract, auditory system, urinary bladder and blood cells have been reported. Extent and severity of organ involvement and response to enzyme replacement therapy (ERT) with

recombinant human acid alpha-glucosidase (rhGAA) also show continuous phenotypic spectrum. With the advent of newborn screening for Pompe disease, there is an urgent need to identify and understand modifiers of phenotypes and immune responses for optimized treatment.

Response to ERT can be compromised by anti-rhGAA antibody formation, which can be averted by immunomodulation. While cross-reactive immunologic material (CRIM)-negative status correlates with high sustained anti-rhGAA antibody titers (HSAT), there is no reliable method to predict a subset of CRIM positive patients who later would develop HSAT.

Over 400 pathogenic GAA mutations have been described. Compound heterozygous mutations with c.-32-13T>G in one allele are associated with mild late-onset phenotype (LOPD), and disease severity generally correlates with the severity of the second mutation qualified in Pompe-Erasmus Rotterdam database. Two null mutations are associated with CRIM-negative infantile-onset (IOPD). However, the correlation is incomplete; some cases do not follow the correlation; variability has been documented between patients with the same GAA; substantial variability exists even within the same family. Recent studies demonstrated that polymorphisms in ACE and ACTN3 genes are associated with disease severity and ERT response. Identification of modifying factors for each phenotype will allow refined prognostication, and may lead to a new therapeutic target or development of adjuvant therapies to ERT.

We aim to 1) reproduce the association of ACE and ACTN3 with disease severity, 2) investigate association of disease severity and ERT response with polymorphisms/variants of genes in autophagy, B-cell regulation, mTORC1 signaling pathways.

Methods: We performed whole exome sequencing (WES) on 99 samples from individuals with Pompe disease, whose clinical phenotype including HSAT and CRIM status have been carefully documented. The dataset represents the largest cohort of its kind in rare autosomal recessive disorders. We will classify the cohort by clinical phenotypes and immune response, control for population stratification by using EIGENSTRAT, and control for GAA mutation type.

Preliminary results: Preliminary analysis of WES data on initial 36 samples revealed that 11/12 CRIM-negative IOPD sample had two null mutations, 8/17 CRIM-positive IOPD samples had one missense and null mutations, consistent with known genotype-phenotype correlation. 4/7 LOPD cases had one heterozygous missense or null mutation, suggesting that the second common intronic mutation was not detected by WES. One CRIM-negative IOPD sample had a frameshift ACE mutation. None of the samples were found with a ACTN3 mutation.

79) BRAIN BIOMARKERS OF LONG TERM OUTCOME OF NEONATAL ONSET UREA CYCLE DISORDER

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Background: The urea cycle disorders (UCDs) are the most common inborn errors of metabolism, with an incidence of approximately one in 30,000 births. Ornithine Transcarbamylase Deficiency (OTCD), the most common UCD, results in impairment of the body's ability to excrete nitrogen, causing toxic buildup of ammonia with resultant encephalopathy. Acute symptoms of hyperammonemia (HA) include vomiting, hyperventilation, seizures, and irritability. Long term neurological changes include deficits in working memory and executive function, ataxia, and failure to thrive.

Objective: To date, there are a lack of specific predictors of prognosis of infants with neonatal onset of OTCD outside of plasma ammonia level at presentation and duration of HA coma. This study provides a comprehensive longitudinal analysis of the case of a 16-year-old male with neonatal onset of OTCD with significant implications on the future clinical work up and management of UCD patients.

Design/Methods: A retrospective chart review of a 16-year-old male patient who presented with neonatal onset of OTCD at University Children's Hospital in Zurich, Switzerland was conducted. The patient had undergone MRI and MR spectroscopy on a 1.5 T Siemens scanner and blood analyses over the course of several years with closer monitoring in infancy period.

Results: The subject's course, which included 75 hospitalizations due to symptomatic HA episodes, produced several noteworthy observations. Plasma and brain glutamine levels both remain elevated following HA episodes even after plasma ammonia levels return to normal. In addition, MR spectroscopy showed a decreased N acetylaspartate peak. Relatively normal brain MRI without signal alterations despite elevated glutamine levels point to the utility of ¹H MRS in the acute and chronic stages of recovery.

Conclusions: This represents the first comprehensive long term analysis of a patient with neonatal onset of OTCD. Elevated levels of plasma and brain glutamine despite normal plasma ammonia levels following HA episodes suggest that glutamine may be a better indicator of neurotoxicity. In addition, plasma ammonia cannot serve as a reliable clinical marker for neuronal damage. Normal MRI results despite delayed development and neurocognitive impairment suggests that routine imaging may not be sufficient to monitor neuronal damage. Based on this fact and findings on ¹H MR spectroscopy, we posture that ¹H MRS should be considered as part of the routine clinical work up in OTCD patients to monitor acute and long term changes.

80) OPA1-PLUS SYNDROMES MAY INCLUDE CARDIOMYOPATHY — EXPANDING THE PHENOTYPE

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Pathogenic variants in the mitochondrial dynamin-related GTPase nuclear-encoded gene OPA1 (Optic Atrophy-1) located on chromosome 3q28–q29, have been known to be the molecular basis of autosomal dominant optic atrophy (DOA). In a subset of patients multi-systemic neurological and neuromuscular features have been described. OPA1 is involved in mitochondrial inner membrane fusion, cristae shaping, oxidative phosphorylation, mitochondrial membrane potential maintenance and the control of apoptosis. Here we report a 60-year-old male with a striking family history of premature cardiac death in his father and brother; who presented with a myopathic left ventricle and conduction system disease (LBBB) without evidence of ischemic or other primary myocardial pathology to explain the LBBB. When corrected, the patient had significant improvement in his left ventricle mechanics, but not his functional status. Subsequent clinical course was consistent with severe myopathy including gait changes, persistent dyspnea, fatigability and restrictive lung disease. Additional clinical features include keratoconus, myopia, tinnitus and left-sided hearing loss. Genetic testing revealed a heterozygous pathogenic variant in the OPA1 gene, c.113_130del18 (p.R38_S43del). Electrophysiological studies raised concern for a myopathic process. Immunohistochemical analysis of skeletal muscle revealed increased cytochrome C oxidase (COX)-deficient fibers over that expected for age. The phenotypic spectrum of OPA1-related disorders is known to have considerable inter- and intra-familial variability and incomplete penetrance. To date, cardiomyopathy has not been associated with the OPA1-plus syndromes except in one family with infantile-onset encephalopathy and progressive hypertrophic cardiomyopathy with a homozygous OPA1 pathogenic variant. Although we cannot exclude the possibility that the patient's non-ischemic cardiomyopathy is unrelated to the OPA1 pathogenic variant, cardiac involvement is increasingly being recognized in disorders of mtDNA maintenance. In addition, OPA1-mutant mice have been shown to have abnormalities of cardiac function. We suggest that this association may further broaden our understanding of the phenotypic spectrum of the OPA1-plus syndromes and that patients with DOA should have comprehensive cardiology evaluations.

81) GLYCOMACROPEPTIDE FOR NUTRITIONAL MANAGEMENT OF PHENYLKETONURIA: A RANDOMIZED, CONTROLLED CROSSOVER TRIAL

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Background: Phenylketonuria (PKU) requires lifelong management with a low-phenylalanine (phe) diet in combination with medical foods comprised primarily of amino acids (AAs) or intact protein from glycomacropeptide (GMP). Glycomacropeptide is a 64 AA glycoposphopeptide isolated from cheese whey and made into palatable, low-phe medical foods as an alternative to phe-free AA formula.

Objective: To assess the efficacy and safety of a low-phe diet containing GMP compared to AA medical foods as the primary protein source in free-living subjects with PKU evaluated at either the University of Wisconsin-Madison or Boston Children's Hospital.

Design and Methods: A randomized, controlled, two-stage crossover trial was conducted in 30 early-treated PKU subjects (aged 15–49 years) randomly assigned to follow low-phe diet treatments of GMP-AA or AA-GMP, each for 3-week at home, with a 3-week washout period using AA formula between treatments. Inclusion criteria included the ability to consume both the subject's usual AA formula and GMP medical foods as the primary source of protein. The protocol with 4 study visits included: nutritional counseling, physical examinations, assessment of fasting plasma AA profiles and phe concentrations in dried blood spots, completion of food records and questionnaires, and neuropsychological tests to assess executive function. The Wisconsin State Lab of Hygiene analyzed AA concentrations in plasma and DBS using ion exchange chromatography and MS/MS, respectively.

Results: Of 156 PKU subjects screened for eligibility during 2012–2015, 32 were enrolled and 30 subjects completed the protocol, 19 in Wisconsin and 11 in Boston. There were no significant carryover effects due to the crossover design. Subject compliance with the GMP diet was 80% (24 of 30 subjects) based on an increase in plasma threonine concentration. Subjects rated GMP medical foods as more acceptable and convenient than AA formula ($p < 0.003$). There were no adverse events associated with either the AA or GMP diets. Subjects reported gastrointestinal symptoms and persistent hunger with the AA diet that improved with the GMP diet.

Conclusions: This is the first randomized, controlled clinical trial to compare the effects of GMP and AA medical foods in the nutritional management of PKU. Results of the primary endpoint, change in plasma concentration of phe in subjects fed the GMP diet compared with change in plasma concentration of phe when fed the AA diet, will be reported.

This study was funded by the Office of Orphan Product Development, of the Food and Drug Administration (FD003711) and is registered at www.clinicaltrials.gov NCT01428258.

82) THE USE OF BRAIN MRS GLUTAMINE AS A BIOMARKER TO GUIDE THERAPY IN A PATIENT WITH GASTRIC-BYPASS RELATED HYPERAMMONEMIC COMA

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Background: Acute idiopathic hyperammonemia in an adult patient is a life-threatening condition often resulting in a rapid progression to irreversible cerebral edema and death. This phenomenon has been reported in association with Roux-en-Y gastric bypass, where patients with malnutrition, particularly zinc deficiency appear to be at risk of developing hyperammonemia. While ammonia-scavenging therapies lower blood ammonia levels, in comparison, clearance of waste nitrogen from the brain may be delayed. We hypothesized that the slower clearance for brain glutamine levels accounts for the delay in improvement following initiation of treatment in cases of chronic hyperammonemia. We also evaluated the use of magnetic resonance spectroscopy (MRS) in monitoring response to therapy.

Methods: In this observational study, we used magnetic resonance spectroscopy (MRS) to monitor cerebral glutamine levels, the major reservoir of ammonia, in a Roux-en-Y gastric bypass patient with hyperammonemic coma, undergoing therapy with high calorie parenteral nutrition along with N-carbamoylglutamate and the ammonia-scavenging agents, sodium phenylacetate and sodium benzoate. We also repleted the vitamin and micronutrient deficiencies.

Results: Improvement in mental status mirrored brain glutamine levels, as coma persisted for 48 hours after plasma ammonia normalized. MRS demonstrated there was no reaccumulation of brain glutamine as therapy was weaned and that levels remained normal one year after coma. No genetic urea cycle defect was identified.

Conclusions: Hyperammonemia after Roux-en-Y bariatric surgery particularly with concomitant nutritional deficiencies is increasingly being recognized. Our patient responded well to standard hyperammonemia therapies and nutritional support. We propose MRS to monitor brain glutamine as a noninvasive approach to be used for diagnostic and therapeutic monitoring purposes in adult patients presenting with idiopathic hyperammonemia.

83) IN VIVO MONITORING OF UREA CYCLE ACTIVITY WITH ¹³C-ACETATE AS A TRACER OF UREAGENESIS

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Background: The hepatic urea cycle is the main metabolic pathway for detoxification of ammonia. Inborn errors of urea cycle function present with severe hyperammonemia and a high case fatality rate. Long-term prognosis depends on the residual activity of the defective enzyme. A reliable method to estimate urea cycle activity in-vivo does not exist yet. The aim of this study was to evaluate a practical method to quantify ¹³C-urea production as a marker for urea cycle function in healthy subjects, patients with confirmed urea cycle defect (UCD) and asymptomatic carriers of UCD mutations.

Methods: ¹³C-labeled sodium acetate was applied orally in a single dose to 47 subjects (10 healthy subjects, 28 symptomatic patients, 9 asymptomatic carriers).

Results: The oral ¹³C-ureagenesis assay is a safe method. While healthy subjects and asymptomatic carriers did not differ with regards to kinetic variables for urea cycle flux, symptomatic patients had lower ¹³C-plasma urea levels. Although the ¹³C-ureagenesis assay revealed no significant differences between individual urea cycle enzyme defects, it reflected the heterogeneity between different clinical subgroups, including male neonatal onset ornithine carbamoyltransferase deficiency. Applying the ¹³C-urea area under the curve can differentiate between severe from more mildly affected neonates. Late onset patients differ significantly from neonates, carriers and healthy subjects.

Conclusion: This study evaluated the oral ¹³C-ureagenesis assay as a sensitive in-vivo measure for ureagenesis capacity. The assay has the potential to become a reliable tool to differentiate UCD patient subgroups, follow changes in ureagenesis capacity and could be helpful in monitoring novel therapies of UCD.

84) MULTIPLE MECHANISMS CONTRIBUTE TO THE ATP DEFICIT IN BIOTIN DEFICIENCY, INCLUDING A NOVEL SIGNALING PATHWAY, AND HAVE IMPLICATIONS FOR INHERITED METABOLIC DISORDERS

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Multiple carboxylase deficiencies (MCD) result from inability to bind biotin to the apoenzymes (HCS–) or to recycle this cofactor (biotinidase–). These disorders are mimicked by dietary absence or insufficiency of biotin. We have previously shown that ATP deficit is a major effect, whose responsible mechanisms have not been thoroughly studied. Here we show that in rats and cultured cells it is a multifactorial process, the result of reduced oxidative phosphorylation observed by a decreased TCA cycle flow, partly due to deficient anaplerotic biotin-dependent pyruvate carboxylase, and by a diminished flow of the electron transport chain, seen by deficient cytochrome c oxidase (complex IV) activity with decreased cytochromes content. There is also severe mitochondrial damage, most likely caused by toxic levels of propionyl and other acyl CoA compounds as shown by carnitine supplementation studies, and marked mitochondrial content decrease, even in the face of the energy sensor AMPK activation, known to induce mitochondria biogenesis. This idea was supported by experiments on AMPK knockout mouse embryonic fibroblasts (MEFs). Additionally, we provide evidence for a novel signaling pathway through which systemic inflammation, concomitant to the toxic state, engender mitochondria deficiency by activating the mitophagic proteins BNIP3 and PINK, mediated by IL-6, STAT3 and HIF-1?. The ATP deficit also provides a plausible basis for the cardiomyopathy in patients with propionic acidemia (PA), a hallmark of MCD. Furthermore our findings, particularly the toxic damage of mitochondria, are very likely an important causal factor of respiratory energy shortage in several inherited metabolic disorders, including PA, methylmalonic acidemia (MMA) and other organic acidemias like Leigh disease, maple syrup urine disease, glutaric acidemia, some of the mitochondrial fatty acid disorders, among others, and possibly some non-Mendelian neurodegenerative diseases. Together our results imply core mechanisms of energy deficit in several inherited metabolic disorders. **Acknowledgment:** This work was supported by research grants from Dirección General de Asuntos del Personal Académico de la Universidad Nacional Autónoma de México (PAPIIT IN208214) and from Consejo Nacional de Ciencia y Tecnología (Fondo SEP-CONACYT 166857).

85) SOLID ORGAN TRANSPLANTATION IN MITOCHONDRIAL DISEASE: PROCEED WITH CAUTION

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Background: Solid organ transplants are occasionally performed in both adult and pediatric patients with primary mitochondrial disease. Poor outcomes have been reported in case reports and small case series. It is unclear whether the underlying metabolic disease has a significant impact on post-transplant morbidity and mortality.

Methods: Data was obtained in 36 patients from 17 mitochondrial disease centers across North America, the United Kingdom and Australia. We assessed the clinical characteristics and outcomes following solid-organ transplant in an international cohort of pediatric and adult patients with primary mitochondrial disease secondary to both mitochondrial and nuclear DNA mutations. Patient outcomes were noted after either liver, kidney or heart transplantation. Descriptive statistics were calculated for demographic, mitochondrial disease-related characteristics, post-transplant symptoms and whether there was worsening of their underlying mitochondrial disease. The overall survival was estimated by the Kaplan–Meier method.

Results: Excluding patients with POLG-related disease, the 1- and 5-year post-transplant survival approached or met statistics seen in non-mitochondrial transplant patients. The majority of non-POLG mitochondrial disease patients did not have worsening of their mitochondrial disease within 90-days post-transplant. Post-transplant complications, including rejection, were generally treatable. Organ rejection was not a common occurrence in mitochondrial disease patients. Over half of our cohort did not have a mitochondrial disease considered or diagnosed prior to transplantation, despite having concomitant manifestations that would have suggested mitochondrial disease to an experienced consultant.

Conclusions: Patients with mitochondrial disease in this cohort generally tolerated solid-organ transplantation. Such patients may not need to be excluded from transplant solely for their mitochondrial diagnosis; additional caution may be needed for patients with POLG-related disease.

Transplant teams should be aware of mitochondrial disease as an etiology for organ-failure and consider appropriate consultation and further investigation to evaluate for these disorders. Outside of a single retrospective analysis of liver transplantation in DGUOK disease, this represents the largest cohort of patients reported with mitochondrial disease that have undergone solid organ transplantation.

86) ASSESSMENT OF METABOLIC MARKERS IN PROPIONIC ACIDEMIA

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Objectives: Despite decades of experience in the treatment of propionic acidemia (PA), the goals of medical and nutritional therapy remain undefined. We assessed multiple biochemical analytes to explore a relationship with illness, age, and other parameters, as an initial step in defining treatment goals.

Methods: Clinical data from 12 individuals with PA was collected along with samples for plasma amino acids, acylcarnitines, carnitine, fatty acid, and quantitative urine organic acid analysis. Sample distribution and mean values were compared for candidate analytes at baseline clinical status and during metabolic decompensation.

Results: A marked accumulation in plasma odd chain fatty acids (OCFA) was observed, specifically C15:0, C17:0, and C17:1 species. In younger individuals with PA (<10 years of age), there appeared to be a greater proportion of C23:0 and C25:0 OCFAs in the plasma total OCFA fraction.

Metabolite analyses performed during periods of metabolic stability (baseline) and acute illness included: plasma amino acids (n = 125, 25), plasma acylcarnitines (n = 75, 11), quantitative urine organic acids (n = 70, 12), and total plasma fatty acids (n = 19, 5). The largest effect sizes with illness corresponded to glutamine (Cohen's d = 1.18), valine (Cohen's d = 0.67), citrate (Cohen's d = 1.17), 2-methylcitrate (Cohen's d = -1.39), and citrate:2-methylcitrate ratio (Cohen's d = 0.764). Statistically significant (p < 0.05) differences in means between baseline and times of illness were observed for glutamine, valine, and intermediates of the tricarboxylic acid cycle.

Conclusions: We observed accumulation of OCFAs in all individuals with PA. There was no apparent relationship between absolute or relative accumulation of OCFAs and metabolic stress. However, the relative accumulation of specific OCFAs appeared to vary between older and younger individuals.

Results also suggest impairment of the tricarboxylic acid cycle. In particular, a reduction in glutamine levels was detected during periods of illness, as previously observed. During times of metabolic stress, glutamine may become a substrate for generation of alpha-ketoglutarate to maintain anaplerosis. These results suggest a role for replenishment of anaplerotic substrates during times of metabolic stress.

87) DIABETES MELLITUS AND GLYCOGEN STORAGE DISEASE TYPE 1A IN AN ADULT FEMALE: A CASE STUDY

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Glycogen storage disease type 1a (GSD 1a) manifests with fasting hypoglycemia with hyperinsulinemia, hepatomegaly, lactic acidemia, hyperlipidemia and hyperuricemia. Long term sequelae include osteoporosis, glomerulosclerosis, gout and hepatic adenomas with a risk of malignancy. Few case reports have brought awareness to the risk of developing diabetes mellitus (DM) as a late complication in GSD 1a. Here we report DM type 2 in a 36-year-old Asian/Indian female who was diagnosed with GSD 1a at 3 years based on decreased glucose-6-phosphatase activity and increased liver glycogen stores with diagnosis later confirmed through genetic testing. She began a high carbohydrate diet with nighttime corn starch (CS) at age 9 years. She reportedly followed the diet for the next 15 years but without being followed at a metabolic center. At age 27 years, she presented with a BMI of 31.5, triglycerides 2232 mg/dL, total cholesterol 341 mg/dL, uric acid 10.6 mg/dL, lactate 4.4 mmol/L, anemia (Hb/Hct 9.4 g/dL/0.30L/L, RBC count 3.55 cells/mcL), and multiple hepatic lesions. She experienced difficulty monitoring her blood glucose (BG) levels and relied on taking CS based on perceived vs actually measured low BG levels. At age 31 years and a BMI of 35, she delivered a normal male child. She developed gestational DM and her metabolic control during pregnancy is unknown. Over the next 4 years she had declining renal function, underwent lithotripsy and continued to gain weight (her BMI increased to 40) in spite of reducing calorie intake and increasing physical exercise. Her maintenance needs for CS decreased from 0.7 g/kg to 0.26 g/kg. She was subsequently diagnosed to have DM type 2 with BG of 310 mg/dL, HbA1C 11% and insulin 32.0 µl U/mL. This case study highlights the challenges in simultaneously treating DM and GSD 1a, two opposite disorders that affect glucose homeostasis.

88) REDUCTION OF BLOOD PHENYLALANINE CONCENTRATIONS AND NEUROPSYCHIATRIC IMPROVEMENTS IN ATTENTION AND EXECUTIVE FUNCTION IN ADULTS WITH PHENYLKETONURIA IN A RANDOMIZED, DOUBLE-BLIND STUDY

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Introduction: High blood phenylalanine (Phe) concentrations have been associated with neuropsychiatric deficits in subjects with phenylketonuria (PKU). Sapropterin dihydrochloride (KUVAN®, BioMarin Pharmaceutical Inc., Novato, CA) has been shown to reduce blood Phe concentrations in individuals with tetrahydrobiopterin-responsive PKU. However, the effects of sapropterin on neuropsychiatric outcomes in adults with PKU is not known. A sub-analysis was performed on adult subjects (>18 years) with PKU from a larger cohort (206 children and adults) of subjects enrolled in the placebo-controlled, double-blind PKU ASCEND study.

Methods: In this study, adult subjects with PKU were randomized to sapropterin (N = 55) or placebo (N = 65) treatment groups for 13 weeks, after which all subjects received open-label sapropterin for an additional 13 weeks. All subjects were asked to continue with their current diets. Outcome measures were changes in blood Phe, symptoms of Attention Deficit Hyperactivity Disorder Adult Self-Report Rating Scale (ADHD-ASRS), self-reports of executive function using Behavior Rating Inventory of Executive Function (BRIEF), and Hamilton Rating Scale for Depression (HAM-D) and Anxiety (HAM-A).

Results: Over 13 weeks, total protein intake decreased for the placebo group (from mean: 68.7 ± 29.8 g to mean: 64.6 ± 26.8 g) but increased slightly for the sapropterin group (mean: 75.3 ± 23.1 g to mean: 75.7 ± 42.7 g). In the sapropterin cohort, a median –10% decrease in blood Phe at week 13 was associated with improvements in ADHD-ASRS total score (mean change in ADHD-ASRS total score: –6.1 points; $p < 0.001$) and executive function (mean change in BRIEF-GEC: –7.7 points; $p < 0.001$). In the placebo cohort, a median –8.1% decrease in blood Phe at week 13 was also associated with improvements in ADHD-ASRS total score (–5.7 points; $p < 0.001$) and executive function (mean change in BRIEF-GEC: –7.9 points; $p < 0.001$). Depression and anxiety scores also improved at 13 weeks for both cohorts. Improvements were generally sustained through week 26 during the open-label period.

Conclusion: Lower blood Phe concentrations in adults with PKU, irrespective of sapropterin therapy, were associated with improvements in neuropsychiatric performance, as assessed by ADHD-ASRS, BRIEF-GEC T-scores, and reductions in anxiety and depression. This study further supports the association between blood Phe, brain function, and cognitive health in adults with PKU.

89) IMPROVED SENSITIVITY AND SPECIFICITY OF URINE-BASED TESTING FOR LYSOSOMAL STORAGE DISORDERS USING TANDEM MASS SPECTROMETRY

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Lysosomal storage disorders (LSDs) are caused by the inability to properly degrade one or more complex macromolecules such as glycoaminoglycans (GAGs), glycoproteins and sphingolipids. The deficiency of a lysosomal enzyme causes the accumulation of partially degraded substrate, resulting in cellular toxicity, and, often, excess urinary excretion. Therefore, urine-based screening methods such as the qualitative separation of GAGs or oligosaccharides by thin layer chromatography or electrophoresis, or the quantitative measurement of total GAGs via DMB binding have historically been used for this group of disorders. However, these traditional methodologies are lacking in both sensitivity and specificity. Our laboratory has utilized UPLC–MS/MS methods to overcome these limitations in 180 urine samples from LSD patients. We have quantified the individual GAG species (heparan sulfate, dermatan sulfate, etc) after chemical or enzymatic digestion in 63 urine samples from untreated patients with MPS I, II, III, IVA or VI. While keratan sulfate was elevated in all 37 urine samples tested from 34 MPS IVA patients, total GAGs were normal in four of these samples. Additionally, the degree to which the individual GAG species are elevated is significantly greater than that observed for total GAGs. Keratan sulfate is on average 4.5-fold elevated in MPS IVA patients versus 1.7-fold for total GAGs and heparan sulfate is 20–30-fold elevated in MPS I, II and III patients versus 2.5–4.5-fold for total GAGs. This provides a much wider dynamic range for therapeutic monitoring. Forty-four samples from 36 patients with MPS I, II, IVA and VI who are receiving treatment have been analyzed and a significant reduction in individual GAG levels has been demonstrated. Using selected-reaction monitoring for disease-specific free oligosaccharides, 71 urine samples have been analyzed from patients with Mucopolidosis, Alpha-mannosidosis, Beta-mannosidosis, Aspartylglucosaminuria, Fucosidosis, Beta-galactosidase deficiency, Sialidosis, Galactosialidosis, Sandhoff disease and Pompe disease, each with clearly abnormal results. Monitoring a specific transition for each condition also dramatically reduces interference, providing improved specificity. In addition, this method is semi-quantitative and can be used for therapeutic monitoring as treatments become available for the glycoproteinoses. An approximately 50% decrease of the disease-specific oligosaccharide was noted in a beta-mannosidosis patient post bone marrow transplantation. In summary, the application of UPLC–MS/MS to the identification and quantification of urinary biomarkers for LSDs has greatly improved the diagnostic utility of non-invasive urine-based testing for this group of disorders.

90) NEWBORN SCREENING FOR LYSOSOMAL STORAGE DISEASES: VIEW FROM ITALY

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Since 2014, newborn screening in North-East of Italy was extended to include aminoacids, fatty acids and organic acids disorders. Recently, four lysosomal storage disorders, namely Fabry, Pompe, Gaucher and Mucopolysaccharidosis Type I (MPS I) diseases, were included in our screening platform.

The enzyme activities of α -galactosidase A (GLA), α -glucosidase (GAA), B-glucocerebrosidase (ABG) and α -L-iduronidase (IDUA), were analyzed in dried blood spot (DBS) by stable isotope dilution flow injection analysis MS/MS (FIA-MS/MS) using the Six-Plex LSD reagents kit (NeoLSD, Perkin-Elmer). Before its inclusion in the routine, a feasibility study was conducted to evaluate performance of the kit. Repeated measured ($n = 10$) of CDC Quality Controls and 4 normal newborn DBS showed a CV % ranging from 5 to 15%. Reference ranges of each enzyme were also determined from the analysis of ~2000 DBS from healthy newborn population. In addition, DBS from patients affected by Fabry disease ($n = 9$), Fabry heterozygotes ($n = 12$) Pompe disease ($n = 5$) Gaucher disease ($n = 18$) and MPS I ($n = 2$) were used to assess disease ranges. We observed an excellent separation from healthy newborns and each of the 4 diseases. Only Fabry heterozygotes overlapped in the healthy ranges, as expected. For newborn screening the first cut off values was fixed at 0.25th percentile and a second cut-off (high risk patients) at 0.2 Multiple Of Median (MOM) (GLA = $2 \mu\text{M/h}$, ABG = $1.83 \mu\text{M/h}$, IDUA = $1.45 \mu\text{M/h}$, GAA = $2.47 \mu\text{M/h}$). For Gaucher and Fabry disease, we add as second tier test, the DBS lysosphingolipids (LysoGL1 and LysoGL3 respectively).

To date 10,000 newborns were analyzed, 4 were positive: Pompe disease ($n = 1$), MPS I ($n = 1$) Fabry disease ($n = 2$). Diagnosis was confirmed by molecular analysis. This preliminary data of LSD screening provided the opportunity to validate the effectiveness of NeoLSD assay system, adjust the cut-off for detection of positive and define the system of recall and follow-up strategy.

91) SHORT-TERM BIOLOGICAL VARIANCE OF PHE IN PATIENTS WITH PHENYLKETONURIA

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Control of blood phenylalanine (PHE) levels is the primary goal in managing phenylketonuria (PKU). Both the overall exposure to PHE and the variation in PHE are thought to contribute to long-term neurocognitive outcome.

The objective of this study was to measure the diurnal variation of PHE in patients ≥ 4 years of age. Four groups were studied: Patients treated with diet alone who were in poor or good control ($>1/3$ or $\leq 1/3$, respectively, of monitoring PHE levels above the target treatment range of ≤ 360 or $\leq 600 \mu\text{M}$ for ages <12 or ≥ 12 years, respectively, in the 6 months preceding enrolment; $n = 8$ "Wide PHE" and $n = 10$ "Target PHE", respectively), patients treated with diet plus Kuvan ($n = 9$ "Kuvan"), and patients with benign/mild hyperphenylalaninemia (not requiring diet; $n = 5$ "Control").

Study subjects were admitted to the short-stay unit in the morning after an overnight fast and then pre-prandial and 1 and 2 hour post-prandial samples were collected for each meal over the next 24 h. Whole blood dried blood spots and plasma were analyzed for amino acids. The maximum, mean and standard deviation of PHE levels were calculated. Significance was calculated by two-way single factor ANOVA with post-hoc Mann-Whitney U test.

The mean PHE levels were 790, 288, 323, 300 μM for the Wide PHE, Target PHE, Kuvan and Control groups, respectively ($p = 0.0003$, Wide PHE significantly higher than the other 3 groups). The maximum PHE levels showed the same pattern ($p = 0.0005$). The standard deviation of PHE was not significantly different between the groups (68, 38, 53, 53 μM , respectively).

In conclusion, PHE levels were higher in patients who historically had poor control of PHE levels, while patients who tended to be in good control on diet or who were on diet plus Kuvan had lower levels that were similar to controls.

92) UNTREATED PHENYLKETONURIA IN ADULT SISTERS WITH DISCORDANT PRESENTATIONS OF NORMAL INTELLIGENCE AND INTELLECTUAL DISABILITY

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Background: Intellectual disability is the cardinal clinical feature of untreated phenylketonuria (PKU). Dietary therapy beginning early in infancy can reduce the blood phenylalanine and prevent the intellectual disability. It is rare to encounter untreated individuals with classic PKU who are not intellectually disabled.

Case Report: A 39-year-old female was diagnosed with PKU at age 7 years during evaluation for intellectual disability. Her IQ was 54 on the Stanford Binet psychological test. She attended special classes in schools, and as an adult she lived at home attending an activity center for the intellectually disabled. She had limited verbal output consisting mainly of echolalia and simple phrases. Her 36-year-old sister was diagnosed with PKU upon family screening. Her developmental milestones were normal. She did well in school, though reported working harder to get good grades than her two non-phenylketonuric siblings. Wechsler Adult Intelligence Scale at age 16 years revealed a full scale IQ 87, verbal IQ 99, and non-Verbal IQ 72. She obtained a bachelor's degree and works as an executive secretary. Both sisters had a normal general physical examination, except for symmetrically increased deep tendon reflexes. Both sisters consumed normal diets. Familial testing demonstrated normal plasma phenylalanine levels in the parents and two siblings. The unaffected siblings full scale IQs were 113 and 91.

Results: Plasma phenylalanine in the proband and her sister were $1839 \mu\text{mol/L}$ (30.4 mg/dL) and $1888 \mu\text{mol/L}$ (31.2 mg/dL) respectively. PAH gene testing in the siblings revealed the same genotype with a null mutation W187X, and a missense mutation R261Q.

Discussion: These siblings share a genotype and biochemical profile, however one sister was largely protected from intellectual disability. Further studies are needed to understand how expression of the genetic defect interacts with other factors to cause intellectual disability in PKU.

93) DIAGNOSIS OF LCHAD IN AN AT RISK FETUS USING UMBILICAL CORD BLOOD

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Objective: To describe the diagnosis of Long-chain hydroxyacyl-CoA dehydrogenase deficiency (LCHAD) using umbilical cord blood at the time of delivery in an at risk pregnancy.

Background: Trifunctional protein deficiency/Long-chain hydroxyacyl-CoA dehydrogenase deficiency (LCHAD/TPD) is a disorder of fatty acid oxidation and ketogenesis. Accumulation of toxic long-chain acylcarnitines in LCHAD/TPD may cause severe neonatal lactic acidosis, cardiomyopathy, and hepatic dysfunction. Patients may also manifest chronic weakness, pain, as well as recurrent rhabdomyolysis. Patients may have acute decompensations with episodes of illness, decreased oral intake, prolonged fasting episodes, surgery, etc. Mothers of affected fetuses can present with acute fatty liver of pregnancy or HELLP syndrome. Patients can be identified through newborn screening with initiation of appropriate interventions. Patients affected with LCHAD have increased hydroxycompounds C14-OH, C16-OH, C18-OH, and C18:1-OH. Urine organic acids demonstrate C6-C14 hydroxydicarboxylic acids. Although patients identified presymptomatically and treated can have a milder course, patients with LCHAD can still develop chronic complications including cardiomyopathy. Treatment consists of limiting dietary fat and providing other essential nutrients in the formula and fasting avoidance. Due to increased renal excretion of bound acylcarnitines, patients may develop a secondary carnitine deficiency. Carnitine can be provided if the patient is found to be deficient. To date, there are no published cases of LCHAD/TPD diagnosed using umbilical cord blood.

Clinical Case: Our patient is a 4 week old female with LCHAD/TPD diagnosed at the time of delivery using umbilical cord blood. Concern for the diagnosis of LCHAD/TPD was suspected due to an affected older sibling diagnosed through routine newborn screening. Her mother was counseled on invasive prenatal testing which was declined. Pregnancy was complicated by HELLP syndrome and delivery at term. Umbilical cord blood was collected at delivery and sent for acylcarnitine analysis. Treatment was started immediately.

Acylcarnitine analysis from the umbilical cord blood was significant for C14-OH 0.14 nmol/mL (normal <0.04 nmol/mL), C16-OH 0.50 (normal <0.10), C18:2-OH 0.23 (normal <0.04), and C18:1-OH 0.39 (normal <0.03). These findings are consistent with a biochemical diagnosis of LCHAD/TPD.

The neonatal course was complicated by transient episodes of tachypnea, which have been improving without intervention. Echocardiogram in the neonatal period was normal. Serum CK was elevated in the neonatal period and normalized. Neutrophils have been mildly decreased, similar to the sibling's recurrent neutropenia with mild stress or illness.

Discussion: Patients with LCHAD/TPD should have treatment initiated as early as possible to avoid or minimize the long-term complications of the disorder including cardiomyopathy. In pregnancies at risk of having a child with LCHAD/TPD, umbilical cord blood sample is an efficient method to diagnose an inborn error of metabolism such as LCHAD/TPD.

94) RARE DISEASES RESEARCH CERTIFICATE PROGRAM: EQUIPPING THE NEXT GENERATION OF RARE DISEASE RESEARCHERS

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Background: Rare disease research is a unique field that requires particular skills not consistently fostered in the current academic research setting. Rare disease research spans multiple medical specialties and funding streams. Rare diseases are defined by the small size of the affected patient populations and multidisciplinary approaches in research. The demand for this research does not match the current supply of researchers prepared to partake in the field. This program is designed to prepare the next generation of researchers for the rare disease field.

Methods: A cohort of early career researchers will be selected from a variety of specialties and sub-specialties. The program includes an in-person seminar at the beginning of the program and an in-person seminar at the end of the program, with semimonthly webinars and asynchronous learning for the months in between. Outcomes are measured by posters submitted for conferences and scored by a rubric, communications skills measured by presentation rubric, their publications post program, and their five-year occupational outcomes.

Results: We have successfully funded this novel program design and have begun the first year of this cohort including 21 participants from multiple specialties. The range of participants includes all types of specialty (pediatric, internal medicine, obstetrics and gynecology) and sub-specialty populations (pulmonary, hematology, oncology, nephrology, genetics, etc.). Cohort members expressed their satisfaction with didactics and one-on-one support of their research and trouble shooting during the monthly series. Short term evaluations of improved satisfaction with research and comfort with rare disease methods of research will be shown; however, long term outcome data is pending.

Conclusions: Given the lack of prepared researchers participating in the rare disease field, this program has been a success by highlighting and identifying funding to support early career rare disease researchers. Specific curriculum to teach the nuances and best practices in rare disease research are essential to effectively equip and encourage early career researchers.

95) NEWBORN SCREENING (NBS) FOR METACHROMATIC LEUKODYSTROPHY (MLD): RESULTS FROM A STUDY OF 100,000 DEIDENTIFIED NEWBORN SCREENING SAMPLES

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MLD is a progressive demyelinating disorder with variable phenotype and caused by arylsulfatase A (ARSA) deficiency. Hematopoietic stem cell transplantation has shown promise and gene therapy is being investigated. Any treatment is expected to have greatest benefit when started before the onset of symptoms, but NBS for MLD has been hampered by the availability of a high-throughput assay. Now, dried blood spot (DBS) based assays have been developed to measure either sulfatides that accumulate in MLD (Spacil Z et al. Clin Chem 2015; pii: clinchem.2015.2451591) or the presence of the ARSA protein (Meikle PJ et al. Mol Genet Metab 2006; 88: 307–142). We pursued the latter approach by immunoassay in a large study that involved screening of nearly 100,000 deidentified NBS samples for MLD and 12 other lysosomal storage disorders, Wilson disease, and Friedreich ataxia by multiplex immunocapture assay. Of 95,163 NBS samples tested 73 samples had ARSA concentrations below a preliminary cut off corresponding to the 1st percentile of the study population. ARSA gene analysis of these cases found 51 individuals carrying mutations associated with ARSA pseudodeficiency, 20 carriers of a known mutation or a variant of uncertain significance, and homozygosity for a possibly pathogenic mutation (c.511G>A, p.D171N) in two cases.

Conclusions: The finding of two potential MLD cases in the study population is consistent with the estimated prevalence of MLD. The test's positive predictive value (2.7%) when applying a simple cut off, however, is problematic. But performance can be improved by post-analytical use of a multivariate pattern recognition software (CLIR, Collaborative Laboratory Integrated Reports; available at <https://clir.mayo.edu>) as has been done previously for other NBS tests (Hall PL et al. Genet Med 2014; 16: 889–93).

96) N-ACETYL CYSTEINE THERAPY IN AN INFANT WITH TRANSALDOLASE DEFICIENCY IS WELL TOLERATED AND ASSOCIATED WITH NORMALIZATION OF ALPHA FETOPROTEIN LEVELS

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Transaldolase deficiency is a rare autosomal recessive disorder of the pentose phosphate pathway that presents clinically with infantile-onset hepatopathy progressing to cirrhosis, nephropathy, connective tissue abnormalities resembling cutis laxa, coagulopathy, cytopenias, congenital heart disease, and increased risk of hepatocellular carcinoma. In many cases, death occurs in infancy or early childhood. There is no established treatment for transaldolase deficiency in humans. Recent work in a knock-out mouse model of transaldolase deficiency has demonstrated a benefit to supplementation with the glutathione precursor N-acetylcysteine (NAC). We describe an infant with genetically confirmed transaldolase deficiency with multisystem involvement, including liver enlargement and markedly elevated alpha fetoprotein. Acetaminophen was strictly avoided. Treatment with oral NAC over a 6-month period was well tolerated and was associated with a sustained normalization of alpha fetoprotein levels and stable clinical course. The clinical significance of normalized serum alpha fetoprotein in this patient is not certain, although it may reflect decreased hepatocyte injury and reduced hepatocarcinogenesis as has been suggested in the mouse disease model. NAC supplementation may provide benefit in humans with transaldolase deficiency. Longer follow-up and data on the response of additional patients with transaldolase deficiency to NAC supplementation will be required to further evaluate efficacy and optimize dosing.

97) CASE REPORT — A CHEDIAK-HIGASHI DISEASE CARRIER PRESENTING WITH SUBTLE CLINICAL FEATURES INCLUDING PARKINSON'S DISEASE

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Background: Chediak-Higashi Disease (CHD) is a rare autosomal recessive disorder caused by mutations in the lysosomal trafficking regulator gene (*LYST*). Patients typically present in early infancy with partial oculocutaneous albinism, immunodeficiency, and a mild bleeding tendency; individuals who survive into adulthood develop a progressive and heterogeneous neurologic dysfunction that includes cerebellar deficits, peripheral neuropathy, and parkinsonism. While these characteristics have been investigated in CHD patients, little is known about how *LYST* heterozygosity influences these clinical features.

Objective: To assess the clinical findings of the paternal grandmother of two brothers diagnosed with atypical CHD, who presented with Parkinson's disease and showed heterozygosity for a missense mutation (c.4361C>A; p.A1454D in exon 12) in *LYST*.

Methods: Family members were evaluated by peripheral blood smear, eye exam, *LYST* mutation analysis, *LYST* mRNA expression, lysosome morphology and localization, and studies of natural killer cell function.

Results: Subtle features of disease segregated with carrier status for the missense *LYST* mutation in this family. Peripheral blood smears showed abnormal inclusions in a subset of neutrophils and lymphocytes in all family members who are carriers, and eye exam showed iris transillumination in only the missense carriers. Quantitative real-time PCR showed intermediate *LYST* mRNA levels in the grandmother ($75 \pm 1\%$) compared with her affected grandson ($54 \pm 1\%$). Furthermore, the grandmother's fibroblasts had lysosomes that clustered near the outer periphery of the nucleus, and natural killer cells exhibited intermediate cytotoxicity.

Conclusions: Family members of CHD patients should be evaluated to gain a better understanding of CHD. Our results indicate that subtle features of disease can be observed in CHD carriers; this could provide insight into disease pathophysiology, specifically pertaining to neurological features.

98) IDENTIFYING PATIENTS WITH VLCAD DEFICIENCY PRESENTING WITH AN ABNORMAL NBS USING INTEGRATED METHODS

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Very long-chain acyl-CoA dehydrogenase deficiency (VLCADD) is the most common disorder of long-chain fatty acid oxidation. It may present from the newborn period to adulthood with hypoketotic hypoglycemia, cardiomyopathy or rhabdomyolysis.

After a positive newborn screen (NBS) suggestive of VLCADD, the current follow up algorithm recommends doing an acylcarnitine profile (ACP). ACP can be normalized in the well fed state and may be uninformative. The aim of this study is to evaluate the utility of using integrated methods including biochemical, molecular and functional studies to help determine true positives.

Methods: We retrospectively reviewed the medical records of 56 cases with a positive NBS consistent with VLCADD, who were seen in Metabolism Clinic at Emory, starting on January 1st of 2007 to date in Georgia.

Results: 32/56 patients had abnormal ACP. All patients had gene sequencing and 53 variants were identified. However, 33 variants were classified as VOUS. 30/56 patients had deletion/duplication analysis and none were positive. Most patients did not have parental studies done.

After biochemical and molecular studies, 10/56 patients still had inconclusive results. Of these 3 had oxidation probe assay in fibroblasts, two were normal and the other had a mild abnormality.

Conclusion: We suggest that if ACP is not conclusive of a diagnosis of VLCADD, a systematic workup should be performed and the cumulative data including biochemical, molecular and functional studies should be conditionally essential to confirm the diagnosis.

99) PHENYLKETONURIA INTESTINAL MICROBIOTA: INSIGHTS FROM *PAH^{ENU2}* MICE FED DEFINED DIETS

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Background: Glycomacropeptide (GMP) is a 64 amino acid (AA) glycosylated peptide that is released from casein during cheese making and is devoid of aromatic AAs. Preclinical and clinical studies have demonstrated the applications of GMP to the nutritional management of phenylketonuria (PKU), obesity and intestinal disorders. GMP is a prebiotic defined as a selectively fermentable ingredient that results in specific changes in the intestinal microbiota that benefit host health.

Objective: To determine the impact of GMP, AA or casein diets on the cecal intestinal microbiota, concentrations of short chain fatty acids (SCFA), immune responses, and the hepatic metabolomics profile.

Design and Methods: Weanling PKU (*Pah^{enu2}*) and wild type (WT) mice were fed isoenergetic AA, GMP or casein diets between 3 to 23 weeks of age. The cecum content was collected for microbial DNA extraction to perform 16S microbiota analysis by Ion Torrent PGM sequencing and to measure concentrations of SCFA by gas chromatography. Splenocyte T cell populations were assessed using flow cytometry. Global metabolic profiles were determined in liver using the Metabolon platform.

Results: Changes in cecal microbiota are primarily diet dependent. The GMP diet resulted in a reduction from 30–35% to 7% in Proteobacteria, genera *Desulfovibrio*, in both WT and PKU mice ($p < 0.002$) with genotype dependent changes in Bacteroidetes or Firmicutes. Cecal concentrations of the SCFA acetate, propionate and butyrate increased with GMP. Spleen mass was significantly larger in PKU mice compared with WT mice. Both WT and PKU mice fed the GMP diet had significantly smaller spleens consistent with reduced inflammation compared with mice fed either casein or AA diet. The percent of stimulated spleen cells producing interferon-gamma was significantly reduced in mice fed GMP compared with casein. PKU mice displayed altered hepatic levels of metabolites derived from phe and tyr and known to be microbial origin compared to WT mice. This suggests that the PKU genotype alters the intestinal microbiota regardless of diet.

Conclusions: The benefits associated with the prebiotic effects of GMP include reduction in sulfate-reducing Proteobacteria (genera *Desulfovibrio*) and increased SCFA known to improve intestinal barrier function and reduce inflammation. The prebiotic effects of GMP may help explain the

improvements in intestinal symptoms reported by subjects with PKU following a diet with GMP medical foods compared to AA formula. This study was funded by Hatch USDA grant WIS01790.

100) THE M405V ALLELE OF THE GLUTARYL-CoA DEHYDROGENASE GENE IS COMMON AMONG MANY GLUTARIC ACIDURIA TYPE I (GA-I) LOW EXCRETORS

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Background: Glutaric aciduria type I is an autosomal recessive organic aciduria resulting from a functional deficiency of glutaryl-CoA dehydrogenase, encoded by the GCDH gene. Two clinically indistinguishable biochemical subgroups of GA-I are known; low and high excretors. Low excretors exhibit minimally elevated or normal levels of glutaryl metabolites. Consequently, these patients can be missed on newborn screen (NBS) and initial metabolic screening. This is notable since pre-symptomatic treatment of both types of GA-I significantly reduces the risk of neurological sequelae and results in better outcomes. We report a 12-year-old African American male who was initially evaluated at 1 year of age for failure to thrive, developmental delay, dystonia and hypotonia, and later biochemically and molecularly confirmed to be a GA-I low excretor (LE) with a p.M405V allele shared by other GA-I LEs.

Methods and Results: Initial MRI of the brain showed basal ganglia changes suggestive of an inborn error of metabolism. Initial biochemical evaluation of our patient (at 12 months of age) included plasma acylcarnitines with glutarylcarnitine at 0.21 μmol . This level was not initially flagged because, at that time, no reference range was available at the reference lab. This range was later set at $<0.15 \mu\text{mol}$. Initial urine acylcarnitine profile revealed a large C5DC acylcarnitine peak, later identified to be glutarylcarnitine. Initial urine organic acids were normal. Our patient was lost to follow-up for about 10 years. His clinical findings now consist of severe neurologic symptoms including; dystonia, dyskinesia, dysarthria, dysphagia and choreoathetosis, severe cognitive impairment and failure to thrive. He is wheelchair bound, G-tube dependent and nonverbal. Plasma acylcarnitines show elevation of glutarylcarnitine up to 0.36 μmol and urine acylcarnitines show a markedly increased glutarylcarnitine concentration. Molecular testing of GCDH identified two deleterious mutations, a known p.M405V and a novel p.V133L. The p.M405V mutation is reported in compound heterozygotes with GA-I and associated with other GA-I low excretors (reviewed here). While the c.397G>T (p.V133L) has not been previously reported, an allelic change, c.397G>A (p.V133M), has been reported in a patient with GA-I, and p.V133L is predicted to be deleterious by in silico algorithms.

Discussion and Conclusions: Glutaric aciduria type I is a devastating disorder but one where early medical and dietary interventions can result in significantly better outcomes and improved quality of life. Early identification of clinically asymptomatic yet at-risk individuals is crucial. We describe a new case of a GA-I LE found to have the p.M405V mutation. We present updated clinical, biochemical and molecular data for the previously described seven other GA-I LEs all sharing the M405V mutation with two cases missed by NBS using tandem mass spectrometry (MS/MS). We provide further evidence for the utility of urine acylcarnitines in diagnosing these patients. Finally, we discuss methods to optimize newborn screening for this disorder including using molecular screening for the M405V variant in certain populations in conjunction with MS/MS in order to identify asymptomatic at-risk GA-I LEs.

101) NEURONAL CEROID LIPOFUSCINOSIS-2 (CLN2) DISEASE, A TYPE OF BATTEN DISEASE CAUSED BY TPP1 ENZYME DEFICIENCY: CURRENT KNOWLEDGE OF THE NATURAL HISTORY FROM INTERNATIONAL EXPERTS

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Background/Objectives: The neuronal ceroid lipofuscinoses (NCLs) are the most common group of neurodegenerative disorders in children and adolescents. CLN2, a type of NCL caused by TPP1 enzyme deficiency, is characterized by seizures, rapid deterioration of language, cognition, motor skills and vision, and premature death. Our aim is to describe expert knowledge of CLN2 disease.

Methods: 18 international NCL experts answered a survey on CLN2 natural history.

Results: Clinical suspicion for CLN2 is low due to its rarity and non-specific presenting symptoms. A 1–4 year delay was reported between first onset of symptoms and diagnosis. Speech delay/decline, developmental delay/regression and seizures/epilepsy were identified as initial presenting symptoms. Symptom onset typically occurs between 1.5–5 years of age, but may occur later (9–12 years). Myoclonic epilepsy was the most commonly reported seizure type. Notably, seizures are refractory oftentimes requiring polytherapy. Cardiac rhythm anomalies, not previously associated with CLN2, were also identified.

Conclusions: CLN2 is a severe, progressive, pediatric-onset neurodegenerative disorder. Disease awareness is low, causing delays in diagnosis. Seizures in concert with a regression of language and/or motor milestones should raise suspicion for CLN2. Knowledge of CLN2 is paramount to ensure timely diagnosis and to enable early initiation of future therapies.

102) IDENTIFYING DRUGS FOR THE TREATMENT OF GUANIDINOACETATE METHYLTRANSFERASE DEFICIENCY (GAMT-D)

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Background: The deficiency of the enzyme GAMT results in the lack of its product creatine but also in the accumulation of its substrate guanidinoacetate (GAA). Accumulation of GAA has been proven to be neurotoxic and epileptogenic in GAMT-D patients. Current treatments, including the supplementation of creatine and ornithine and the restriction of arginine, fail to normalize GAA. Our goal is the identification of drugs that can prevent the accumulation of GAA through inhibition of the upstream enzyme L-arginine:glycine amidinotransferase (AGAT) that synthesizes GAA.

Methods: We set up two high-throughput screening assays; one to screen for inhibitors of AGAT gene expression and the other to screen for inhibitors of AGAT enzyme activity. Transcriptional inhibitors are found from drug library screening using a permanent HeLa cell line stably expressing AGAT promoter luciferase. Direct enzymatic inhibitors are validated from the thermal shift denaturation profiles of the enzyme in the presence of a ligand.

Results: Inhibition assays of AGAT transcription and AGAT enzyme activity were established and validated. From the screening of a small subset of libraries we found several compounds as potential AGAT transcription inhibitors. Two of the best hits were further tested for combinatory effects with drugs from another library. The effectiveness of one of those hits was validated in selected cell lines by RT-PCR.

Conclusion: We demonstrated that our established assays can be used as powerful tools towards finding new treatments for metabolic disorders.

103) RATE OF LEUCINE CLEARANCE IN MSUD

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Maple Syrup Urine Disease (MSUD), or branched-chain alpha-keto acid dehydrogenase deficiency, is associated with encephalopathy and neurologic dysfunction due to abnormal accumulation of leucine in the brain. Acute management relies on efficiently lowering leucine levels either by leucine-free diet with aggressive caloric support (i.e., IV glucose and lipids) to avoid catabolism, or by hemodialysis. For patients managed via nutritional support, we hypothesized that the decline in plasma leucine concentration follows a predictable kinetic model that could help inform management of future patients. We retrospectively reviewed plasma amino acid data from 17 MSUD patients over a total of 31 acute episodes. All were managed with sufficient calories and leucine free formula, following the protocol of Morton, et al. Combining all values over all patient encounters, the leucine decayed exponentially at an average rate of $-0.51 \times X$ in 24 h (X = starting leucine concentration; $SD = 0.17$). Variability was reduced when patients were analyzed separately based on indication for referral, with the lowest variability seen for patients immediately following an abnormal newborn screening result (leucine decay constant 0.48 ± 0.10). Follow-up patients with hyperleucinemia associated with illness showed a more variable decay rate, presumably secondary to other mitigating clinical factors. This kinetic model may serve as a guide to anticipate leucine concentrations during clinical management of patients with MSUD.

104) VERY LONG CHAIN ACYL CoA DEHYDROGENASE DEFICIENCY (VLCADD) AND CARDIAC INVOLVEMENT

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Background: Very Long Chain Acyl CoA Dehydrogenase Deficiency (VLCADD) is a long chain fatty acid oxidation disorder that can present with significant clinically variability: in the neonatal period with severe hypoglycemia, cardiomyopathy, and arrhythmias; in early childhood with hypoketotic hypoglycemia with fasting; or a later-onset disorder with rhabdomyolysis, myalgia, and exercise intolerance. Due to newborn screening, VLCADD patients are more likely to be identified prior to symptoms since treatment including fasting precautions and a low fat diet with medium chain triglyceride (MCT) oil can be initiated early. For individuals with cardiac involvement, MCT oil has improved existing cardiomyopathy. Routine cardiac evaluation is recommended, but there is a paucity of data regarding cardiac involvement in VLCADD patients who are identified with newborn screening and started on early treatment regimens.

Objective: Evaluate for cardiac involvement in patients with early diagnosis and treatment of VLCADD.

Methods: A comprehensive chart review was performed on all new patients presenting to the Biochemical Genetics clinic at the University of Wisconsin/Waisman Center between 2009–2015 with a newborn screen concerning for VLCADD or a prior diagnosis. All patients had cardiac assessment with standard echocardiography (and some with electrocardiography) performed at the time of diagnosis and had repeat cardiac evaluation scheduled annually.

Results: A total of 12 patients with VLCADD between 2009–2015. All but one patient were diagnosed based on a positive newborn metabolic screen for elevated long chain fats including C14:1. The one patient was diagnosed by biochemical and molecular DNA testing due to a family history of two siblings with cardiomyopathy. The patients had significant variability in apparent disease burden based on clinical symptoms, biochemical profiles, and genotype. All patients are treated, but the degree of intervention is variable depending on biochemical markers. No patients had evidence of cardiomyopathy on initial cardiac screening or at the time of follow up.

Conclusions: The natural history of cardiac involvement in VLCADD patients is unclear. Early diagnosis with newborn metabolic screening may identify at-risk infants and allow early treatment to help prevent development of cardiomyopathy, arrhythmias, and sudden death events. The results above suggest a low incidence of cardiomyopathy in those identified and treated early; however, continued cardiac surveillance in VLCADD is important to better understand those at risk of late-onset cardiac involvement as well as potential risks of treatment protocols starting in these young patients.

105) COMPROMISED MITOCHONDRIAL OXYGEN CONSUMPTION AND INCREASED REACTIVE OXYGEN SPECIES PRODUCTION IN LONG CHAIN FATTY ACID OXIDATION DEFECTS

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Background & objective: Acyl-CoA dehydrogenases are enzymes that catalyze the first step of mitochondrial fatty acid β -oxidation (FAO). Among them, very long-chain acyl-CoA dehydrogenase (VLCAD) and long chain acyl-CoA dehydrogenase (LCAD) are involved in the chain shortening of long chain fatty acids. Long chain FAO disorders, including VLCAD deficiency, are clinically characterized by hypoglycemia, hepatic dysfunction, rhabdomyolysis, and cardiomyopathy, whose pathophysiology is not yet established. In this study oxygen consumption, mitochondrial homeostasis and reactive oxygen species (ROS) production were assessed.

Methods: Oxygen consumption in fibroblasts from patients with VLCAD deficiency was monitored by a Seahorse XF analyzer. Mitochondrial mass was examined by fluorescent stain. ROS generation was monitored in patient fibroblasts by mitoSOX dye. To maximize the dependence on oxidative phosphorylation, fibroblasts were cultured in the absence of glucose for at least 48 h before the experiments. To further investigate the effect of the absence of VLCAD or LCAD on various organs, ROS generation was evaluated in liver mitochondria from VLCAD- and LCAD-deficient mice, as well as from wild type mice and rats using DCF-DA oxidation.

Results: Fibroblasts from patients with various VLCAD mutations showed a decrease of basal respiration (49%) and reserve capacity (53%) as measured by oxygen consumption. Superoxide production was increased by 75% and mitochondrial mass was increased by 18% in fibroblasts containing a c.1707_1716dup (L540P) mutation in the VLCAD gene. ROS production was increased (40%) in liver mitochondria from wild type rats when incubated with palmitoyl-CoA plus acetyl-CoA. DCF-DA oxidation was greater using liver mitochondria from LCAD-deficient mice as compared to wild type mice, though VLCAD deficient mice were unchanged.

Conclusions: Our findings identify increased ROS production with disrupted long chain FAO. In addition, respiratory chain function is decreased in VLCAD deficient fibroblasts, indicating that the energy metabolism dysfunction in VLCAD deficiency exceeds that of FAO alone. These novel pathophysiological mechanisms in FAO disorders also suggest new possibilities for therapeutic strategies.

106) INCIDENTAL MEDULLOBLASTOMA IN ADOLESCENT WITH KNOWN GLUTARIC ACIDEMIA TYPE I

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Glutaric acidemia type I is an inborn error of metabolism that leads to accumulation of glutaric and 3 hydroxyglutaric acid in situations of metabolic stress and prolonged fasting leading to central nervous system injury and dystonia as sequelae. The detection by newborn screening and implementation of emergency room protocols have been successful in minimizing the sequela of this condition.

We report the unusual occurrence of a medulloblastoma in a long term survivor.

Patient is a 14-year-old male originally from Korea, diagnosed at one month of age with respiratory distress and increasing head size. Molecular genetic testing revealed two mutations denominated c.641C>T and c.1204C>T. Enzyme activity on cultured skin fibroblasts was reported to be zero.

He was followed over the years with protein restricted diet, levo-carnitine and emergency instructions in case of acute illness. Brain MRI at the age of 9 showed prominence of the bilateral subarachnoid space in the sylvian fissure and anterior middle cranial fossa with bilateral and symmetric increase in the T2 signal in the centrum semiovale with no diffusion abnormality. At the age of 14, he did not have any neurological deficits or signs of dystonia. In anticipation to liberalize his diet, follow-up brain MRI with contrast was requested. A left cerebellar mass measuring 4.1 × 3.2 cm × 2.4 cm was identified incidentally. Prior MRI findings remained unchanged. In preparation to the resection, he was admitted the day prior to the surgery and started on Dextrose 10% at 150% maintenance while being nil per os for surgery.

With a surgical time of 6 h and total nil per os time of 40 h there was no documented evidence of metabolic acidosis, hypoglycemia or ketosis before or after the surgery. Special parenteral nutrition was not required. Pathology studies showed the mass to be a medulloblastoma, nodular/desmoplastic variant WHO grade IV. Further imaging and lumbar puncture did not demonstrate distal metastases. Post-operatively there were expected difficulties with gait but there has been no evidence of dystonia. Radiotherapy and chemotherapy are being planned at this time.

107) CO-EXPRESSION OR PHENYLALANINE HYDROXYLASE VARIANTS AND EFFECTS OF INTERALLELIC COMPLEMENTATION ON IN VITRO ENZYME ACTIVITY

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Background: In phenylketonuria (PKU) patients, the combination of two phenylalanine hydroxylase (PAH) alleles determines residual enzyme activity in vivo. Inconsistencies in genotype-phenotype correlations have been observed in compound heterozygous patients. A particular combination of two PAH alleles may produce a phenotype different from expected, due to interallelic complementation.

Methods: Two eukaryotic vector systems with two distinct PAH proteins N-terminally fused to different epitope tags were used. Transient co-expression of two distinct PAH variants was investigated in COS-7 cells. PAH activity was measured by LC-ESI-MS/MS, and expression levels of PAH were monitored by western blot. Genotypes were compared with phenotypes from the BIOPKU database (www.biopku.org).

Results: Through the expression and co-expression of 17 variant alleles we demonstrated that interallelic interaction could be both positive and negative. The co-expressions of p.[Ile65Thr];[Arg261Gln] and p.[Ile65Thr];[Arg408Trp] with 24% and 12%, respectively, lower PAH activity than predicted are examples of negative interallelic interaction. The co-expressions of p.[Glu178Gly];[Gln232Glu] and p.[Pro384Ser];[Arg408Trp] (19% and 15% higher PAH activity, respectively) are examples of positive subunit interactions. Inconsistencies of PAH residual enzyme activity in vitro and of PKU patients' phenotypes were observed as well. As expected, genotypes exhibiting lower in vitro PAH activity in the range below 15% are almost exclusively associated with the classic PKU phenotype and BH4 non-responsiveness, while those with a relatively high residual PAH activity of more than 50% correlate with the MHP phenotype and BH4 responsiveness.

Conclusion: The co-expression of two distinct PAH variants revealed possible dominance effects (positive or negative) by one of the variants on residual PAH activity as a result of interallelic complementation.

108) N-CARBAMYL-L-GLUTAMATE IS A CHAPERON FOR CPS1 E1034G MUTANT: CORRELATION WITH AUGMENTED UREAGENESIS AND CLINICAL IMPROVEMENT

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Background: It has been well established that N-carbamyl-L-glutamate (NCG), a stable analog of N-acetyl-L-glutamate (NAG), can substitute for NAG in the activation of carbamyl phosphate synthetase 1 (CPS1) and restoring ureagenesis to rescue the patients with the inherited NAG synthase (NAGS) deficiency. Our recent studies demonstrate that some patients with CPS1 deficiency respond favorably to the NCG supplementation. One patient with CPS1 deficiency caused by a homozygous E1034G CPS1 mutation showed decreased blood ammonia levels, increased ureagenesis and a marked clinical improvement when given NCG. This investigation was aimed to define the molecular and biochemical basis for the favorable effect of NCG in this patient.

Methods: The recombinant wild-type (WT) and E1034G mutant CPS1 were prepared using baculovirus/insect cells expression system. The enzymatic activities were measured of both WT and E1034G CPS1 mutant with and without the presence of NAG or NCG or both using an ornithine transcarbamylase (OTCase) coupling assay and LC-MS. The thermal stability of the proteins with and without NAG or NCG was monitored by the fall in the activity of the enzyme after heating at different temperature. Protection of WT and E1034G CPS1 mutant by NAG or NCG against thermal unfolding were followed by the changes of the fluorescence of Sybro Orange, which was added before temperature ramp.

Results: Activity assay indicated that E1034G CPS1 mutant had about half of enzyme activity of WT CPS1 in the presence of NAG, and only about 5% of activity of WT CPS1 in the presence of NCG. It appears that NAG no longer protects the stability of the E1034G CPS1 mutant as WT CPS1. Instead, NCG but not NAG protects the E1034G CPS1 mutant from early thermal unfolding.

Conclusions: NCG is able to bind to the E1034G mutant enzyme at a site different from that of NAG binding CPS1. We propose that NCG binds to the pocket occupied by the side chain of 1034G left empty by the mutation stabilizing the protein. Additional studies to confirm this novel NCG mechanism are in progress.

109) THE FIRST REPORTED OUTCOMES OF STEM CELL THERAPY IN BETA-MANNOSIDOSIS: A CASE STUDYKatrina Simmons¹, Sara Cathey², Paul Orchard³, Laura Pollard¹, Tim Wood¹¹Greenwood Genetic Center, Greenwood, SC, United States²Greenwood Genetic Center, Charleston, SC, United States³Division of Blood and Marrow Transplant, University of Minnesota Medical School, Minneapolis, MN, United States

We report a case of a 5-year-old male, OM, who was recently diagnosed with Beta-mannosidosis. Beta-mannosidosis is a very rare autosomal recessive lysosomal disease caused by mutations in the beta-mannosidase gene (MANBA, 4q24, OMIM #248510). Only approximately 20 cases of Beta-mannosidosis have been reported. Patients diagnosed with Beta-mannosidosis can present with intellectual disability, speech impairment, hypotonia, and demyelination. OM was appropriately sized and had no abnormalities at birth. By 9 months of age he presented with moderate hypotonia, global motor delay, and tremors. An initial MRI at one year of age appeared normal with no structural abnormalities. OM continued to display gross motor delays, tremors, and ataxia which prompted another MRI (at age 4) that showed severe demyelination. Laboratory testing identified a deficiency of beta mannosidase and two heterozygous, previously reported mutations in the MANBA gene: a frameshift mutation on exon 5 (c.563_572dup10) and a missense mutation on exon 12 (c.1499G>A, p.R500H). Thin-layer chromatography of urinary oligosaccharides at diagnosis was considered normal. However, UPLC MS/MS oligosaccharide analysis, identified extremely high levels of mannose beta-1,4-acetylglucosamine (Man(β1-4)GlcNAc). At 4.5 years old OM received a stem cell transplant (SCT) from an umbilical cord sample at the University of Minnesota. After his SCT, his beta mannosidase activity increased dramatically in leukocytes, dried blood spots, and plasma. Significant reductions of Man(β1-4)GlcNAc were also noted in two separate urine samples taken 1 and 6 months after SCT. Overall OM, to our knowledge, is the newest case of Beta-mannosidosis since 2009 and may be the first Beta-mannosidosis patient to receive SCT. His case is also unusual in that he presents with only a few of the common traits reported in children with Beta-mannosidosis.

110) A CASE OF METAPHYSEAL CHONDROMATOSIS WITH D-2-HYDROXYGLUTARIC ACIDURIAG. Simon¹, A. Wierenga¹, E. Struijs², S.G. Prabhu⁴, B. Lettenmaier¹, H. Lawrence³, K.J. Wierenga¹¹Department of Pediatrics, Section of Genetics, OUHSC, Oklahoma City, OK, United States²Metabolic Unit, Department of Clinical Chemistry, VU University Medical Center, Amsterdam, The Netherlands³Department of Dermatology, OUHSC, Oklahoma City, OK, United States⁴Department of Radiology, OUHSC, Oklahoma City, OK, United States

An apparently normal, full term male infant with a birth weight of 5 lbs, from an uncomplicated pregnancy, was born to a 36-year-old G2P1 mother. Unusual skin pigmentation was identified at 1 month of age and at 6 months of age, congenital cataracts in the right eye requiring surgery were diagnosed. Clinical genetics evaluation at 1 year of age, reported a patient with normal development, lop-shaped ears, brachydactyly, short stature, and irregular long bones on palpation. Whorled skin hypo-pigmentation was noted on trunk and extremities. Skeletal survey revealed extensive metaphyseal enchondromatosis. A potential genetic etiology of metaphyseal chondromatosis with D-2-hydroxyglutaric aciduria was considered (MIM #614875). Urine organic acid investigations revealed marked excretion of 2-hydroxyglutaric acid, later quantitatively identified as D-2-hydroxyglutaric aciduria. Molecular studies of D2HGDH and IDH2 by Sanger sequencing were normal. Next Gen Sequencing of IDH1 in DNA from peripheral blood revealed no mutations. Possible explanations may include genetic heterogeneity, though somatic mosaicism below detection range for a mono-allelic change in IDH1 or IDH2 may be more likely (Vissers et al 2011, Nota et al 2015). Vissers et al (2011) identified in 2 out of 4 unrelated patients somatic mosaicism for p.R132H and p.R132S, with 23% and 10% mutant reads respectively. The other 2 patients remained without identified sequence abnormality, similar to our patient.

Somatic mosaicism may hinder the confirmation of clinically and biochemically diagnosed disorders. The pigmentary abnormality observed in this patient may be consistent with this hypothesis.

111) MUTATIONS IN MITOCHONDRIAL TRANSCRIPTION FACTOR A (TFAM), CAUSE NEONATAL LIVER FAILURE ASSOCIATED WITH mtDNA DEPLETIONMariella Simon^{1,2}, Ashley R. Stiles^{3,4}, Negar Khanlou³, Hanlin L. Wang³, Shino Magaki³, Hane Lee^{3,4}, Kate Partynski¹, Naghmeh Dorrani^{3,4,5}, Richard Chang^{1,7}, Julian A. Martinez-Agosto^{4,5,6}, Jose E. Abdenur^{1,7}¹Division of Metabolic Disorders, CHOC Childrens, Orange, CA, USA²Department of Developmental and Cellular Biology, School of Biological Sciences, University of California Irvine, Irvine, CA, USA³Department of Pathology and Laboratory Medicine, David Geffen School of Medicine at University of California Los Angeles, Los Angeles, CA, USA⁴UCLA Clinical Genomics Center, University of California Los Angeles, Los Angeles, CA, USA⁵Department of Human Genetics, David Geffen School of Medicine at University of California Los Angeles, Los Angeles, CA, USA⁶Department of Pediatrics, University of California Los Angeles, Los Angeles, CA, USA⁷Department of Pediatrics, University of California Irvine, Orange, CA, USA

Background: The mitochondrial transcription factor A (TFAM) is essential for transcription, replication and packaging of mitochondrial DNA (mtDNA). TFAM knockout mice display embryonic lethality, secondary to severe mtDNA depletion. mtDNA depletion in humans has been associated with a group of disorders that arise as a consequence of defects in nuclear encoded genes, involved in mtDNA replication or nucleotide synthesis. These syndromes are usually classified by their clinical presentation as encephalomyopathic, myopathic, neurogastrointestinal or hepatocerebral.

Aim: Here we describe the clinical, molecular and pathological results in two siblings presenting with neonatal onset liver failure caused by homozygous mutations in *TFAM* (MIM 600438).

Methods: Whole exome sequencing (WES) was performed at the Clinical Genomics Center at UCLA for patient 1 and her parents. *TFAM* Sanger sequencing was performed for patient 2, the deceased brother of patient 1. mtDNA copy number analyses in liver and muscle and electron transport chain (ETC) activity tests in muscle from patient 1 were performed at Baylor Miraca Genetics laboratories. Muscle and liver tissues were studied by standard light and electron microscopy at the Department of Pathology at UCLA. Research testing for *TFAM* protein levels was performed for patient 1 in lysates from primary fibroblasts using a rabbit polyclonal antibody.

Results: Two siblings born to consanguineous parents of Colombian and Basque descent presented with intrauterine growth retardation (IUGR), elevated transaminases, conjugated hyperbilirubinemia and hypoglycemia. The disease rapidly progressed to liver failure with pronounced ascites. Both siblings had abnormal Newborn Screening (NBS) test results with confirmatory testing showing elevated tyrosine and methionine in plasma and increased 4-hydroxy-phenyl derivatives and N-acetyltyrosine in urine. Patient 2 died at 2 months of age due to complications of liver failure, while patient 1 is alive, with supportive treatment at 4 months of age. No cardiac, kidney or neurological involvement was noted. Liver biopsy in patient 1 revealed cirrhosis with microvesicular steatosis, cholestasis and prominent iron deposition. Electron microscopy of muscle revealed abnormal mitochondrial morphology and distribution while enzyme histochemistry was unremarkable. mtDNA content in liver and muscle was reduced to 11% and 21% of normal controls, respectively. ETC testing in muscle showed increased citrate synthase activity suggesting mitochondrial proliferation, while respiratory chain activities, normalized by citrate synthase, were at the lower end of normal. WES revealed a homozygous variant in *TFAM* at position c.533C>T; p.Pro178Leu. The mutation was confirmed in patient 2. This mutation is located in the high-mobility group (HMG) box 2 domain of the protein, which is involved in mtDNA binding and compaction. Several of the employed *in silico* pathogenicity prediction tools deemed the variant as damaging. Immunoblotting revealed decreased *TFAM* protein levels in patient fibroblasts as compared to controls.

Discussion: We report a novel mtDNA depletion syndrome caused by mutations in *TFAM*, which presents with neonatal-onset hepatic failure and mtDNA depletion in liver and muscle. While *TFAM* has been described almost 30 years ago, this is, to our knowledge, the first direct correlation between mutations in this gene and human disease.

112) ORTHOTOPIC LIVER TRANSPLANTATION IN A PATIENT WITH COMPOUND HETEROZYGOTE S135L/NULL GALT MUTATIONS AND LIVER FAILURE

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Background: The galactose-1-phosphate uridyltransferase (GALT) activity in erythrocytes is absent or barely detectible in patients with classic galactosemia. Yet, both classic and clinical variant galactosemia may present with failure to thrive, cataracts, anemia and liver failure unless a galactose-restricted diet is implemented in the neonatal period. Theoretically, African Americans with clinical variant galactosemia and liver GALT activity of 8–12% of normal control values may also develop liver failure.

Case: We report a case of an African American male on an unrestricted diet who presented in fulminant liver failure at age 2 months after he was not started on a galactose-restricted diet nor referred to genetics following positive Perkin-Elmer newborn screening (NBS). His initial NBS at age 1 day revealed a total galactose of 39.8 mg/dL (N < 15), and GALT of 18.7 μ M (N > 40.0). Confirmatory GALT testing revealed activity of <2.0 U/g Hb (N \geq 18.5). DNA sequencing of the GALT gene identified two mutations: p.S135L (c.404C>T) and a novel frameshift variant c.576_589delCAGTTTCTGCCAG, which results in a downstream stop codon. The common S135L mutation, prevalent in Africa, in homozygous form is associated with clinical variant galactosemia and no long term complications if treatment is implement in the first week of life. There is limited phenotypic data on persons who are compound heterozygotes of the S135L and a null allele, as well as limited data on the prevalence of liver failure in patients with one or two copies of S135L.

Our patient received an orthotopic liver transplant at age 68 days as he had sustained irrecoverable liver injury. There are only 3 published cases of patients with galactosemia who have received liver transplant. After the liver transplant, on a galactose-restricted diet, galactose-1-phosphate (Gal-1-P) was 2.6 mg/100 mL RBC (N 0–1) and urine galactitol 182.3 mmol/mol Cr (N 0–45). Following two weeks of an unrestricted diet, Gal-1-P was 2.3 mg/100 mL RBC and urine galactitol was 285.6 mmol/mol Cr.

At 3 years of age, our patient remains on a galactose restricted diet with consistently elevated Gal-1-P levels. Brain MRI demonstrated changes consistent with classic galactosemia, and neuropsychological testing at 2 years revealed a 12-month developmental delay.

Conclusions: Patients who are compound heterozygous for S135L/classic or null mutation may develop liver failure without galactose restriction in the neonatal period and exhibit a long-term phenotype that is intermediate between classic and clinical variant galactosemia. Following orthotopic liver transplantation and on a galactose-restricted diet, RBC Gal-1-P and urine galactitol remain elevated.

113) GNE MYOPATHY: DISEASE PROGRESSION DETERMINED BY LONGITUDINAL NATURAL HISTORY DATA AND MATHEMATICAL MODELING

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GNE myopathy is a rare autosomal recessive inborn error of metabolism caused by mutations in *GNE*, the gene that encodes the rate-limiting enzyme in sialic acid biosynthesis. GNE myopathy is characterized by adult-onset, slowly progressive skeletal muscle weakness and atrophy leading to significant disability including wheelchair use and dependent care. There is no approved therapy, but promising therapeutic candidates have been identified. Clinical trial designs for GNE myopathy are complicated by the small patient population and slow disease progression. To overcome this challenge, we generated a disease progression model to understand the rate of muscle decline using longitudinal quantitative strength data collected from a diverse cohort of 42 patients enrolled in “A Natural History Study of GNE Myopathy” (ClinicalTrials.gov NCT01417533). Quantitative muscle assessment (QMA, Aeverl Medical) was determined to be the most sensitive measure of disease progression; it assesses force (kg) in individual muscle groups and is expressed as the proportion of strength predicted for subjects' age, gender and BMI. ‘Disease age’ was determined by fitting a logistic decay model to knee flexion (KF) strength; disease age was defined as zero when KF strength equalled 0.5.

Of the 42 patients enrolled, 38 (90%) were able to complete the QMA, compared to only 27 (64%) who completed the 6-minute walk test (6MWT). When strength for individual muscle groups was plotted as a function of disease age, a characteristic pattern of sequential muscle involvement became evident, with the anterior tibialis muscle (ankle dorsiflexion; ADF) affected first, followed by KF, shoulder abduction (SA), grip and elbow flexion (EF). Individual muscles had different rates of progression, with the quadriceps (knee extension; KE) progressing the slowest. The model estimated proportions of strength at disease age 0 as: ADF 0.03, KF 0.5, SA 0.55, KE 0.7, grip 0.78 and EF 0.84. The disease ages at which each muscle has strength of 0.5 are: ADF – 8.1, KF 0, SA 2.6, EF 9.6 and grip 11.2. Distance walked on the 6MWT was <400 m at disease age ~5 and <200 m at disease age ~20. The median disease ages at which patients reported functional deficits were: falls 0.2; braces 8.8; cane or walker 12.8; lost ambulation 28; dependent on activities of daily living (ADLs) 32.7. Clinical trial designs were simulated to calculate power under different endpoints, where a treatment effect of $\gamma = 0.5$ corresponds to a 50% reduction in the rate of decline. A trial with QMA as the primary endpoint and the primary analysis based the progression model, can determine efficacy in 2 years with 50 subjects (98% power; $\gamma = 0.5$), compared to 370 and 232 subjects with the 6MWT or composite upper extremity strength as primary endpoints, respectively (80% power; $\gamma = 0.5$). Novel endpoints are needed to catalyze the development of therapies for rare diseases, particularly those that are slowly progressive. The GNE myopathy progression model allowed us to determine the rate of disease progression, predict progression across the spectrum of the disease, and design evidence-based clinical trials.

114) A UNIQUE CASE OF EARLY DIAGNOSED MUCOPOLYSACCHARIDOSIS TYPE I

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Background: Mucopolysaccharidosis type I (MPS I; Hurler syndrome) is a lysosomal storage disorder characterized by deficiency in the enzyme, alpha-L-iduronidase, responsible for the degradation of the glycosaminoglycans (GAGs) dermatan and heparan sulfate. Chronic accumulation in the lysosome leads to the progressive multisystem disease. Early detection and therapy result in improved outcome. While MPS I is typically diagnosed in early childhood, the variable severity in clinical phenotype makes it often difficult to diagnosis under the age of one year. Objective: We report clinical, histologic, and molecular findings on a patient diagnosed with MPS I at 2 months of age after being identified to have interstitial lung disease defined as pulmonary interstitial glycogenosis (PIG). We also review the literature linking MPS to PIG.

Case Report: Our patient was born at 36 weeks of gestation with a history of mild dysmorphic features, hypotonia, failure to thrive, and chronic respiratory insufficiency requiring NICU admission. An initial lung biopsy was positive on periodic acid Schiff (PAS) staining resulting in a working diagnosis of PIG. The metabolic service was consulted to address potential glycogen storage disease. Review of the literature revealed a single case report associating MPS II with PIG, prompting urine MPS screening with Alcian blue. This was positive, with follow-up thin layer chromatography indicating the presence of dermatan and heparan sulfate most suggestive of MPS I or MPS II. Simultaneously, continued analysis by pathology revealed electron microscopic findings suggestive of a lysosomal storage disorder and subsequent Alcian blue and lysosomal immunostain positivity in lung tissue suggested the storage material was GAG and not glycogen. Follow up alpha-L-iduronidase enzyme activity analysis was abnormal (0.30 nmol/4 h/mL, normal 6–71.4) and IDUA sequence analysis identified two known pathogenic mutations (c.208C>T and c.1205G>A), confirming the diagnosis of MPS I. Our patient was started on weekly Laronidase enzyme replacement therapy at 3 months of age with the plan for bone marrow transplant at 6–9 months of age.

Discussion: This case represents an early and unusual presentation of interstitial lung disease in MPS I. Screening was prompted by the pathologic and clinical suspicion of PIG. Initial PAS staining was non-specific and represented the storage of GAGs rather than glycogen. This is likely the case in the only other report of MPS with PIG in the literature. Definitive diagnosis of MPS I allowed for targeted and early therapy which should relatively improve outcome, although this case may be severe based on age of initial presentation. Respiratory symptoms, growth, and development have improved on treatment. Odontoid hypoplasia, discovered because of the diagnosis, prompted potentially life-saving precautions in potential future intubations.

Conclusions: This case highlights the importance of considering MPS as part of the differential for PIG, an index of suspicion for MPS in early interstitial lung disease, and the importance of very early diagnosis of MPS I as a potential modifier of clinical outcome.

115) ADULT ONSET LEUKODYSTROPHY AND INCREASED SURVIVAL AS A PRESENTATION OF AARS2 RELATED DISEASE

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Defects in AARS2, encoding mitochondrial alanyl-tRNA synthetase, result in a spectrum of findings, ranging from infantile cardiomyopathy to progressive leukoencephalopathy. To date, there are 8 such reported cases. In this report, we present a 50-year-old man with progressive cognitive and motor decline, beginning in his fifth decade of life, as well as evidence of a leukodystrophy on brain MRI, found to have compound heterozygous AARS2 mutations. The patient presented for evaluation in Neurogenetics clinic at the age of 48 years. His childhood and early adulthood course were significant for two seizures (one between 2–4 years of age and another between 14–16 years of age), fine tremor which developed around 25 years of age, and anxiety attacks starting in his early 30s. His neurological deterioration commenced when he was 42 years old, when he developed fine motor difficulties, worsening tremor, depression, and unsteady balance. Thereafter, he progressively declined in cognitive and motor abilities. By around 46 years of age, he had lost all speech, became immobile, and developed dysphagia. MRI at age 47 years showed bilateral, symmetric white matter changes in the frontal regions, occipital regions, and brain stem, as well as cortical and cerebellar atrophy; these findings were progressive compared to MRI at 42 years, which revealed prominent frontal white matter changes. After extensive prior genetic and metabolic testing, he underwent whole exome sequencing, which revealed compound heterozygous changes in AARS2: c.595C>T (p.R199C); c.2557C>T (p.R853W). The p.R199C variant has been previously reported in association with leukoencephalopathy and is predicted to impact synthetase function based on published structural modeling analysis. The p.R853W variant occurs at a position that is evolutionarily conserved, and in silico pathogenicity tools predict that this variant is probably damaging. Our case expands the clinical heterogeneity of AARS2 related disorders, as he is the oldest known survivor of this disease.

116) GENERATING A MURINE MODEL FOR HERMANSKY-PUDLAK SYNDROME USING CRISPR-Cas9

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Hermansky-Pudlak syndrome (HPS) is a rare, autosomal recessive disorder characterized by oculocutaneous albinism and a bleeding diathesis due to defects in intracellular vesicle formation and trafficking. Of the nine subtypes, HPS1 is the most common, with a prevalence as high as 1:1800 in NW Puerto Rico due to a probable founder mutation. HPS-1, HPS-2 and HPS-4 patients develop pulmonary fibrosis (HPSPF) in their third to fifth decade of life. HPSPF is lethal, and to date the only treatment option is lung transplantation. Development of therapy relies on understanding the pathogenesis of HPS, especially the development of HPSPF. Research into this mechanism is further hindered by the limited models for the study of pulmonary fibrosis. The development of animal models that recapitulate the disease may provide a means for studying the underlying processes in HPS and lead to novel therapeutic targets and treatment options.

Here, we generated an *Hps1* null mouse model by using the clustered regularly interspaced short palindromic repeats – CRISPR associated protein 9 nuclease (CRISPR-Cas9) system, a genome-editing tool that utilizes nucleases (Cas9) guided by a programmable RNA known as the single guide RNA (sgRNA), to make double stranded DNA breaks in the third coding exon of the mouse *Hps1* gene. Genotypic and phenotypic selection of the mice over successive generations generated two founder lines: a homozygous 5 base-pair deletion and a homozygous 1 base-pair insertion, both of which are predicted to be frame-shift truncating mutations.

Our two *Hps1* null founder lines have discernible phenotypes that suggest defects in biogenesis of lysosomal-related organelles, including melanosomes (varying levels of light-colored coats, tails, and ears), endolysosomes (deposition of lipofuscin-like granules in the proximal renal tubular epithelial cells), and lamellar bodies (enlargement in type-II pneumocytes). All these phenotypic presentations are observed in human patients with HPS-1, thus confirming our model's fidelity to the human disease. Overall, our *Hps1*-CRISPR null mice will be a valuable new tool for investigation into disease mechanism and development of therapeutic options for HPS.

117) “BENIGN” HYPERPHENYLALANINEMIA? A CASE SERIES OF FIVE TREATED PATIENTS

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Benign hyperphenylalaninemia (BHP) is a disorder diagnosed only incidentally by newborn screening, a by-product of screening for phenylketonuria (PKU), a disorder resulting in elevated phenylalanine levels which if untreated cause irreversible neurologic sequelae. In contrast, BHP is thought to have a benign phenotype because phenylalanine levels are insufficiently elevated to cause neurological damage, thus obviating the need for rigorous dietary phenylalanine restriction. Phenylalanine levels below 360 $\mu\text{mol/L}$ are generally considered safe, thus this threshold is not only the upper therapeutic range for treated PKU, but is also the upper threshold for the diagnosis of BHP. However, the published literature provides little guidance on long-term follow-up of phenylalanine levels in BHP, in particular how frequently to monitor phenylalanine levels to evaluate for subsequent elevations above the ‘safe’ range. Additionally, there are few publications that address how to treat patients initially diagnosed with BHP who subsequently present with phenylalanine levels above the therapeutic range, in particular, whether to institute the conventional PKU treatment of strict protein-restriction, which is expensive, may impede linear growth and can be unpalatable to children not treated from infancy. Upon retrospective review we have identified 14 patients with BHP, ascertained via newborn screen and currently aged 4 months to 35 years. All patients had an initial untreated level of phenylalanine between 90 $\mu\text{mol/L}$ (our upper limit of normal) and 360 $\mu\text{mol/L}$. Of these patients, five subsequently demonstrated either fluctuating or sustained increases in phenylalanine above 360 $\mu\text{mol/L}$. Three have been treated successfully with sapropterin (Kuvan) therapy without dietary intervention and two have been treated with mild to moderate protein-restriction. Our experience demonstrates successful treatment of these children without the traditional highly restrictive PKU diet. However, a better understanding of this disorder is necessary to more safely and appropriately identify, monitor and manage children with BHP.

118) A 'CRISPR' VIEW OF PEROXISOMES: DISSECTING THE METABOLIC CROSSTALK WITH MITOCHONDRIA

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Mitochondrial long-chain fatty acid beta-oxidation (mFAO) plays a key role in energy homeostasis as it constitutes the main fuel during fasting. Patients with a defect in mFAO may present with low blood sugar upon fasting as well as heart and skeletal muscle disease. Even though most mFAO disorders have been included in newborn screening programs, the treatment options for affected children are scarce and the underlying mechanisms remain largely undefined. We have recently demonstrated that when mitochondrial acyl-CoA import is impaired, peroxisomes can also degrade fatty acids that are typically handled by mitochondria.

To identify the individual players in the peroxisomal degradation of these fatty acids, we adopted the CRISPR/Cas9 genome editing technique, which can be used for the targeted disruption of any gene of interest. We established this method in HEK293 cells, which enables us to identify clones with biallelic nonsense mutations (KOs). In our HEK293 model, mFAO is genetically disrupted through CPT2 KO or pharmacologically inhibited with L-aminocarnitine. Next, we targeted several peroxisomal candidate genes; PEX13 (crucial in peroxisome biogenesis), EHHADH and HSD17B4 (involved in peroxisomal FAO) and ABCD3 (a peroxisomal membrane transporter). These cell lines were incubated with the medium-chain fatty acid lauric acid (C12:0), followed by acylcarnitine analysis in the extracellular medium. Upon CPT2 inhibition, control cells showed production of the intermediate C10-carnitine. Accumulation of C10-carnitine was abrogated in PEX13 KO cells, demonstrating the peroxisomal origin of this metabolite. Upon loading with lauric acid, ABCD3 and HSD17B4 KO cells also fail to accumulate C10-carnitine, while the EHHADH KO cell lines mirror the control cells. This suggests that ABCD3 and HSD17B4 are required for degradation of medium-chain fatty acids in peroxisomes, while EHHADH is either not involved, or at least not essential in this process. Through the use of CPT1A and CPT2 double KO cells, we also provide evidence that lauric acid not only enters as a CoA ester (canonical route), but also as a carnitine ester. Importantly, there is substantial evidence that a similar pathway is functional in vivo. Upon treatment with L-aminocarnitine, WT mice do not only accumulate C16- and C18-, but also C10-, C12- and C14-acylcarnitine species. C10, C12 and C14 fatty acids are uncommon in diet and not stored in fat, therefore these acylcarnitine species are likely metabolites of peroxisomal origin. Similarly, it has been reported that patients with CPT2 deficiency display a marked increase of plasma C12- to C18-carnitine species.

Thus, we have uncovered a novel pathway by which peroxisomes accept typical mitochondrial substrates. This pathway may represent a novel modifier and therapeutic target in mFAO disorders.

119) INTERIM RESULTS FROM AN OPEN-LABEL PHASE 2 STUDY TO ASSESS SAFETY AND CLINICAL EFFECTS OF INVESTIGATIONAL UX007 (TRIHEPTANOIN) IN SUBJECTS WITH LONG-CHAIN FATTY ACID OXIDATION DISORDERS (LC-FAOD)

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LC-FAOD are a group of autosomal recessive genetic disorders characterized by metabolic deficiencies in which the body is unable to convert long-chain fatty acids into energy causing serious liver, muscle, and heart disease. This single-arm, open-label Phase 2 study evaluated 29 patients (aged 10 months to 58 years) with LC-FAOD demonstrating severe ongoing disease despite standard-of-care treatment. The majority of patients qualified based on severe musculoskeletal disease. After a four-week run-in period on current regimen (27 of 29 patients on MCT oil), UX007 (triheptanoïn), a medium odd-chain fatty acid, was titrated to a target dose of 25–35% of total daily caloric intake. Patients were evaluated over 24 weeks on several endpoints, including cycle ergometry, 12-minute walk test (12MWT), and quality of life (QoL). Patients performed only the assessments appropriate for their age and clinical status.

At week 24 cycle ergometry testing, the seven age- and condition-eligible patients showed a mean 60% (+446.8 W) increase (median: +127.5 W; min, max: –388, +2438) over a baseline of 744.6 W. In the last assessment for 12MWT (week 18), the eight eligible patients showed a mean increase of +188 m (median: 93.5; min, max: –80, 880) from a baseline mean of 673.4 m, an increase of 28%. Additionally, adult self-reported, health-related QoL (SF-12v2) physical component summary scores were significantly improved, mean increase 8.9 points, supporting the clinical meaningfulness of the observed changes in exercise tolerance tests. No difference was seen in parent-reported scores (SF-10) for the pediatric patients. Overall, 18 patients (62%) had treatment-related adverse events, predominantly gastrointestinal (55%), which were mild-to-moderate in severity. In patients suffering with ongoing severe LC-FAOD, treatment with UX007 showed improvements in exercise tolerance and muscle function, and were associated with positive changes in self-reported QoL. Further investigation in a controlled confirmatory study is warranted.

120) AN EXPLORATION OF GENETIC TEST UTILIZATION, GENETIC COUNSELING, AND CONSANGUINITY WITHIN THE INBORN ERRORS OF METABOLISM COLLABORATIVE

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The Inborn Errors of Metabolism Collaborative (IBEMC) encompasses clinicians from 29 institutions collecting longitudinal data to enhance understanding of metabolic conditions diagnosable by newborn screening. Member institutions enter data about individually rare but collectively common conditions, including hospitalizations, laboratory results, provision of genetic counseling, clinic visits, and long-term outcomes. Through evaluation of compiled data, we assessed how frequently genetic counseling had been provided to families with metabolic genetic conditions identified by newborn screening, and how often genetic testing had been performed either to confirm a diagnosis or for other purposes. We also assessed changes in utilization of genetic testing following newborn screening over time and determined the rate of consanguinity in this population as compared to historic data.

For this retrospective study, a data query from the Inborn Errors of Metabolism Information System (IBEM-IS) was performed for 1553 subjects consented and enrolled prior to April 28, 2014. The following elements were abstracted for analysis: current age, metabolic condition, if genetic counseling was provided (and by whom), if molecular genetic testing was performed, and any reported parental consanguinity. In the dataset, 1123 subjects had values entered for the question "Has the family received genetic counseling?" Of these, 1076 (95.8%) reported genetic counseling had been provided. The initial dataset was expanded after data collection was initiated and a question was added to ask who provided the genetic counseling, with data available for 210 subjects. In 144 (68.6%) cases, a genetic counselor was involved in the counseling while in 66 cases (31.4%) the counseling was provided by a physician, nurse practitioner, dietician, or medical student. Thus, while the majority of families are receiving genetic counseling, in about 1/3 of cases a genetic counselor did not provide this service.

Regarding genetic testing for metabolic conditions, 691 (68.0%) of 1017 subjects had undergone DNA testing, with usage especially high for fatty acid oxidation disorders. Genetic testing was performed greater than 90% of the time for three conditions: carnitine palmitoyltransferase I (CPT1) deficiency, very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency, and long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency. Genetic testing was least likely to be performed for the diagnoses of 3-methylcrotonyl-CoA carboxylase deficiency (3-MCC), tyrosinemia, and Hyperphenylalaninemia — PKU.

The average ages of individuals in the database who have had genetic testing is younger (8.70 years) than those who have not (12.10 years). Finally, as most metabolic conditions diagnosable by newborn screening are autosomal recessive, the data set includes basic information on consanguinity. In the database, consanguinity was reported in 26 of 1093 cases (2.38%).

In summary, within the IBEMC there is very high frequency of genetic counseling for metabolic conditions, though in one-third of cases a genetic counselor was not the primary counseling professional. Additionally, while metabolic conditions have historically been diagnosed by biochemical methods, there is now high utilization of DNA testing for these conditions. This is especially true for fatty acid disorders where the underlying genotype helps predict clinical presentation.

121) DEVELOPMENTAL IMPLICATIONS OF COBALAMIN C DISEASE IN HISPANIC TODDLERS DETECTED THROUGH NEWBORN SCREENING

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Background: Cobalamin C (CblC) disease is the most common inborn error of cobalamin metabolism. Recent data indicates higher prevalence among children of Hispanic heritage, with an estimated incidence of 1:46,000. We report the cognitive outcomes of five Hispanic patients detected through newborn screening (NBS).

Methods: Five Hispanic subjects with CblC disease ages 2–4 years (M = 39.4 months; SD = 13.4 months) underwent developmental testing (with a bilingual examiner). Broad cognitive ability (calculated using mental over chronological age), language, and adaptive functioning scores were converted to z-scores and compared to normative data (z = 0.0, SD = 1.0). Additionally, correlations between biochemical levels and ability were evaluated using corrected probability cutoffs and false discovery rates.

Results: The mean cognitive age-equivalent was 20.6 months (SD = 11.8), with z-scores below normative expectations (M = -2.96; SD = 2.27), $t(4) = -2.91$, $p = .022$. Similarly, language (M = -2.16, SD = 0.92) and adaptive functioning (M = -1.9, SD = 1.68) were below normative expectations, $t(4) = -5.24$, $p = .003$; $t(4) = -2.54$, $p = .032$, respectively. There were significant correlations between language ability and homocysteine (Hcy) at diagnosis ($r = -.99$, $p = .001$), most recent Hcy ($r = -.98$, $p = .001$), and plasma methionine at diagnosis ($r = .93$, $p = .011$). Hcy (at diagnosis and most recently) and plasma methionine at diagnosis were also strongly correlated with cognitive ability ($r = -.88$, $.77$, respectively); however, given limited sample size, these relationships were not significant after applying corrections.

Individual case analysis revealed one of the subjects to have significantly higher cognitive and language skills than the rest of the sample. This subject's Hcy at diagnosis (70.43 $\mu\text{mol/L}$) and most recently (19.96 $\mu\text{mol/L}$) was significantly lower than the other subjects' (201.06–225.36 $\mu\text{mol/L}$ at diagnosis; 68.06–74.2 $\mu\text{mol/L}$ most recently), while plasma methionine at diagnosis was significantly higher (30.3 nmol/mL) than other subjects' (2.3–10 nmol/mL).

Conclusions: Hispanic toddlers with CblC disease detected by NBS and treated early experience cognitive, language, and adaptive functioning delays despite current standard treatments; however, lower Hcy and higher plasma methionine (at diagnosis) may predict better cognitive and language development.

122) EXPERT OPINION ON THE MANAGEMENT OF CLN2 DISEASE

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Background and objectives: CLN2 disease, an inherited, rare, pediatric-onset, rapidly progressive neurodegenerative lysosomal storage disorder caused by TPP1 enzyme deficiency, is characterized by language delay, seizures, movement disorder, motor deterioration, dementia, blindness and early death. To date, no guidelines exist for managing this condition. Our aim is to gain insight into current management strategies.

Methods: 24 disease experts (healthcare professionals and patient advocates) from ten countries and from multiple disciplines completed an on-line survey comprising questions on management of CLN2. A smaller group subsequently met to discuss management practices.

Results: Experts share common goals in the management of patients and their families. Goals and interventions evolve as the disease progresses, with a shift in focus from maintenance of function (with emphasis on ambulation and communication) early in the disease to maintenance of quality of life (QoL). The goal of antiepileptic medication is to achieve sufficient seizure control to support function balancing the side effects. Antiepileptic medications may have unique response in patients with CLN2. Carbamazepine and phenytoin should be used with caution. School as well as home environments should be adapted to accommodate physical and cognitive/behavioral impairments as affected children benefit from maintaining social interactions. Physical, occupational and speech therapies are recommended to be initiated early and assessed frequently. Early use of adaptive devices should be considered to support function and independence. Palliative care team engagement is essential to the family soon after diagnosis is made. Appropriate tools to better assess neurobehavior, sleep and pain in this disease are needed.

Conclusions: CLN2 management practices are consistent among experts worldwide. A multidisciplinary approach is critical for optimizing care and QoL of patients and families throughout the disease course. Although gaps in knowledge remain, this effort to identify common management practices represents an initial step towards development of consensus-based management recommendations.

123) SWEET SECRETS OF GALACTOSE: A SIMPLE SUGAR'S THERAPEUTIC EFFECT IN PGM1-CDG AND REGULATORY ROLE IN GLYCOSYLATION

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Deficiency in phosphoglucomutase-1 (PGM1) causes PGM1-CDG, a recently discovered subtype of congenital disorder of glycosylation (CDG). In addition to maintaining glucose homeostasis, PGM1 also plays a critical role in protein N-linked glycosylation, an important post-translational modification. Hypoglycosylation disrupts the functions of many transport proteins, coagulation factors, and hormones, resulting in a complex phenotype with multi-system involvement. Unlike most CDG, PGM1-CDG is treatable: dietary supplementation with D-galactose alone has shown to improve glycosylation and mitigate most symptoms. The remarkable clinical success of galactose raised many questions; in particular, what are the mechanisms underlying the biological effects of galactose?

We evaluated the effect of galactose supplementation on glycosylation in cultured skin fibroblasts derived from three PGM1-CDG patients and one healthy individual. Cells were cultured with or without galactose supplementation. Changes in protein expression of ICAM-1, a validated marker for N-linked glycosylation, was detected by immunoblotting and immunohistochemistry. Across all patient cells, glycosylation increased with 5-day

galactose supplementation in a dosage-dependent manner. Additionally, global changes in glycosylation were assessed with lectin histochemistry, which revealed increased Golgi secretion of glycans. In parallel, total cellular glycomics determined by tandem MS supported improvement in glycosylation.

To investigate whether galactose acts as PGM1's chaperone, we performed thermal shift assay (TSA) of wild-type PGM1 with galactose. We also assayed the PGM1 enzyme activity in patient fibroblasts. While TSA showed that galactose did not stabilize wild-type PGM1, we observed a two-fold increase in PGM1 enzyme activity following galactose supplementation in cell extracts of Patient 1, who carries a homozygous missense mutation, but no change, in Patients 2 and 3, who carry nonsense mutations. It is possible that galactose may stabilize misfolded PGM1, as immunoblotting showed increased PGM1 protein level in Patient 1 only. However, improvement in glycosylation across all patient cells, regardless of PGM1 protein level or enzyme activity, is strongly indicative of a mechanism that is independent of the PGM1 enzyme.

Our findings clarified some aspects of the regulatory effect of galactose supplementation on glycosylation. Future studies include TSA of mutant PGM1, measurements of LLO and glycan fractions, and microarray-based gene expression analysis of enzymes downstream to PGM1.

124) A PATIENT WITH HYPER-BETA-ALANINEMIA

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We report an infant with plasma beta-alanine in the millimolar range. Plasma amino acids were measured when a newborn screen by tandem mass spectrometry at 9 days of age revealed a blood spot tyrosine of 480 $\mu\text{mol/L}$ (normal < 300). Follow-up plasma samples at 3 and 4 weeks of age showed that tyrosine was normal, but that beta-alanine was greater than 3000 $\mu\text{mol/L}$ (normal < 10). The identity of beta-alanine was confirmed by mobility and color reaction with ninhydrin on one dimensional paper chromatography in butanol:acetic acid:water (12:3:5). Beta-alanine and beta-aminoisobutyric acid were increased in urine, but taurine and gamma-aminobutyric acid were not. The parents are Native Americans and deny consanguinity, and the baby appeared to be normal at one- and two-month well child visits. These levels of beta-alanine are more than 50-fold greater than those reported by Scriver et al. in 1966, and further investigations of the child are ongoing and will be reported.

Scriver CR, Pueschel SM, Davies E. Hyper-beta-alaninemia associated with beta-aminoaciduria and gamma-aminobutyricaciduria, somnolence and seizures. *N Engl J Med* 274:635–643, 1966.

125) A CRISPR MODIFIED HEPARG CELL MODEL FOR THE STUDY OF ORNITHINE TRANSCARBAMYLASE DEFICIENCY

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Ornithine transcarbamylase (OTC) is a mitochondrial enzyme expressed in hepatocytes and enterocytes. OTC deficiency (OTCD), an X-linked disorder, causes recurrent and life-threatening episodes of hyperammonemia. Although several OTCD mice models are available, an in vitro model is still needed for several reasons: 1- all mice models are partially deficient (either hypomorphic, or only maintained in a heterozygous background); 2- Cell culture is more amenable for evaluating drug efficacy tests in vitro, especially for high throughput screening; 3- A homogeneous in vitro system would provide much clearer results for in situ assessments. Primary hepatocytes are the most relevant in vitro systems for studying various aspects of the liver biology, including ammonia elimination and drug metabolism. However, large variations in functional activities, the relative shortage and unpredictable availability of human biopsies, limited growth and stability in culture, considerably limit the use of this model for OTCD study. A recently established cell model, human HepaRG cell, is a bipotent hepatic progenitor with high proliferation potential and is capable of differentiating into biliary and hepatocyte-like cells. Liver-specific urea production and drug metabolism capacity were found in HepaRG hepatocyte-like cells. In this study, we constructed 3 GFP-tagged CRISPR/Cas9 gene modification plasmids, which targeted different regions in OTC exon 1 (OTC-KO), then employed electroporation to transfer them or a null control (NC) into HepaRG cells. After flow cytometry sorting and several rounds of screenings, we successfully obtained at least 3 OTC-KO stable clones that had no OTC expression while still retained the expression of carbamoyl phosphate synthase (CPS1), which is a highly expressed urea cycle enzyme in hepatocytes, and cytochrome P450 enzyme (CYP) 3A4, which is found in most activated hepatocyte-like HepaRG cells. At the same time, a NC clone that had all 3 enzymes activities was also selected. The following experiments are in progress in both OTC-KO and NC clones to investigate the detailed effects of N-carbamylglutamate treatment in this OTCD model.

126) THE QUESTIONNAIRE SURVEY OF QUALITY OF LIFE OF CHILDREN WITH INHERITED METABOLIC DISORDERS AND THEIR FAMILY MEMBERS

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Objectives: There are about 19,000 patients with an inherited metabolic disorder (IMD) in Japan in 2013. There is no research mainly focused on QoL of not only children also family members in Japan. We examined the related factors of QoL of children and their families.

Methods: We conducted a questionnaire survey for Japanese children with IMD and their mothers, fathers and siblings from August to October 2015. To reveal the related factors of QoL, we set the parents' scores of WHOQOL26 as the objective variable and 14 factors as explanatory variables

for multiple regression analysis. We conducted correlation analysis for the scores of KINDL of children with IMD and their siblings, and for assessing relation of each family members' QoL. The significant level was set at less than 5%.

Results: The related factors of mothers' QoL were parental stress ($B = -.589$) and family empowerment ($B = .194$) and cognition of household economy ($B = .162$) and a degree of burden of raising a child with IMD ($B = -.103$). The related factors of fathers' QoL were cognition of household economy ($B = .348$) and cognition of relationship between his child with IMD ($B = .392$) and anxiety about raising his child with IMD ($B = -.276$).

Conclusion: We revealed parents' QoL were affected by raising a child with IMD, and directly. We will investigate how parent's QoL would be affected by the severity of IMD and the condition of illness of children.

127) RECURRENT KETOTIC HYPOGLYCEMIA, LACTIC ACIDOSIS, AND HYPERAMMONEMIA DUE TO UBIQUINOL-CYTOCHROME C REDUCTASE CORE PROTEIN II DEFECTS RESULTING IN RESPIRATORY CHAIN COMPLEX III DEFICIENCY

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Background: Mitochondrial ubiquinol-cytochrome c reductase (UQCR) core protein II, encoded by the nuclear gene UQCRC2, is an essential component of respiratory chain complex III. Three patients (5-year female, 4 year male, and 18-month female) with a homozygous UQCRC2 deleterious mutation (p.Arg183Trp) from a Hispanic family have been reported. Their clinical presentations were unique and characterized by neonatal/early infantile onset recurrent metabolic acidosis, ketosis, hypoglycemia, lactic acidosis, and hyperammonemia.

Cases: Patient 1 was a full term Hispanic female and a product of a second cousin marriage. She weighed 2329 g (5–10th%iles) with a length of 46 cm (5–10th%iles), and her OFC was 34 cm (25–50th%iles). Her Apgar scores were 81, 95, and 910. She developed a severe metabolic acidosis within 1 day, requiring admission to a neonatal intensive care unit. Blood lactate and pyruvate on admission were 25.5 mM and 0.436 mM, respectively (L/P ratio = 58). Blood ammonia was 126 μ M (ref: < 80 μ M). The patient responded promptly to supportive therapy with IV glucose infusion and a sodium bicarbonate drip, improving the blood lactate levels within 24 h. Urine organic acid analysis on admission was remarkable for massive lactic and pyruvic aciduria, as well as ketonuria. Plasma amino acids were remarkable for a high alanine level (1,519 μ M). She was hospitalized more than 10 times because of episodic metabolic decompensation with lactic acidosis (highest value was 10.8 mM at age 3 years and 10 months), hyperammonemia (highest value was 346 μ M at age 3 years and 3 months), ketosis, and hypoglycemia, which were triggered by intercurrent illnesses including fevers, vomiting, and diarrhea. The patient is now age 8 years, with normal growth and no signs of intellectual disability. Patient 2 was a younger full sibling of patient 1. His Apgar scores were 81 and 95. He developed tachypnea, grunting, and poor feeding within 1 day with hypoglycemia and lactic acidosis. He was intubated for 2 days and treated with intravenous glucose infusion and a bicarbonate drip to correct the metabolic acidosis. At age 8 months, he was found unresponsive after 6 h of fasting owing to decreased appetite associated with mild URI. At a local ER, ketotic acidosis (pH 7.23), hypoglycemia (3 mg/dl), and hyperammonemia (463 μ M) were noted. He had five episodes of generalized seizure in this episode. He had more than 10 similar episodes triggered by intercurrent illnesses. He is now 7 years of age with normal growth and no signs of intellectual disability. Both siblings have been treated with high carbohydrate diet and fasting precautions.

Conclusions: Two patients with UQCRC2 defect showed episodic ketotic hypoglycemia, lactic acidosis and hyperammonemia, which promptly responded to IV glucose infusion therapy. UQCRC2 defects may involve multiple metabolic pathways including fatty acid oxidation and urea cycle. It is noteworthy the normal growth and development of both patients despite repeated severe metabolic decompensations.

128) THE STATE OF NEWBORN SCREENING FOR HEREDITARY TYROSINEMIA TYPE 1 (HT-1) IN THE UNITED STATES IN 2015

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Introduction: Hereditary tyrosinemia type 1 (HT-1) is a rare autosomal recessive disease that can cause liver failure and death within the first years of life if not detected early. Newborn screening (NBS) is necessary for early asymptomatic diagnosis and ensuring an improved HT-1 patient prognosis. While elevated tyrosine can be a marker for HT-1, it is not specific for the disease and can be falsely normal in early infancy. Succinylacetone (SUAC) is a specific diagnostic marker for HT-1 that represents a significant improvement over screening via tyrosine levels. However, across the US, NBS programs continue to use both tyrosine and SUAC assays. We are interested in understanding the shift amongst NBS programs from tyrosine to SUAC as an HT-1 assay, noting how this shift can enable accurate timely diagnosis and potentially reduce symptomatic HT-1 presentation.

Method: We conducted online research, augmented by telephone and email correspondence with state newborn screening programs, during the 2nd quarter of 2015 to develop a comprehensive picture of the current state of HT-1 newborn screening throughout the United States.

Results: Of 50 states, 39 currently test for SUAC as a primary marker for HT-1, including Virginia and Indiana, which have implemented SUAC since 2013. Five states use SUAC as a secondary marker. The other six states use tyrosine alone as an assay for HT-1 NBS. Further adoption of SUAC testing is on the horizon, with Arizona and North Carolina currently piloting the assay.

Conclusion: While progress has been made toward uniform NBS via SUAC for HT-1, the use of the SUAC assay is still not consistently implemented in the United States. Broader implementation of SUAC testing is contingent upon the priority a given state assigns to funding the upgrade, piloting, validating, and optimization of the assays. Additionally, positive HT-1 patient specimens are required for successful validation of the assays, a challenge faced by at least one state. Piloting and validation of SUAC testing could be expedited through collaboration and sharing of information between all states. Only when all 50 states are testing for SUAC as a primary marker in newborn screening can we be confident in effectively eliminating the symptomatic presentation of HT-1.

129) SIMULTANEOUS QUANTIFICATION OF ALPHA-AMINOADIPIC SEMIALDEHYDE/PIPERIDEINE-6-CARBOXYLATE AND PIPECOLIC ACID IN PLASMA AND URINE

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Increased levels of pipecolic acid, alpha-aminoadipic semialdehyde (AASA) and its cyclic form delta-1-piperideine-6-carboxylate (P6C) are characteristic of pyridoxine dependent epilepsy (PDE), a rare disorder of inborn error of metabolism. Recent studies showed the effectiveness of dietary therapy in PDE patients and emphasized the importance of the assessment of these metabolites for monitoring treatment efficacy. We developed a robust and sensitive method for simultaneous quantification of AASA-P6C and PA in plasma and urine. Plasma and urine samples were derivatized with HCl-butanol and injected onto ACQUITY BEH-C18 column. A gradient of water/methanol containing 0.1% formic acid was used for the chromatographic separation of AASA, P6C and pipecolic acid. The analytes' concentrations were calculated using their calibration curves and the sum of AASA and P6C (AASA-P6C) was calculated. To evaluate the clinical utility of this test, samples from unaffected controls and patients with confirmed PDE were analyzed. The performance characteristics of the assay as well as sample stability and interferences were determined. The intra- and inter- assay imprecisions were <2.9% and <10.9% for AASA-P6C, and <3.3% and <12.6% for pipecolic acid, respectively. Reference ranges for AASA-P6C and pipecolic acid in plasma and urine were established. Comparison of values obtained from unaffected controls and PDE patients showed high clinical sensitivity and specificity of the assay. Thus, this novel method for the simultaneous quantification of AASA-P6C and pipecolic acid in plasma and urine can be used in a clinical laboratory setting for the diagnosis and monitoring of patients with PDE.

130) LYSINE RESTRICTION AND ARGININE SUPPLEMENTATION IN TREATMENT OF TWO PATIENTS WITH PYRIDOXINE DEPENDENT EPILEPSY

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Pyridoxine Dependent Epilepsy (PDE) is a recessive disorder of lysine metabolism characterized by intractable seizures controlled by the administration of pharmacological doses of vitamin B6. Despite seizure control with pyridoxine, intellectual disability and developmental delays are often observed in PDE patients, likely due to the accumulation of toxic intermediates in the lysine catabolic pathway: alpha-aminoadipic semialdehyde (AASA), delta-1-piperideine-6-carboxylate (P6C) and pipecolic acid. Dietary therapies, such as lysine-restriction and/or arginine supplementation, can reduce the accumulation of these PDE biomarkers and appear to improve neurodevelopmental outcomes in some PDE patients. Here we evaluate biochemical and clinical parameters in two PDE patients treated with a lysine restricted diet and arginine supplementation (100–150 mg/kg). Lysine restriction through the use of reduced protein intake and/or medical formula resulted in decreased accumulation of PDE biomarkers and developmental improvements. The levels of AASA-P6C and pipecolic acid directly correlated with lysine concentrations in plasma ($p = 0.0015$, $r^2 = 0.6136$ and $p = 0.0013$, $r^2 = 0.6234$, respectively). Although there was no inverse correlation of PDE biomarkers with plasma arginine, additional improvements in biochemical and neurocognitive outcomes were observed with the initiation of arginine supplementation. In addition, plasma concentrations of threonine strongly correlated with the levels of AASA-P6C and pipecolic acid ($p = 0.0003$, $r^2 = 0.7133$ and $p = 0.0008$, $r^2 = 0.6587$, respectively), suggesting a sensitivity of threonine catabolism to pyridoxine availability. Thus, our results further support the use of dietary therapies in combination with pyridoxine for the treatment of PDE.

131) THE N-ACETYL-L-GLUTAMATE BINDING SITE OF HUMAN CARBAMYL PHOSPHATE SYNTHETASE 1: STRUCTURAL STUDIES OF N-ACETYL-L-GLUTAMATE BINDING DOMAIN

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Background: Carbamyl phosphate synthetase 1 (CPS1) is active only in the presence of its essential allosteric activator, N-acetyl-L-glutamate (NAG), which is produced by N-acetyl-L-glutamate synthase. It was proposed that NAG binding site is located at the last allosteric domain of CPS1. However, the exact NAG binding site and the mode of binding remains unknown.

Methods: The crystal structure of the NAG binding domain of human CPS1 deposited in the Protein Data Bank (PDB ID: 2YVQ) was re-visited. The structure was re-refined against the deposited data. The recombinant NAG binding domains of human and mouse CPS1 were expressed in *E. coli*, purified and crystallized.

Results: An unrecognized electron density was found at a site proposed to be corresponding to the NAG binding site based on the deposited data from protein data bank (2YVQ). Structure modeling suggested that the bound chemical is threo-3-hydroxy-D-aspartate. Moreover, the missing loop, Gly1415-Gln1416-Asn1417-Pro1418-Ser1419, could be reasonably modeled into the density. The structural and enzymatic studies of our recombinant CPS1 protein confirm the NAG binding site, mode of binding of threo-3-hydroxy-D-aspartate.

Conclusions: NAG binding site was elucidated out from the structural studies of the NAG binding domain of CPS1. Threo-3-hydroxy-D-aspartate binds CPS1 using the NAG binding site.

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